

INFLUENCE OF THE ORAL SURGICAL INTERVENTION TO THE ACUTE PHASE PROTEIN (CRP) VALUES IN CARDIOSURGICAL PATIENTS

Sadeta Šečić^{1*}, Selma Zukić², Amra Ahmić²,
Amila Zukanović³

¹ Department of Oral Surgery with Dental Implantology, Faculty of Dentistry with Clinics, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

² Department of Dental Morphology, Anthropology and Forensics, Faculty of Dentistry with Clinics, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

³ Department for Pediatric and Preventive Dentistry, Faculty of Dentistry with Clinics, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

*Corresponding author

Sadeta Šečić, Ph.D, Professor
Faculty of Dentistry with Clinics
University of Sarajevo
Bolnička 4a
71000 Sarajevo
Bosnia and Herzegovina
Phone: +387 33 214249
Email: sadetasecic@gmail.com
ssecic@sf.unsa.ba

ABSTRACT

Modern research, confirming the role of infection in pathogenesis of atherosclerosis, is based on proofs regarding the presence of microorganism inside the wall of an atherosclerosis blood vessel. Data show the influence of oral cavity bacteria in forming a thrombus as well as the presence of high values of acute phase proteins. Our research was conducted on patients whom were, during preoperative cardio surgical procedure, diagnosed with dental bacterial focus which required oral surgical intervention. All patients were tested for acute phase protein, C-reactive protein (CRP) before oral surgical intervention, two days after and three weeks upon cardio surgical procedure. Kruskal Wallis test indicated that there is no significant statistical difference when the sex, number of extracted teeth as well as presence of certain microorganism tested for C reactive protein values, were compared. Statistically, most significant differences were in C reactive protein values in situations between, before and after tooth extraction ($p < 0,01$), and CRP drops in certain measure after cardio surgery. It may be concluded that after removing teeth the CRP values are decreasing.

Key words: oral surgery, CRP, cardiosurgical patients

Introduction

Previously described as a degenerative disease, atherosclerosis is nowadays described in literature as a chronic inflammatory disease in which development participate humoral and cell immune reactions. Atherosclerosis is a progressive disease where big and medium sized muscle and big elastic arteries are occluded by fibro-lipid changes. It occurs as excessive inflammatory-proliferative answer to different forms of endothelium damages. In its essence, this is a chronic process which gradually progresses by maturing of body passing through more stadiums. [1] Atherosclerosis is a systematic dysfunction of endothelium, chronic inflammatory fibro-proliferation and pro-thrombotic focal disease of artery intima. Atherosclerosis is a consequence of atherogenesis, inflammation and thrombosis. There is no precise data regarding the incidence of atherosclerosis but the epidemiology data are related to the clinical appearances or to its most important consequences: coronary disease, cerebrovascular and periphery vascular atherothrombosis. [2, 3]

Development of fundamental sciences revealed the role of inflammation in occurrence of all phases of atherosclerosis, starting from the initiation of the process, over its progression to superimposed thrombosis complications. It is a reason that the blood vessels damage and accompanied inflammatory response to damage are nowadays quoted as the essential components of atherogenesis. Numerous researches' data indicate that a chronic, weakly expressed inflammation is an important factor in atherosclerotic cardiovascular disease. [4] Studies confirming this may be found in different medical branches such as cell biology, epidemiology, clinical researches and experimental researches on animals. They unanimously speak in favor of the fact that inflammatory components are included in atherosclerotic lesions. Cell interaction in atherogenesis is similar to the one present at chronic and inflammatory-proliferative disease. Atherosclerotic lesion represents a series of highly specific cell and molecular responses and it could be described as inflammatory disease. [5]

Still, factors initiating and supporting these inflammatory processes have not been thoroughly revealed. Many researchers believe that oxidized and heat-shock proteins (HSPs) may provoke inflammatory response and contribute to munching inflammation in atherosclerotic plaque. Certain authors quote a possibility of autoimmune damage of the blood vessel wall that develops as an answer to the presence of these proteins. Potential factor, being a subject of growing interest because of the possibility to induce both inflammatory and autoimmune answer, is infection as well. [6] Facts of infection's contribution in pathogenesis of atherosclerosis are based on the presence of

infective agents inside the wall of atherosclerotic blood vessel and on seroepidemiological researches indicating the connection of specific IgG antibodies and atherosclerosis. [7]

It is possible to found infective agent in atherosclerotic lesion as a consequence of direct infection of the blood vessel. Later on, pathogen microorganism persists in the vessel's wall in latent stage producing abortive infection. Infection agents may also participate in development of endothelium dysfunction by changing phenotype of endothelium cells from normal anticoagulation into pro-coagulation stadium with deterioration of vasodilatory response being dependent on endothelium. [8, 9]

Although all pathophysiology mechanisms bringing to cardiac and cerebral complications are not fully known, researches indicate the role of certain bacteria in oral cavity (*streptococcus sanguis*, *porphyromonas gingivalis*, etc.) and their connection to thrombus production. It is proved that periapical processes and paradontopathy are connected to the series of systemic diseases and have influenced coagulation factors because they represent a mixed tissue infection. [10]

Infection does not have to participate in pathogenesis of atherosclerosis through mechanisms of local blood vessel wall demanding. Infection causes whole specter of systemic effects, including the changes in circulation level of cytokine, reactants of the acute phases and responses mediated by immune system. Mechanisms through which system inflammation response may express effects to damaged blood vessel cell are different. Interaction of cardiovascular diseases and oral infections is well known and it is specifically related to bacteremia of oral origin as the source of microorganisms which may damage swings causing bacterial endocarditis. [9] The answer of acute phases is a common name for events in the organism during the infection but also some other conditions such as: tissue damage, immunology reactions and inflammatory processes. Human organism contains large number of different proteins. It is assumed that 30 000 to 50 000 structural genes participate in coordinating synthesis of those proteins. Between 3.000 and 5.000 different proteins can be identified in one cell while more than 300 and more may be isolated from plasma. Some of them are in specific physiological or pathological states. Many proteins are structural elements of cells or tissue organizations while the other are soluble, free in intra cell and/or extra cell liquids. Majority of plasma proteins are synthesized in liver with the exception of immunoglobulins and protein hormones. Numerous extra vascular liquids contain certain quantities of plasma proteins. Type and relation of certain proteins in extra vascular liquids is determined by molecule masse and by specific nature of their transport mechanism. It is important to point out that

different diseases contribute to characteristic exchange of the quantity and relations of certain proteins. The most examined proteins in clinical practice are blood plasma proteins, or in other words, serum. Plasma proteins are complex mixture of not only simple proteins but also the complex ones such as glycoproteins and lipoproteins. In plasma, they express a series of common functions, although almost each and every of present protein has certain number of specific functions.

One of important function of serum protein is the maintenance of osmotic balance between blood and interstitial liquid. Plasma proteins are responsible for about 25 mmHg of total osmotic plasma pressure. Drop in serum protein concentration leads to disturbance of balance between osmotic and hydrostatic pressure and edema appearance. Proteins, then, participate in regulating electro-chemical blood reactions. As amphoteric compounds, they may react with acid and base equivalents. [11] In circulation, proteins transport a series of substances which are by their nature insoluble in water. Proteins of acute phases are structural and functional diverse group of proteins being synthesized in liver and their level extremely increases inside first hours and days of infection or any other condition associated with tissue damaging. Existence of the acute phase proteins and their activity in human plasma was found in 1894 by Fermi and Pernossi. [12] They include a series of proteins: C-reactive protein (CRP), G-2 globulin, fibronectin, haptoglobin, ceruloplasmin. Proteins of acute phase are indirect markers of monocytes' biological activity because their synthesis and serum concentration are changed as the response to production of pro-inflammatory cytokine. C-reactive protein is the most important positive protein of acute phase and non-specific marker of systemic inflammation on low level. CRP is a member of pentraxin family of proteins composed from five identical non-covalently bound subunits of molecularly mass from 25 kDa. It has molecular mass of 110 kDa and its concentration is determined by biochemical tests in serum and plasma. [13] To evaluate concentration of C-reactive protein, different methods are used: ELISA, quick immune diffusion and visual agglutination but mostly immune-turbidimetry is applied.

CRP is mostly used as the indicator of the acute inflammatory reaction. It is important for both evaluation of illnesses and effectiveness of treatment. Indications for measuring concentration of CRP are: search for inflammatory processes especially in primary care; confirmation of the presence of acute organic disease or chronic conditions; diagnosis and follow up of the infection when microbiology testing is slow or impossible. Increased CRP values appears in blood after 6-9 hours from the infection's beginning and the highest values at 1-3 days later. CRP values at inflammation grow faster than sedimentation and leucocyte number in blood and in safer way differ

virus from bacterial infection because at bacterial infection the growth is significantly more expressed. In the case of virus infections with increased sedimentation and increased number of leucocyte, CRP remains at lower values from those expected for bacterial infection. During successful therapy with antibiotics, values of CRP in blood drop faster than the level of sedimentation thus it is possible to void unnecessary consumption of ineffective drugs. Factors which importantly change the CRP values in serum may be biologic (high age, high elevation, increased physical efforts, pregnancy, smoking) and analytic (lipemia, rheumatism factors). [14] Important growth of the C-reactive protein concentration in plasma, although nonspecific, happens during the myocardium infarct, stress, trauma, inflammatory processes and neoplastic proliferation. Values may grow even 2000 times in relation to normal level. Determination of the C-reactive protein concentration is of the clinical importance to screening organic diseases, evaluation of the activities of certain inflammatory diseases, detection of inter-current infections. CRP values are especially important for differentiation of bacterial from virus infection. In early 1980ies, researches started to find out whether the basal level of C-reactive protein under 10 mg/l at currently healthy individuals may be used as a prognosis factor. Only in 1990ies methods for accurate measuring of very low levels of CRP characteristic for healthy individuals were developed. Researchers searched for correlation between a series of inflammatory markers and the occurrence of heart diseases. CRP levels have not been only faithful indicator for the disease appearance but CRP proved to be significantly better marker than all others. Almost all inflammatory molecules including interleukin-6, cell adhesion molecules, factors of tissue necrosis, were increased at patients suffering from heart diseases but neither of those molecules was not biologically stable such as CRP nor has such a wide range of concentration. Even among healthy individuals, CRP levels have wide spectrum of values ranging from 0.01 mg/l to 10 mg/l providing the evaluation of low, middle and high predisposition for illness. [15,16]

Aim of this paper is to evaluate the influence of the oral surgical procedures on values of acute phase protein (CRP).

Material and methods

During our research, we processed 100 patients who have had need for oral surgery procedures with indication for cardio vascular procedure afterwards. Based upon clinical examination and radiographic recordings evaluation, diagnosis for potential dentogenic focal points

was set. In cooperation with competent cardiologist, existing therapy was included or corrected followed by the first test of C-reactive protein values.

According to the protocol from the Institute for clinical bio-chemistry of the Clinical Center University in Sarajevo the values of C- reactive protein were determined by applying latex method. After 24 hours, the planned tooth extraction was performed and two days after the procedure of determining the values of quoted parameter was repeated. The same procedure of determining the values of this protein of acute phase was performed on the twenty first day after cardio surgical intervention. The very oral surgical procedure included a tooth extraction in local anesthesia. Prior to tooth extraction, the patients rinsed oral cavity with 0, 05 % solution of chlorine hexidine digluconata. At each extracted tooth, by fissure drill, we resected root top and with sterile stick took smear for microbiological examination. Smears for aerobe were placed in enriched liquid tioglicolat base and for anaerobe we applied the same procedure adding to the tioglicolat base a paraffin oil layer. Such seeded bases in 15 minutes time were transported to Microbiology ward of the General Hospital Sarajevo where they performed the processing of biologic material. Bases were kept in laboratory within thermostat at 37 degrees C through 24 hours. After that, the bases for aerobe were seeded on blood agar and then to Mc Conkey agar. All preparations were colored by Gram. Gram negative bacteria were proved by biochemical seeding on shortened biochemical series composed from doubled Kligler sugar, urea agra, peptone water, methyl red, Simson citrate, one percentage saccharose and one percentage manit. On the basis of biochemical activity and presence of certain enzyme, identification of Gram negative bacteria was performed. Parallel, antibiogram disc was done on Muller-Hinton bases. Gram positive bacteria were identified by microscope preparations colored by Gram from colonies growing on blood agar. The following tests were performed: bacitracin test, optohin test and if necessary the growth on esculine bases. For Gram positive bacteria, antibiogram was done on blood agar also by disc method. For the sake of quantification of bacteria and additional bacteriological control with blood agar which was incubated in Gass Pak anaerobe system along with additional Gas generating box "H2+CO2". Certain forms of bacteria were identified on preparation and for the others the incubation in Gas pack chamber was extended to 5 days. After that, the chamber was opened and the growth of colonies or their morphological characteristics was followed. Preparation by Gram was made again from them being compared with previous preparations. On the basis of morphology, length of incubation and coloring by Gram, certain types of anaerobe bacteria were identified. Along with seeding on anaerobe bacteria in Gas pack chamber, the seeding on blood agar

was performed being incubated in aerobe conditions caused by eventual presence of facultative anaerobe-aerobe bacteria. After completion of oral-surgical procedure with following microbiological processing, patients were directed to further treatment into Clinic for heart of the Clinical Center of University in Sarajevo where within regular protocol for cardio surgery the values of same parameters have been determined. The values of quoted parameters were measured again three weeks after performed cardio surgery.

Results

Results of research obtained by Kruskal Wallis Test represented in **Table 1** indicate that quantity of reactants at men and women in different experimental situations is not statistically significant because neither parameter p is not lower than 0.05 being the same as when we tested the influence of the number of extracted teeth and the presence of certain type of bacteria (**Table 2, Table 3**).

Table 1. Testing the CRP presence in different experimental situations regarding the sex

Kruskal Wallis Test	CRP-prior to tooth extraction	CRP-after tooth extraction	CRP-after cardio surgery
Chi-Square	0,248	1,645	0,326
df	1	1	1
Asymp.Sig.(p)	0,619	0,200	0,568

Table 2. Testing the CRP presence in different experimental situation regarding the number of extracted teeth

Kruskal Wallis Test	CRP-prior to tooth extraction	CRP-after tooth extraction	CRP-after cardio surgery
Chi-Square	1,606	0,254	0,163
df	1	1	1
Asymp.Sig.(p)	0,205	0,614	0,686

Table 3. Testing the CRP presence in different experimental situations regarding bacteria presence

Kruskal Wallis Test	CRP-prior to tooth extraction	CRP-after tooth extraction	CRP-after cardio surgery
Chi-Square	2,003	4,432	4,640
df	3	3	3
Asymp.Sig.(p)	0,572	0,218	0,200

Table 4. Results of Friedman test

FRIEDMAN TEST	
N	100
Chi-Square	100,34
df	2
Asymp.Sig.(p)	0,01

Table 5. Results of post-hoc test for Friedman test regarding significance of differences in CRP quantity between three experimental situations

Wilcoxon Signed Ranks Test	CRP-after tooth extraction- CRP-prior to extraction	CRP-after cardio surgery- CRP-prior to tooth extraction	CRP-after cardio surgery CRP-after tooth extraction
Z	-3,91	-7,89	-7,21
Asymp.Sig.(p)	0,01	0,01	0,01

Results of Friedman test showed that statistical differences between experimental situations for the CRP quantity indicates that arithmetic mean of CRP is significantly decreased after the tooth extraction and also after the cardio surgery where p is lower than 0,001. (Table 4)

Analyzing post hoc test in Table 5, all possible parameters of three experimental situations were placed into relation and it may be concluded that after removing teeth the presence of CRP drops in certain measure and it is especially the case after cardio surgery.

Discussion

Having in mind the fact that the number of cardio vascular diseases are increasing, the scientists find out epidemiological and experimental proves which on daily basis confirm extremely important role of inflammation in development of all phases of atherosclerosis, starting from initial processes, over progression to later thrombolytic complications. [17] Many pathophysiological mechanisms may explain the association between immunity, infection, inflammation and atherosclerosis with all its complications. It is probably about complex and multi-factorial mechanisms which differ from patient to patient demanding synergy with classic risk factors in order to bring to acute coronary syndromes having cardio surgery as justified consequence. [18,19] Numerous researches indisputably confirm the existence of mutual influences between periodontal and certain systematic diseases. Contem-

porary studies show that there is connection between dentogenic infection and cardio vascular diseases. It is well known that dentogenic diseases represent a mixed infection caused by primarily anaerobe bacteria.

In their researches Kweider with assistants and then in separate researches Loss and Wu with their assistants studied and confirmed the connection of periodontitis and risk from cardio vascular diseases. They proved, by their researches, that the patients with periodontal diseases have increased CRP values and increased number of leucocytes in comparison to healthy respondents in the control group. [20, 21, 22]

Researches from different scientific fields revealed data which explicitly show that chronic, or continuous inflammation is an important factor in atherosclerosis occurrence and they all identically point out that atherosclerotic lesions include inflammatory component. Dentogenic infection, caused by its capacity to induce both inflammatory and autoimmune response, represent an issue of increasing interest of numerous contemporary researches.

Wu and assistants in 2000 pointed out the results of their research of connection between periodontal and cardio vascular diseases where in tested group of patients were found increased values of lipoproteins of high density and cholesterol, CRP and fibrinogen in plasma. [21]

In the same period, Slade and assistants published similar results confirming that the patients with periodontitis have one third higher values of CRP than the respondents from the control group without mentioned diseases. [23] Beck and assistants in 1996 acknowledged that atherosclerosis and periodontal diseases have similarities, and they found that both appear more often in male. In our research, we did not record statistically important difference between male and female respondents. [24] Our results show statistically important differences in concentration of tested proteins of acute phase in all periods of measurement. Researches from Wu and assistants based upon a decade long follow up of almost ten thousand respondents proved that there is a connection of periodontitis phase and diseases of cardio vascular system. [21]

Glurich and Grossi with assistants in their research in 2002, compared values of protein of acute phase with cardio vascular and periodontal disease in patients who had only one from quoted diseases thus representing a control group. By results of their research, they confirmed that the values of CRP ($p < 0.028$) are bigger at patients with both diseases in comparison to other tested groups. Statistically important increase of the CRP value was previously recorded only at patients with cardio vascular disease, but modern clinical studies prove that such

increased values are present also in periodontal disease. This research confirmed that CRP is tripled increased in patients with cardio vascular and periodontal diseases. [25]

Conclusion

Values of evaluated parameter (C-reactive protein) through experimental situations did not show significant statistical differences regarding the gender of respondents. Test of the independent samples showed no statistically important difference in testing the value of the CRP in relation to the presence of certain types of microorganisms. Statistically, the most important difference of the value of C – reactive protein is found before and after oral surgical intervention and after cardio vascular intervention ($p=0,01$) thus the results of our researches showed the feasibility of our set goals.

References

- Ross R Atherosclerosis an inflammatory disease. *N Engl J Med* 1999;340:115
- Beck JD, Elter JR, Heiss G, Couper D, Mauriello SM, Offenbacher S. 2001. Relationship of periodontal disease to carotid artery intima-media wall thickness: The Atherosclerosis Risk in Communities (ARIC) study. *Arterioscler Thromb Vasc Biol.* 21(11): 1816–1822
- Libby P, Ridker PM, Maseri A: Inflammation and atherosclerosis. *Circulation* 2002;105: 1135-1143
- D'Auto F, Parkar M, Andreou G, Suvan J, Brett P.M, Ready D, Tonetti M.S. Periodontitis and Systemic Inflammation: Control of the Local Infection is Associated with a Reduction in Serum Inflammatory Markers. *J Dent Res* 83(2): 156-160, 2004
- Epstein SE, Zhou YF, Zhu J: Infection and atherosclerosis: Emerging Mechanistic Paradigms. *Circulation* 1999; 100: 20-28
- Ford PJ, Gemmell E, Chan A, Carter CL, Walker PJ, Bird PS, West MJ, Cullinan MP, Seymour GJ. Inflammation, heat shock proteins and periodontal pathogens in atherosclerosis: an immunohistologic study. *Oral Microbiol Immunol.* 2006 Aug;21(4):206-11.
- Haraszthy VI, Zambon JJ, Trevisan M, Zeid M, Genco RJ. Identification of periodontal pathogens in atheromatous plaques. *J Periodontol.* 2000;71: 1554–1560
- Nakamura Y, Tagusari O, Seike Y, Ito Y, Saito K, Miyamoto R, et al. 2011. Prevalence of periodontitis and optimal timing of dental treatment in patients undergoing heart valve surgery. *Interact. Cardiovasc. Thorac. Surg.* 12:696–700
- Slade GD, Offenbacher S, Beck JD, Heiss G, Pankow JS. Acute-phase inflammatory response to periodontal disease in the US population. *J Dent Res.* 2000;79: 49–57
- Darveau R. P. 2010. Periodontitis: a polymicrobial disruption of host homeostasis. *Nat. Rev. Microbiol.* 8:481–490
- Salminen A, Kopra K.A, Hyvärinen K, Paju S, Mäntylä P, Buhlin K, et al. 2015. Quantitative analysis of salivary pathogen burden in periodontitis. *Front Cell. Infect. Microbiol.* 5:69.
- Matilla K, Nieminen M, Valtonen V, Rasi V, Kesaniemi Y, Syrjala S, Jungul P, Isoluoma M, Hietaniemi K, Jokinen M, I Huttunen J. (1989). Association between dental health and acute myocardial infarction. *British Medical Journal* 298,779-782
- Ridker PM, Hennekens CH, Buring JE, Rifai N. C-Reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 2003;342:836-43
- Pepys MB, Gideon M. Hirschfield - C-reactive protein: A critical update. *J Clin Invest* 2003;111:1805-12.
- Chalmers JD, Singanayagam A, Hill AT. C-Reactive Protein is an independent predictor of severity in community - acquired pneumonia. *Am J Med* 2008;121:219-25.
- Ross, R. Mechanisms of disease: atherosclerosis-an inflammatory disease. *New England journal of Medicine* 340,115-126
- Tushika Bansal, Anita Pandey, Deepa D, and Ashish K Asthana J C-Reactive Protein (CRP) and its Association with Periodontal Disease: A Brief Review *Clin Diagn Res.* 2014 Jul; 8(7)
- Kapellas K, Jamieson L, Do L, Bartold P, Wang H, Maple-Brown L, Sullivan D, O'Dea K, Brown A, Celmaj DS, Slade GD, Skilton MR Associations between periodontal disease and cardiovascular surrogate measures among Indigenous Australians. *Int J Cardiol.* 2014 May 1;173(2):190-6. doi: 10.1016/j.ijcard.2014.02.015. Epub 2014 Feb 22.
- Ross R. Atherosclerosis-an inflammatory disease. *New England Journal of Medicine* 340,115-126
- Haffajee A.D, Socransky S.S. Microbial etiological agents of destructive periodontal diseases. *Periodontology* 2000 5,78-111

20. Kweider M, Lowe G.D, Murray G.D, Kinane D.F, McGowan D.A. Dental disease, fibrinogen and white cell count; links with myocardial infarction? *Scottish Medical Journal* 38,73-74
21. Wu T, Trevisan M, Genco R.J, Falkner K.L, Dorn J.P, Sempos C.T. Examination of the relation between periodontal health status and cardiovascular risk factors: serum total and high density lipoprotein cholesterol, C-reactive protein, and plasma fibrinogen. *American Journal of Epidemiology* 151,273-282
22. Loos B.G, Craandijk J, Hoek F.J, Wertheim-van Dillen P.M, van der Velden U. Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. *Journal of Periodontology* 71,1528-1534
22. Johansson CS, Ravald N, Pagonis C, Richter A. Periodontitis in patients with coronary artery disease: an 8-year follow-up. *J Periodontol.* 2014;85:417-425
23. Slade G.D, Offenbacher S, Beck J.D, Heiss G, Pankow J.S. Acute phase inflammatory response to periodontal disease in the US population. *Journal of Dental Research* 79,49-57
24. Beck J, Garcia R, Heiss G, Vokonas P, Offenbacher S. Periodontal disease and cardiovascular disease. *Journal of Periodontology* 67,1123-1137
25. Grossi SG, Skrepcinski FB, DeCaro T, et al. Treatment of periodontal disease in diabetics reduces glycated hemoglobin. *J Periodontol.* 1997;68:713-719.