Reduced systemic inflammatory mediators after treatment of chronic gingivitis

Ahmed S. Alzahrani, BDS, MSD, *Nabil F. Bissada,* DDS, MSD, *Richard J. Jurevic,* DDS, PhD, *Sena Narendran,* BDS, PhD, *Imad E. Nouneh,* DDS, MSD, *Mohammad S. Al-Zahrani,* BDS, PhD.

ABSTRACT

الأهداف: تقييم التغيرات في مستوى البروتين المتفاعل و عامل نخر الورم الفا في السائل اللثوي الفلعي بعد علاج التهاب اللثة المزمن في الأفراد الأصحاء.

الطريقة: هذه تجربة سريرية أجريت في جامعة كيس ويسترن ريزرف، كليفلاند، أوهايو، الولايات المتحدة الأمريكية خلال الفترة من فبراير إلى ديسمبر 2011م. تم تعيين مجموعه 41 من الاشخاص الأصحاء إلى مجموعتين وفقا لشدة التهاب اللثة لديهم. تألفت المجموعة الأولى من 18 شخصاً ممن كانوا يعانون من التهاب لثوي خفيف، وتألفت المجموعة الثانية من 23 شخصاً ممن كانوا يعانون من التهاب لثوي أشد. تألف تقييم اللثة من قياس المؤشر اللثوي وعمق السبر وحجم السائل اللثوي الفلعي. كررت نفس القياسات بعد 6–4 أسابيع على أثر العلاج الوقائي وإعطاء إرشادات لحفظ صحة الفم و تم تحديد مستوى البروتين المتفاعل وعامل نخر الورم الفا في السائل اللثوي الفلعي باستخدام مقايسة الممتز المناعي المرتبط بالإنزيم.

النتائج: وجد انخفاض ذات دلالة إحصائية في مستويات البروتين المتفاعل وعامل نخر الورم الفا بعد العلاج في المجموعة التي كانت تعاني من التهاب لثوي شديد خلافاً للمجموعة التي كانت تعاني من التهاب لثوي خفيف. وأظهرت كلا المجموعتين انخفاض ذات دلالة إحصائية قي قياسات المؤشر اللثوي وعمق السبر وقراءات جهاز البريوترون بعد العلاج.

خاتمة: يقلل علاج التهاب اللثة الشديد المزمن من مستويات البروتين المتفاعل وعامل نخر الورم الفا في السائل اللثوي الفلعي في الأفراد الأصحاء وهذا قد يكون له تأثيراً على منع أو التحكم بحالات الأمراض الجهازية القائمة أو التي قد تحدث مستقبلا.

Objectives: To assess the changes in the level of C-reactive protein (CRP) and tumor necrosis factor-alpha (TNF-α) in gingival crevicular fluid (GCF) after treatment of chronic gingivitis in systemically healthy individuals.

Methods: This is a clinical trial conducted at Case Western Reserve University, Cleveland, Ohio, United States of America from February to December 2011. A total of 41 systemically healthy subjects were assigned to 2 groups according to the severity of gingival inflammation. Group I consisted of 18 subjects who had mild gingival inflammation; and group II consisted of 23 with more severe gingival inflammation. Periodontal assessment consisted of gingival index (GI), probing depths (PD), and GCF volume. Four to six weeks after prophylaxis and oral hygiene instruction, the same measurements were repeated. The level of CRP and TNF-α in the GCF was determined using enzyme-linked immunosorbent assays.

Results: A statistically significant reduction in the mean CRP and TNF-α levels after the treatment was found in the severe, but not in the mild gingivitis group. Both groups showed a statistically significant reduction in GI, PD, and periotron readings after the treatment.

Conclusion: Treatment of severe chronic gingivitis reduces the levels of CRP and TNF-α in GCF of otherwise systemically healthy individuals, which could have an impact on preventing or controlling future or existing systemic disease conditions.

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From the Departments of Periodontics (Alzahrani, Bissada, Nouneh), Biological Sciences (Jurevic), and Community Dentistry (Narendran), Case Western Reserve University, Cleveland, Ohio, United States of America, and the Department of Periodontics (Al-Zahrani), King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia.

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Address correspondence and reprint request to: Dr. Nabil F. Bissada, Department of Periodontics, School of Dental Medicine, Case Western Reserve University, 2124 Cornell Road, Cleveland, Ohio 44106-4905, United States of America. Tel. +1 (216) 3686752. Fax. +1 (216) 368-3204. E-mail: nxb4@case.edu

Plaque-induced gingivitis is a common chronic condition affecting approximately 90% of American adults.[1] It is a reversible inflammation of the gingiva caused by bacterial plaque, and may progress to affect deeper periodontal structures if not treated. Periodontal infection is a potential risk factor for cardiovascular diseases, adverse pregnancy outcomes, and poor glycemic control of diabetics.[2] Patients who suffer from chronic periodontitis, have elevated systemic markers such as C-reactive protein (CRP) and tumor necrosis factor-alpha (TNF-α).[3-5] Bazile et al[6] reported that gingival inflammation may be considered a significant risk factor for coronary artery diseases. C-reactive protein is a circulating protein that may have significant proinflammatory effects resulting in complement activation, and may exacerbate tissue damage, leading to increased severity of an existing disease.[7] Several studies had shown a beneficial effect of periodontal treatment on reducing CRP in patients with periodontitis.[8-13] In 2007, Lalla et al[14] found a 37% decrease in serum CRP level after periodontal therapy. Tumor necrosis factor-alpha increases synthesis of prostaglandins, which lead to amplification of the inflammatory response that degrades connective tissue and causes osteoclastic bone resorption.[15] Navarro-Sanchez et al[16] found a significant reduction in gingival crevicular fluid (GCF) concentrations of interleukin (IL)-1β and TNF-α 90 days after periodontal treatment in patients with type 2 diabetes and chronic periodontitis.

Gingival crevicular fluid flow is primarily the result of increased permeability of the blood vessels underlying the junctional and pocket epithelium. The GCF shows a similar immuno-electrophoretic pattern in comparison with blood serum and its composition mirrors that of the plasma. Therefore, biochemical analysis of the GCF may be used to assess how periodontal disease influences systemic host response.[17] There is evidence supporting the association between periodontal diseases and systemic inflammatory marker levels.[3-5] Several investigators reported a positive association between mild, moderate, and severe types of chronic periodontitis and changes in the inflammatory markers. The increase of CRP and TNF-α levels in the blood serum may play a role in the progression of systemic diseases.[18] Furthermore, there are reports indicating that the levels of these markers decrease after periodontal treatment.[8-14] These previous studies, however, focused on periodontitis, and a literature search of PubMed and Scopus databases revealed no specific study that evaluated the effect of chronic gingivitis treatment on CRP and TNF-α. The objective of the present study was therefore, to examine the impact of the treatment of gingivitis on the levels of the TNF-α and CRP in GCF among systemically healthy subjects with mild or severe chronic gingivitis.

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Methods. The Institutional Review Board at Case Western Reserve University approved the protocol for this study that was carried out between February and December 2011, and which was in compliance with principles of the Helsinki Declaration. One hundred and nine subjects were screened at Case School of Dental Medicine from the dental admitting clinic. After screening, 41 subjects agreed to participate in the study and signed a written consent form. Subjects' age, gender, and medical and dental history were obtained and recorded. Inclusion criteria were subjects who were 18 years of age or older, having at least 20 natural teeth, and had no clinical attachment loss, bone loss or probing depth greater than 4 mm. Exclusion criteria were subjects who had undergone periodontal treatment within one year prior to the study, pregnancy, patients on nonsteroidal anti-inflammatory drugs, patients with known systemic diseases, and current or former smokers. A single calibrated examiner recorded probing depths (PD) at 6 sites per tooth and gingival index (GI) scores according to Loe and Silness.[19] Gingival crevicular fluid samples were obtained from the 8 most inflamed sites based on the GI score using PerioPaper Strips (OraFlow Inc., Smithtown, NY, USA), and the volume of the fluid absorbed by each strip was determined using a chair-side located Periotron (eBioscience, Inc., San Diego, CA, USA). Before sampling, supragingival plaque was removed, and the teeth were air-dried and isolated with cotton rolls. The strips were inserted into the gingival pockets until slight resistance was felt, and left for 30 seconds, strips with blood contamination were discarded. All samples from each subject were placed together in a tube containing 300 uL of phosphate-buffered saline (PBS) and protease inhibitors and shook for one minute at 1800 RPM at room temperature. The paper strips were mixed for 30 minutes, then the strips were removed and the fluid was immediately frozen at -80°C for later determination of the amount of CRP and TNF-α. After periodontal examination and GCF collection, oral hygiene instructions and prophylaxis

were performed on each subject. Four to six weeks later, the same clinical parameters and GCF samples were again collected from the same sites and processed in the same way (**Figure 1**). Enzyme-linked immunosorbent assay (ELISA) was used to determine the levels of CRP and TNF-α according to the manufacturer's specifications (eBioscience, Bender Med Systems GmbH, Vienna, Austria). The human CRP instant ELISA with a sensitivity of 3.0 pg/ml and standard curve range of 78-5000 pg/ml and the human TNF-α instant ELISA with a sensitivity of 1.65 pg/ml and standard curve range of 7.8-500 pg/ml were utilized. Both ELISA kits were designed to detect protein in a wide range of body fluids and sample media including GCF. All GCF samples were diluted in 300 uL sterile PBS to elute the material from the periopaper strips.

Statistical analysis. Descriptive analysis such as means and standard deviations described the age, GI, PD, Periotron readings, CRP, and TNF-α for mild (MG) and severe gingivitis (SG) groups. The frequency and percentage distribution described the gender of the subjects. Differences between the groups in the age and pre-treatment values of GI, Periotron readings, and TNF-α were assessed using 2-sided t-tests and differences in PD and CRP were assessed using the Mann-Whitney U test. The chi-squared test investigated any gender differences between the groups. Tests for the changes between pre- and post-treatment measurements of GI, Periotron readings, and TNF-α level within each group were carried out using paired 2-sided t-tests, and changes in these measurements between the 2 groups were carried out using unpaired 2-sided t-tests. The changes in PD and CRP measurements between pre- and post-treatment within each group were assessed utilizing the Wilcoxon signed-rank test and the changes between the 2 groups were evaluated using the Mann-Whitney U test. Further, the number of patients with a decrease in CRP and TNF-α levels after treatment were identified and the difference between mild and severe gingivitis groups were tested using chi-squared tests and Fisher's exact tests where applicable. Pearson's correlation coefficient and the p-value for significance of correlation assessed the association between the changes in periodontal parameters and CRP and TNF-α. Data were analyzed using the Statistical Package for Social Sciences software for Windows Version 16 (SPSS Inc., Chicago, IL, USA). All hypothesis testing was carried out at the 0.05 alpha level for statistical significance.

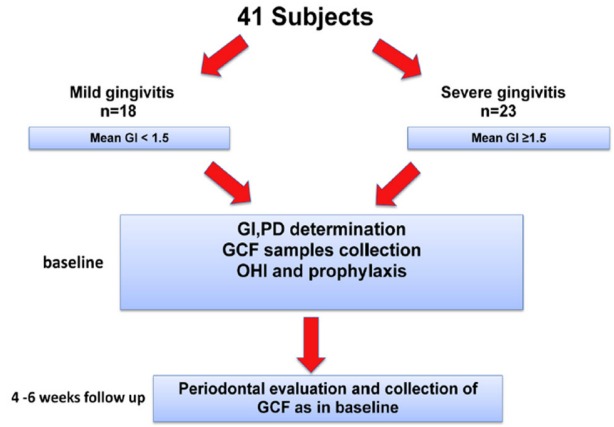

Figure 1 - Experimental design of 41 subjects.

Results. The mean age of the study subjects was 26.5±6.7, and 39% of the study population were females. Gingival index and periotron readings (PR) were significantly greater in the SG than MG groups (p=0.001). Probing depths and the levels of CRP and TNF-α were not significantly different between the 2 groups. Baseline measurements are presented in **Table 1**. Means and standard deviations of clinical parameters, CRP, and TNF-α pre- and post-gingival treatment are presented in **Table 2**. In the MG group, PD, GI, and PR significantly decreased after treatment, whereas no significant changes were found in the levels of CRP and TNF-α. In the SG group, all the clinical parameters and CRP and TNF-α values were significantly decreased

Table 1 - Clinical parameters, tumor necrosis factor-alpha (TNF-α) and C-reactive protein (CRP) values for mild and severe gingivitis groups at baseline.

Clinical parameters	Mild gingivitis (n=18)	Severe gingivitis (n=23)	*P*-value
Gingival index	0.83±0.31	2.01±0.27	0.001
Probing depth (mm)	2.66±0.26	2.72±0.28	0.563
Periotron	96.07±7.86	114.06±8.77	0.001
CRP (ng/ml)	0.24±0.06	0.26±0.08	0.958
TNF-α (pg/ml)	54.45±7.36	58.08±14.66	0.34

Data are expressed as mean ± standard deviation.

Table 2 - Clinical parameters, tumor necrosis factor-alpha (TNF-α) and C-reactive protein (CRP) values pre- and post-gingival treatment.

Clinical parameters	Mild gingivitis			Severe gingivitis		
	Pre-treatment	Post-treatment	*P*-value	Pre-treatment	Post-treatment	*P*-value
Gingival index	0.83±0.31	0.19±0.05	0.001	2.01±0.27	0.47±0.28	0.001
Probing depth (mm)	2.66±0.26	2.47±0.15	0.001	2.72±0.28	2.54±0.20	0.001
Periotron	96.1±7.86	76.9±4.13	0.001	114.1±8.8	87.13±6.90	0.001
CRP (ng/ml)	0.24±0.06	0.28±0.16	0.338	0.26±0.08	0.22±0.04	0.033
TNF-α (pg/ml)	54.5±7.36	50.5±6.92	0.13	58.1±14.7	46.6±11.50	0.006
Data are expressed as mean±standard deviation.						

Table 3 - Number of subjects that showed a decrease in gingival index and laboratory parameters after treatment.

Clinical parameters	Total sample n (%)	Mild gingivitis group n (%)	Severe gingivitis group n (%)	Between groups *P*-value
Gingival index	41 (100)	18 (100)	23 (100)	-
C-reactive protein ng/ml	21 (51.2)	6 (33.3)	15 (65.2)	0.043
Tumor necrosis factor-α pg/ml	32 (78.1)	12 (66.7)	20 (87.0)	0.147
Data are presented as number and percentage (%).				

after treatment. In regard to the differences between the pre- and post- treatment measurements, there was a significantly greater reduction (p=0.01) in the GI and Periotron values in the SG group (1.54±0.30 for GI and 26.92±9.25 for Periotron) compared with the MG group (0.64±0.29 for GI and 19.10±5.13 for Periotron). A greater reduction in the level of CRP (p=0.039) was found and a trend towards TNF-α reduction (p=0.06) was also noted.

Table 3 presents the number and percentage of subjects with decreased GI, CRP, and TNF-α after treatment. All patients showed a decrease in GI, whereas 78% showed a decrease in the TNF-α level, and 51% showed a decrease in the CRP level. When the changes in clinical parameters after periodontal treatment were compared to the changes in the levels of CRP and TNF-α, GI was found to be significantly correlated with TNF-α (r=0.4; p=0.011), and there was also a trend toward a correlation between GI and CRP (r=0.4; p=0.056). The PR was significantly correlated with TNF-α (r=0.6; p=0.001).

Discussion. The present study examined the effects of treatment of mild and severe gingivitis on the levels of TNF-α and CRP in GCF in systemically healthy subjects. The results showed a significant decrease in both TNF-α and CRP levels after treatment of severe gingivitis. A decrease in the level of inflammatory markers after 1-3 months of treatment of periodontitis was previously reported.[8,9,20,21] The reported reduction in the levels of the inflammatory markers after periodontal treatment ranged between 21-38%. In the present study, the reduction in the inflammatory markers was assessed 4-6 weeks after prophylaxis and enforced oral hygiene in systemically healthy subjects with mild or severe gingivitis. The reduction in the inflammatory marker levels ranged between 15-19%, which is less than the changes in previous studies in patients with chronic periodontitis. Periodontitis is a severer form of periodontal disease than gingivitis, which may explain the reduction of inflammatory markers to a lesser extent in gingivitis patients. In the present study, a correlation was noted between the decrease in gingival inflammation after treatment and the changes in the TNF-α and CRP levels. This implies that the decrease in the levels of TNF-α and CRP after treatment is due to the reduction in the infection burden. This further supports the effect of gingival inflammation on the GCF levels of the assessed inflammatory markers, which may also be a reflection of its effect on their systemic levels. Bazile et al[6] demonstrated a statistically significant association between gingival inflammation and coronary artery disease in chronic periodontitis patients. The authors suggested that the gingival inflammation per se, may play a more significant role than the periodontal attachment and bone loss associated with periodontitis. The GCF cytokine profile is governed by the intensity of periodontal inflammation and is directly proportional to the amount of bone loss and attachment destruction.[22] The increase in gingival inflammation is associated with a marked increase in vascular permeability of the vessels underlying junctional and sulcular epithelia. This indicates that the blood vessels of chronically inflamed gingiva are more likely to show an increase in permeability than the vessels of clinically healthy gingiva.[17,22] This may explain the presence of higher

levels of inflammatory markers in the GCF in severe gingivitis than in clinically healthy gingiva or mild gingivitis. Treatment of severe gingivitis in this study was associated with lowered levels of inflammatory markers in the GCF. Biochemical analysis of the GCF offers a means of assessing the nature of the host response in the periodontal tissues. Gingival crevicular fluid composition in its broad features is similar to blood serum; therefore, it may be used as a non-invasive alternative method to assess the level of systemic inflammatory markers instead of a blood sample.

An important limitation of this study is its small sample size. Therefore, future studies with a larger sample size and longer follow up time are needed to verify the present findings.

In conclusion, treatment of severe chronic gingivitis reduces the levels of TNF-α and CRP in the GCF of systemically healthy subjects. Therefore, reduction of these inflammatory mediators may play a role in reducing existing systemic inflammation.

References

1. Li Y, Lee S, Hujoel P, Su M, Zhang W, Kim J, et al. Prevalence and severity of gingivitis in American adults. ***Am J Dent*** 2010; 23: 9-13.
2. Kim J, Amar S. Periodontal disease and systemic conditions: a bidirectional relationship. ***Odontology*** 2006; 94: 10-21.
3. 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: 1172-1179.
4. Pejcic A, Kesic LJ, Milasin J. C-reactive protein as a systemic marker of inflammation in periodontitis. ***Eur J Clin Microbiol Infect Dis*** 2011; 30: 407-414.
5. Passoja A, Puijola I, Knuuttila M, Niemelä O, Karttunen R, Raunio T, et al. Serum levels of interleukin-10 and tumour necrosis factor-α in chronic periodontitis. ***J Clin Periodontol*** 2010; 37: 881-887.
6. Bazile A, Bissada NF, Nair R, Siegel BP. Periodontal assessment of patients undergoing angioplasty for treatment of coronary artery disease. ***J Periodontol*** 2002; 73: 631-636.
7. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. ***J Clin Invest*** 2003; 111: 1805-1812.
8. D'Aiuto F, Nibali L, Parkar M, Suvan J, Tonetti MS. Short-term effects of intensive periodontal therapy on serum inflammatory markers and cholesterol. ***J Dent Res*** 2005; 84: 269-273.
9. D'Aiuto F, Ready D, Tonetti MS. Periodontal disease and C-reactive protein-associated cardiovascular risk. ***J Periodontal Res*** 2004; 39: 236-241.
10. Offenbacher S, Beck JD, Moss K, Mendoza L, Paquette DW, Barrow DA, et al. Results from the Periodontitis and Vascular Events (PAVE) Study: a pilot multicentered, randomized, controlled trial to study effects of periodontal therapy in a secondary prevention model of cardiovascular disease. ***J Periodontol*** 2009; 80: 190-201.
11. Taylor B, Tofler G, Morel-Kopp MC, Carey H, Carter T, Elliott M, et al. The effect of initial treatment of periodontitis on systemic markers of inflammation and cardiovascular risk: a randomized controlled trial. ***Eur J Oral Sci*** 2010; 118: 350-356.
12. Hussain SA, Khan AA, Tatakis DN, Azhar M, Hanif M, Izhar M. Non-surgical periodontal therapy lowers serum inflammatory markers: a pilot study. ***J Periodontol*** 2009; 80: 1574-1580.
13. Al-Zahrani MS, Alghamdi HS. Effect of periodontal treatment on serum C-reactive protein level in obese and normal-weight women affected with chronic periodontitis. ***Saudi Med J*** 2012; 33: 309-314.
14. Lalla E, Kaplan S, Yang J, Roth GA, Papapanou PN, Greenberg S. Effects of periodontal therapy on serum C-reactive protein, sE-selectin, and tumor necrosis factor-alpha secretion by peripheral blood-derived macrophages in diabetes. A pilot study. ***J Periodontal Res*** 2007; 42:274-282.
15. Braun T, Schett G. Pathways for bone loss in inflammatory disease. ***Curr Osteoporos Rep*** 2012; 10: 101-108.
16. Navarro-Sanchez AB, Faria-Almeida R, Bascones-Martinez A. Effect of non-surgical periodontal therapy on clinical and immunological response and glycaemic control in type 2 diabetic patients with moderate periodontitis. ***J Clin Periodontol*** 2007; 34: 835-843.
17. Lamster IB, Ahlo JK. Analysis of gingival crevicular fluid as applied to the diagnosis of oral and systemic diseases. ***Ann N Y Acad Sci*** 2007; 1098: 216-229.
18. D'Aiuto F, Parkar M, Andreou G, Suvan J, Brett PM, Ready D, et al. Periodontitis and systemic inflammation: control of the local infection is associated with a reduction in serum inflammatory markers. ***J Dent Res*** 2004; 83:156-160.
19. Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. ***Acta Odontol Scand*** 1963; 21: 533-551.
20. Ortiz P, Bissada NF, Palomo L, Han YW, Al-Zahrani MS, Panneerselvam A, et al. Periodontal therapy reduces the severity of active rheumatoid arthritis in patients treated with or without tumor necrosis factor inhibitors. ***J Periodontol*** 2009; 80: 535-540.
21. Correa FO, Gonçalves D, Figueredo CM, Bastos AS, Gustafsson A, Orrico SR. Effect of periodontal treatment on metabolic control, systemic inflammation and cytokines in patients with type 2 diabetes. ***J Clin Periodontol*** 2010; 37: 53-58.
22. Armitage GC. Analysis of gingival crevice fluid and risk of progression of periodontitis. ***Periodontol 2000*** 2004; 34: 109-119.