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C – reactive protein in saliva of non-smoking patients with periodontitis (a pilot study)

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ABSTRACT

Introduction: C-reactive Protein (CRP) as an inflammatory biomarker can be easily determined in saliva, but the values of salivary CRP in periodontitis are not well-studied. The aim of this study was to analyze and determine the values of salivary CRP in non-smokers with periodontitis stage 3 or 4 before and after supragingival and subgingival full-mouth periodontal therapy.

Methods: Standard periodontal parameters and saliva samples were collected in 12 non-smoking patients. Patients in the test group (n = 6) underwent supragingival and subgingival full-mouth periodontal therapy, and the control group (n = 6) received only supragingival full-mouth therapy. Both groups received the same oral hygiene instructions in addition to therapy. After 3 months, re-registration of periodontal parameters and re-sampling of saliva for analysis of salivary CRP were done for both groups.

Results: Statistical analysis revealed large differences in the values of clinical periodontal parameters and CRP levels in the test group after therapy. Values of salivary CRP in the test and control groups were lower 3 months the therapy; however, the results were not statistically significant. The correlation of clinical periodontal parameters and salivary CRP varied in both groups.

Conclusion: Our pilot study reveals decreased concentrations of salivary C-reactive protein in non-smoking patients following non-surgical periodontal therapy. Further studies are needed to prove the reliability of salivary CRP as a biomarker for periodontitis.

Keywords: C-reactive protein; periodontitis; saliva; biomarkers

INTRODUCTION

Periodontitis is a multifactorial chronic inflammatory disease initiated by specific biofilm bacteria that compromises the integrity of the periodontal tissues including the gingiva, periodontal ligament, dental cementum, and alveolar bone (1). It is well known that periodontitis causes an inflammatory response and inflammatory parameters such as cytokines, interleukins, and C-reactive protein (CRP) can be found in elevated concentrations in serum, gingival tissue, sulcus, and saliva (2). When the periodontal disease develops, bacteria as well as mediators of the inflammatory response including cytokines and prostaglandins, enter the systemic circulation where proteins of acute inflammatory reaction, such as CRP, fibrinogen, and haptoglobin, are considered biomarkers of systemic inflammatory diseases (3). The onset of biomarkers for systemic inflammatory diseases in periodontitis tells us about the impact of periodontitis on the whole organism (4). In the field of periodontology, traditional clinical criteria are sometimes not sufficient to determine the site of active periodontal disease, to monitor a patient's response to therapy, or to measure the degree of susceptibility to future disease progression. In the past 10 years, numerous studies have focused on the examination of biomarkers for periodontitis from serum and saliva, such as IL-6 and suPAR (5-7). Recent studies using the serum and salivary biomarker Galectin-3 have confirmed the link between periodontitis and coronary heart disease (CHD) (8), and the results showed that patients with periodontitis and a group of patients with periodontitis and CHD had significantly higher levels of Galectin-3 in serum and saliva compared to patients with CHD and a healthy control group.

Saliva is a valuable source for clinically relevant information because it contains biomarkers specific to the unique physiological aspects of periodontal disease, and may be used as a mirror of oral and systemic health (9). Proposed diagnostic markers of saliva for periodontitis include components found both in serum and saliva such as immunoglobulins,

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cytokines, enzymes, bacterial components or their products, and interleukins.

CRP is an acute phase pentameric protein that mediates innate immunity by binding to foreign pathogenic and damaged cells, initiating the classical complement cascade pathway. It is a circulatory biomarker of inflammation and CRP is a key indicator of inflammation used daily in clinical medical practice (10). Altered serum CRP values in patients with periodontitis have been shown in numerous studies, while salivary CRP in periodontitis has been insufficiently documented.

Higher values of CRP have been earlier associated with chronic and aggressive periodontitis, and with other biomarkers like IL-6 (11), and it has been measured in saliva using a lab-on-a-chip method in patients with periodontitis (2).

Early detection and the possibility of monitoring periodontitis, as well as the possibility of observing the effects of therapy on periodontitis, are interesting for many researchers, and CRP is one of the mediators of inflammation whose level can be determined in patients' saliva. The aim of the study was to determine and analyze the values of salivary CRP in non-smokers with periodontitis Stages 3 and 4, before and after the supragingival and subgingival full-mouth periodontal therapy.

METHODS

All subjects have signed a written consent according to the Helsinki Declaration II for entering the study, which was approved by the Ethics Committee of the School of Dental Medicine of the University of Zagreb (05-PA-30-XIII-1/2020). The study group consisted of 12, otherwise healthy, subjects (6 females and 6 males) aged 35–54 years (median 41, 97 years) with Stage 3 or 4 periodontitis with a minimum of 20 teeth, randomly selected from the patients referred for opinion and periodontal treatment. None of the subjects had any systemic disease or condition, nor did they take any medications that might have influenced the periodontal treatment 6 months before baseline. All patients were Caucasians and non-smokers. Excluding factors were: History of periodontal therapy (non-surgical and surgical), the existence of systemic illnesses or any oral mucosal diseases, presence of infectious diseases, need of antibiotic prophylaxis before examination, mental disability, use of antibiotic, corticoid or immunosuppressive therapy within the 6 months before the study, pregnancy, lactating women, minors, patients undergoing endodontic treatment, patients with periapical lesions, the use of antiseptics, and antimicrobial drugs.

Criteria used for the diagnosis of periodontal disease were according to the Classification of periodontal and peri-implant diseases from 2017 (12). A dental mirror and a standard periodontal probe (PCP-15, Hu-Friedy, Chicago, IL, USA) were used to diagnose periodontitis. The same calibrated researcher performed the periodontal examination and non-surgical periodontal therapy in all patients. Patients had standard periodontal parameters and indices measured at 6 sites per tooth, excluding third molars, and these included probing depth (PD), gingival recession (RE), clinical attachment level (CAL), full mouth plaque score (FMPS), and full mouth bleeding score (FMBS).

Supragingival and subgingival periodontal therapy were performed with machine and hand instruments using local anesthesia as full-mouth therapy in the test group (n = 6). The control group of patients (n = 6), who received only supragingival periodontal therapy (full-mouth) for the study, underwent subgingival therapy after the end of the study, or 3 months after supragingival therapy was conducted for the study. Both groups of subjects received the same detailed instructions for oral hygiene in addition to therapy. The third and last phase was performed after 3 months and included re-registration of all periodontal parameters (PD, RE, CAL, FMPS, and FMBS) and re-sampling of saliva for analysis of salivary CRP for study and control group.

Saliva samples for analysis of all patients were collected in the morning on working days (Monday to Friday) between 8 and 10 p.m. A sample of unstimulated saliva was collected from the participants before the periodontal treatment in a calibrated sterile vial of 5 mL. Saliva samples were collected on the volume base according to the modification of the saliva collecting method described by the authors Navazesh and Kumar (13).

All samples were refrigerated within 5 min of sampling and sent to the laboratory for analysis. Whole saliva samples were stored at –70°C until analysis. Salivary CRP was analyzed by ELISA Human C Reactive Protein ELISA Kit (PTX1, Abcam, Cambridge, United Kingdom) for detection and quantification.

All data collected in the study were aggregated in Microsoft Excel. Results were distributed according to the normal distribution, and continuous variables were presented as mean ± SD/median. ANOVA test and t-test were used for comparisons of means and proportions between the test group and the healthy control group. Pearson's correlation coefficient was used to assess the correlation of salivary CRP and clinical periodontal parameters. Only p values lower than 0.05 were considered significant. Empower stats software was used for data analysis.

RESULTS

The mean age of the patients in the test group was 39.67 and in the control group 44.67 years. Patients in the test group had an average of 6 teeth extracted, while in the control group the average number of extracted teeth was 5.83.

Baseline values of clinical parameters showed higher values compared to the values after 3 months, and a statistically significant difference was found for PD and FMBS in the test group (Table 1). Values of CRP in the saliva of patients in the test and control groups were lower after 3 months; however, the results were only marginally significant for the test group ($p = 0.05$) (Table 2).

Table 3 presents the correlation between CRP and clinical parameters at baseline and after 3 months. The strongest correlation values were found between C-reactive protein and CAL after 3 months in the control group (R = 0.78) but the result was not statistically significant ($p = 0.07$).

TABLE 1. Clinical findings data (mean ± standard deviation)

	PD		CAL		FMPS		FMBS	
	BL	3M	BL	3M	BL	3M	BL	3M
Test group	6.12±0.10	4.98±0.13*	9.52±0.89	8.89±1.04	64.51±10.91	53.21±9.40	61.26±10.19	46.91±10.43*
Control group	6.40±0.39	6.16±0.33	9.61±0.90	9.5±0.84	59.17±8.74	50.43±5.26	58.19±9.91	51.39±6.17

*Significant difference between baseline (BL) and after 3 months (3M) measurement; p < 0.05). PD: Probing depth, CAL: Clinical attachment loss, FMPS: Full mouth plaque score, FMBS: Full mouth bleeding score, BL: Baseline, 3M: Values after 3 months

TABLE 2. CRP levels in saliva (mean and standard deviation) on baseline and 3 months after therapy (ANOVA)

	Baseline		3 Months		
	Mean	SD	Mean	SD	p value
Test group	5036,46	2178,92	2693,18	1467,8	0.05
Control group	2315,18	1088,17	1914,19	427,56	0.42

TABLE 3. Pearson correlation coefficients R with p values between CRP and clinical periodontal parameters

		PD	CAL	PI	BOP (%)
	Baseline	p=0.39	p=0.39	p=0.23	p=0.87
Test group		R=0.43	R=−0.43	R=0.58	R=−0.09
		p=0.40	p=0.36	p=0.80	p=0.87
	3M	R=0.43	R=−0.46	R=−0.13	R=−0.09
	Baseline	p=0.92	p=0.35	p=0.95	p=0.74
Control group		R=−0.06	R=0.47	R=-0.04	R=−0.17
		p=0.75	p=0.07	p=0.32	p=0.57
	3M	R=0.17	R=0.78	R=0.50	R=−0.30

PD: Probing depth, CAL: Clinical attachment loss, FMPS: Full mouth plaque score, FMBS: Full mouth bleeding score, BL: Baseline, 3M: Values after 3 months

DISCUSSION

This study was planned to serve as a pilot study for future research in the field of salivary CRP findings in patients with periodontitis. There have been some studies on CRP values in saliva in periodontitis but the results are confusing. One of the studies did confirm that the values of CRP can be increased by the acute inflammatory reaction in periodontal tissues (14). Our pilot study determined that concentrations of salivary CRP in non-smoking patients with periodontitis Stages 3 and 4 were lower 3 months after supragingival and subgingival full-mouth periodontal therapy, but the results were statistically not significant.

In earlier research, concentrations of salivary CRP in periodontitis were found to be higher in patients with gingivitis, and moderate to severe periodontitis compared to the healthy control group (15,16).

In the study by Pederson et al., the participants were distributed into five groups: Healthy, gingivitis, moderate periodontitis, severe periodontitis, and a group of edentulous patients. CRP levels ranged from 0 to 472 pg/ml, and CRP was significantly lower in a group of healthy patients than in all other groups. Pederson et al. suggest that levels of salivary CRP are directly related to an individual's periodontal status (9). The mean salivary CRP levels in the study by Shojaee et al. were 5332.62 ± 5051.63pg/ml in periodontitis patients, and the results showed a significant difference in salivary CRP concentrations between the periodontitis patients and the healthy control group. The author of this study also stated that there is a significant association between periodontitis and salivary CRP concentrations (10). Salivary CRP levels in our study were far higher than the values from the study of Pederson et al. and closer to the average values shown by Shoajee et al. in the results of their study.

In the study by D'Aiuto et al., serum Il-6 and CRP were monitored in patients with severe generalized periodontitis after non-surgical periodontal therapy, and the patients who had a better oral response to non-surgical periodontal therapy were more likely to have decreased inflammatory risk category and lower CRP in serum. In the conclusion, the authors state that periodontitis may add to the patient's burden of inflammation with serum CRP levels increasing the risk of cardiovascular disease (17).

Earlier research also considers the presence of elevated salivary CRP to be a link between periodontitis and some of the systemic diseases (18). New review reports have shown that patients with periodontitis have higher levels of CRP compared to healthy controls, but that most of these clinical studies had poor control of potential contributing factors such as obesity, hypertension, smoking, and systemic inflammatory diseases. These factors should be very strictly controlled in clinical studies to assess values of salivary CRP as an indicator of inflammation in periodontitis (19). In studies where patients with periodontitis had a history of systemic inflammatory diseases, salivary CRP values were higher (20,21), confirming the need to consider the existence of chronic inflammatory diseases when evaluating salivary CRP as a marker of periodontal inflammation.

In the study by Lee et al. with 34 participants with severe chronic periodontitis in the test group and 20 participants without periodontal destruction in the control group, participants in the test group received nonsurgical periodontal therapy with saliva samples collected before and after non-surgical periodontal therapy. The study observed several salivary biomarkers, including CRP. Salivary CRP values were lower after non-surgical periodontal therapy, but the results were not statistically significant (22), which corresponds to the findings of our pilot study.

We have to address that FMPS and FMBS in our study did not have a satisfactory decrease 3 months after therapy in both groups, although patients from both groups received equal and very detailed instructions for performing oral hygiene measures. Due to this result, all patients were re-instructed to carry out oral hygiene measures and their periodontal therapy and monitoring of oral hygiene were continued. According to the latest recommendations for periodontal treatment, full-mouth bleeding scores after treatment should be <30% (23). FMBS in the test group 3 months after therapy was 46.91 ± 10.43, and in the control group in which patients had only full-mouth supragingival therapy, the reduction of this parameter was

even smaller (51.39 ± 6.17). Similarly, FMPS of 53.21 ± 9.40 in the test group and 50.43 ± 5.26 in the control group was recorded 3 months after therapy.

A possible cause of unsatisfactory oral hygiene and high FMPS and FMBS values in subjects 3 months after nonsurgical periodontal therapy, was the epidemiological situation and economic consequences caused by SARS-CoV-2 pandemic. We found that all patients had a low level of interest in the conducted periodontal treatment, poor motivation to implement oral hygiene measures after therapy, as a result of anxiety and fear caused by the poor epidemiological situation in the country during the study period. It is possible that poor oral hygiene and relatively high values of other periodontal parameters 3 months after therapy affected the values of CRP saliva in the second measurement. Although the mean value of CRP saliva in the test group decreased from 5036.46 pg/ml to 2693.18 pg/ml 3 months after therapy, we believe that the result could have been even better if the level of oral hygiene and patient cooperation was at a higher level.

CONCLUSION

Our pilot study reveals that concentrations of salivary CRP in non-smoking patients with periodontitis Stage 3 and 4 were lower 3 months after supragingival and subgingival full-mouth periodontal therapy, but the results were statistically not significant. FMPS and FMBS did not have a satisfactory decrease 3 months after nonsurgical periodontal therapy, which possibly negatively affected the salivary CRP finding on the second measurement. The study sample was small and further studies are needed to prove the reliability of salivary CRP as a biomarker for periodontitis.

CONFLICT OF INTEREST

No conflict of interest to declare.

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