

Evaluation of serum and gingival crevicular fluid C-reactive protein and IL-6 levels in patients with periodontitis and transient ischemic attacks

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Abstract

Background: Recent advances have suggested that periodontitis (PD), the paradigm of chronic infection in dental pathology, shares several pathogenic pathways with cardio- and cerebro-vascular disorders (CVD), based on inflammatory mediators including IL-1, IL-6, TNF- α . **Aim:** To assess pro-inflammatory biomarkers (C-reactive protein – CRP, IL-6) in serum and gingival crevicular fluid (GCF) in patients with PD and with transient ischemic attacks (TIAs). **Materials and Methods:** Prospective observational study on 143 patients classified as follows: 40 healthy subjects (group A), 50 PD patients (group B) and 53 PD-TIAs patients (group C). The predefined assessment protocol has included: current medical data, risk factors for CRP changes, periodontal status (clinical, orthopantomography, Schei Ruler technique), inflammatory biomarkers (CRP, IL-6). **Results:** High serum CRP and IL-6 have been reported in both TIAs and PD, while statistically significant increase in GCF CRP only in PD-TIAs ($p < 0.05$). Moreover, both generalized and localized chronic PD may be at higher risk for CVD, since CRP level was higher in these subgroups. However, no significant differences were reported in serum IL-6 between generalized and localized PD. A score function was demonstrated, including bone loss degree, bleeding index, collection site depth, serum and GCF IL-6 and CRP, tooth loss, allowing the classification of PD based on risk for developing TIAs. **Conclusions:** CRP and IL-6 are commonly involved in the pathways of PD and TIAs. Interdisciplinary assessment should be promoted in order to implement the stratification of PD patients according to the risk for TIAs as suggested by the proposed algorithm.

Keywords: CRP, IL-6, periodontitis, transient ischemic attack.

Introduction

Chronic periodontitis is an inflammatory disease affecting the supporting tissues of teeth. The expression of the disease results from the interaction of host defense mechanisms, microbial agents, environmental, and genetic factors [1]. Various compounds such as inflammatory mediators (C-reactive protein, CRP) and pro-inflammatory cytokines have been detected in gingival crevicular fluid (GCF) [2–6] and may be especially beneficial for diagnosing of current periodontal status and addressing the effects of periodontal treatment. Although recent immunohistochemistry studies focus on the capacity of the inflamed periodontal for the synthesis of CRP, high levels of CRP in the GCF appear to be of both systemic and local

origin [2, 3, 7, 8]. Moreover, based on cross-sectional and prospective epidemiological studies, periodontitis has been linked to cardiovascular disease (CVD), cerebrovascular ischemia and also with immune-inflammatory rheumatic conditions [4, 9–12].

The acute-phase response is a nonspecific process that may occur in the initial host response to injuries, infections, ischemic necrosis, or malignancy. It is initiated by the activation of local macrophages and other cells (including fibroblasts and endothelial cells), leading to the release of mediators such as TNF- α , IL-6 and IL-1 β . These, in turn, cause systemic changes including hepatic release of a range of plasma proteins, activation of complement and various metabolic changes [13].

CRP is a plasma protein synthesized by the liver and adipocytes, being actually recognized as an important biomarker of a wide spectrum of conditions such as systemic inflammation, inflammatory disorders, infections, neoplasias (lymphoma), osteomyelitis, inflammatory bowel diseases and immune-mediated rheumatic disorders including rheumatoid arthritis and vasculites [2, 4].

In addition, high-sensitivity CRP (hsCRP) measures cardiac and cerebrovascular risk (transient ischemic attack, TIA) being of special interest as risk factor. Therefore, the American Heart Association (AHA) has established cut-off values defining risk categories according to hsCRP level as follows: hs-CRP lower than 1.0 mg/L for low risk for developing CVD, values between 1.0 and 3.0 mg/L for an average risk, while values higher than 3.0 mg/L are commonly reported in high risk subjects [14].

On the other hand, interleukin-6 (IL-6) is an important pro-inflammatory cytokine involved in the regulation of host response to tissue injury and infection. It is produced by a variety of cells, such as monocytes, fibroblasts, osteoblasts and vascular endothelial cells in response to inflammatory challenges [8, 15, 16]. Moreover, it is widely accepted that IL-6 induces CRP production [13, 17, 18]. Since elevated plasma levels of IL-6 have been associated with unstable angina and CVD, IL-6 is actually related to other cardiovascular risk factors [14, 19].

The primary end points of the current work were to assess pro-inflammatory biomarkers, CRP and IL-6, in serum and gingival crevicular fluid and to evaluate the periodontal damage in patients with periodontitis and patients with transitory ischemic attacks.

☐ Materials and Methods

We have performed a prospective observational study on 143 patients, aged between 20 to 50 years, randomized in three groups as follows: group A or control group including 40 healthy subjects (20 women) without periodontal disease and TIAs; group B including 50 patients (23 woman) diagnosed with different degrees of periodontal disease, and group C including 53 patients (26 woman) with periodontal disease and TIAs, hospitalized in the Neurology Department, “Prof. Dr. Nicolae Oblu” Emergency Clinical Hospital in Iassy, Romania.

Informed consent was obtained from each patient prior to enrolment in the study.

All patients were assessed according to a predefined protocol including: (i) current medical data (General Medical Questionnaire); (ii) risk factors for CRP changes (representing the exclusion criteria); (iii) periodontal status (clinical and imagistic) and (iv) inflammatory biomarker assessment, CRP and IL-6, in both gingival crevicular fluid and serum.

Periodontal evaluation was performed by three examiners.

Samples of GCF were currently collected from the most damaged sites, while peripheral venous blood by venipuncture at the antecubital fossa using non-

anticoagulation vacuum container systems, followed by serum centrifugation and frozen at -20°C until further processing. The same procedure was performed in all patients, including: isolation of the sample site with sterile cotton rolls, moderated air-drying using air stream for 30 seconds, gentle insertion for 30 seconds of the paper points into the gingival sulcus/periodontal pocket. After imbibition, each paper point was conserved in an Eppendorf tube containing 300 μL PBS (pH 7.2) and freeze at -20°C . If multiple damaged sites, GCF was collected separately from each periodontitis-affected site. Each sample was analyzed separately.

CRP and IL-6 were evaluated by Enzyme-Linked Immunosorbent Assay (ELISA) in both GCF and blood samples. The serum normal values of the interest molecules were established according to the data reported in the literature and the manufacturer’s reference range (IL-6 <12.5 pg/mL; CRP <0.6 mg/dL).

Orthopantomography was performed and analyzed by a single examiner in the Radiology Department, Dental Medicine Education Base at the University of Medicine and Pharmacy in Iassy; due to overlapping of the adjacent teeth’s roots, the complexity of the maxillary molars root morphology and the root proximity of the maxillary premolars, we have evaluated only the mandibular teeth. Alveolar bone assessment was performed by a method, which expresses the bone loss as a proportion of the root’s length, using the Schei Ruler composed by 10 straight lines converging at the same angle. Orthopantomography scanning was done and we have assessed by computerized means the level of bone loss, overlapping the Schei Ruler over the scanned images. The relative bone loss in percentage was evaluated in two different sites of the same tooth (mesial and distal) using the above-mentioned method (Figure 1). The Schei Ruler measurement starting point for each site was the cement–enamel junction (CEJ) of the tooth, parallel to the occlusal plane. Later, the line was moved so that the 10th line was tangent to the apex.

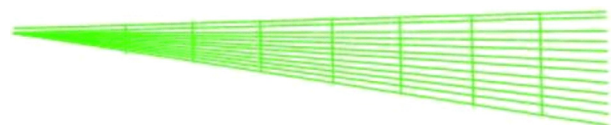


Figure 1 – The Schei Ruler.

A cut-off value of 10% of the root length bone loss is suggested by its placement between two coronary lines, while 10 to 20% bone loss is registered when it is localised between the second and the third line.

If the CEJ was destroyed or overlapped by the interproximal restorations, the reference point became the margins of the restorations. If the alveolar crest could not be determined due to the adjacent tooth overlapping, the interproximal site was classified as “unevaluable”. If an interproximal surface presented a double contour, it was classified as a pit on the interproximal surface of the root. Thus, the periodontal bone loss was evaluated on the radiographs by direct measurement from the cement-enamel junction (CEJ) (Figure 2).

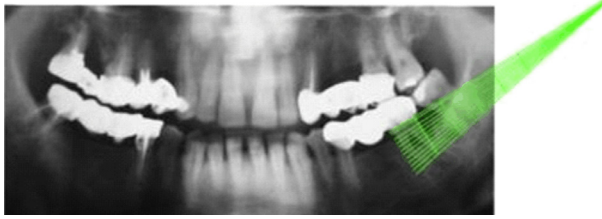


Figure 2 – Alveolar bone loss percent measurement, using the Schei Ruler.

Data were analysed in SPSS-19 software, $p < 0.05$ being considered statistically significant.

Results

The majority of patients in both periodontal group and periodontal with TIAs group presented with early and moderate aggressive periodontal involvement (96% and 92.5% respectively).

Mean serum levels of CRP detected in our study were: 183.17 ± 197.54 mg/L in healthy subjects, 1776.21 ± 1898.46 mg/L in periodontal disease and 1800.92 ± 2033.24 mg/L in patients with both periodontal and cerebrovascular disease (TIAs), while mean levels of CRP in the GCF were 1.89 ± 8.22 mg/L in controls, 36.94 ± 224.76 mg/L in patients with periodontal involvement and 46.99 ± 232.69 mg/L in patients with periodontal disease and TIAs.

On the other hand, mean serum levels of IL-6 were 3.69 ± 6.97 pg/mL in healthy subjects, 8.46 ± 12.58 pg/mL in periodontal disease and 12.17 ± 27.54 pg/mL in patients with periodontitis and TIAs, respectively. Mean IL-6 levels in GCF were 3.42 ± 2.3 pg/mL in the control group 9.78 ± 29.71 pg/mL in periodontitis and 7.79 ± 22.62 pg/mL in periodontitis and TIAs patients, respectively.

Statistical significant differences have been demonstrated only in serum CRP levels among the three groups of patients (ANOVA, $F=13.23$, $p=0.000$), whereas no statistical significant differences among groups for LGC CRP concentration and both locally and systemic levels of IL-6 ($p > 0.05$).

We were also interested in identifying relationships between studied inflammatory biomarkers in different patient populations. Thus, both systemic and locally assessed CRP correlates with serum IL-6 in patients known with periodontal disease; although weak, the correlation is statistical significant (Spearman correlation rank test: $r=-0.36$, $p=0.011$ for GCF IL-6, and $r=0.35$, $p=0.016$ for serum IL-6, respectively).

The same weak but statistical significant correlation has been reported in patients with periodontitis when talking about gingival IL-6 level and serum CRP level (Spearman rank test: $r=-0.35$, $p=0.013$).

The same weak but statistical significant correlation has been reported in patients with periodontitis when talking about gingival IL-6 level and serum CRP level (Spearman rank test: $r=-0.35$, $p=0.013$). Besides, it seems that the same type of correlation (weak, negative, statistically significant) has been validated for GCF IL-6 and serum CRP (Spearman rank test $=-0.44$, $p=0.001$) in patients with periodontitis and TIAs. On the other hand, serum IL-6 also correlates with serum CRP

in this particular patient population (Sperman rank test: $r=0.30$, $p=0.027$).

A positive statistical significant correlation was described for serum and gingival level of IL-6 in patients with only periodontal involvement ($r=0.29$, $p=0.035$), but also in those with periodontal disease and TIAs ($r=-0.27$, $p=0.047$).

No statistical significant relation between local and systemic CRP concentration in both groups of patients.

We have evaluated a score function which allows the classification of patients with periodontitis and a high risk for developing TIAs based on different parameters. Therefore, several variables have been used, including demographics (sex, age), parameters defining periodontal status (degree of bone loss, bleeding index, collection site depth, tooth loss), serum and GCF inflammatory biomarkers (IL-6, CRP) and smoking status. We have obtained a statistically significant score function (Wilks' Lambda = 0.677, $p < 0.005$) based on the following parameters: bone loss degree (Wilks' Lambda = 0.925, $p=0.001$), bleeding index (Wilks' Lambda = 0.864, $p=0.000$), collection site depth (Wilks' Lambda = 0.891, $p=0.000$), serum IL-6 (Wilks' Lambda = 0.972, $p=0.054$), periodontal disease (Wilks' Lambda = 0.844, $p=0.000$) (Table 1).

Table 1 – Score function in studied patients

	Wilks' Lambda	F	df1	df2	Sig.
Sex	.992	1.101	1	141	.296
Age [years]	1.000	.049	1	141	.826
Bone loss degree	.925	11.435	1	141	.001
Bleeding index	.864	22.222	1	141	.000
Collection site depth	.891	17.208	1	141	.000
Serum IL-6 level [pg/ml]	.972	4.096	1	141	.045
Serum CRP level [mg/L]	.981	2.691	1	141	.103
Gingival crevicular fluid IL-6 [pg/ml]	.999	.157	1	141	.692
Gingival crevicular fluid CRP [mg/L]	.994	.787	1	141	.377
Smoking status	.996	.615	1	141	.434
Tooth loss	.994	.900	1	141	.344
Periodontal disease	.844	26.035	1	141	.000

Moreover, according to this score function a patient with periodontitis will be allocated to one or another risk group for developing cerebrovascular disease with high-level accuracy (77%).

Discussion

Various epidemiological studies have implicated periodontitis, a mixed infection of the supporting structures of the teeth, as a risk factor for the development of cardiovascular disease [15, 20–22]. Persistent infection such as chronic periodontitis may induce immune-inflammatory responses, which, in turn, may contribute to early accelerate atherogenesis and vascular diseases in conjunction with other risk factors. It may be speculated that periodontitis may predispose affected patients to cardiovascular diseases by increasing the levels of acute phase proteins, which may lead to increased inflammatory activity in atherosclerotic lesions [2, 15, 22].

Several inflammatory biomarkers have already been validated as cardiovascular risk factors, particularly CRP, an emerging and reliable biomarker of the acute phase response to infectious burdens and/or inflammation; elevated serum CRP >0.3 mg/dL is actually regarded as a risk predictor for CVD. In addition, IL-6 may also be listed among factors contributing to the association between chronic infections and cardiovascular diseases, displaying pro-inflammatory and pro-coagulant properties [4, 14].

Moreover, there is strong evidence that serum CRP is elevated in periodontitis as compared to controls [4], supporting the hypothesis that periodontitis may enhance the process of vascular inflammation and lead to sub-clinical and clinical atherosclerosis [15]. Furthermore, elevated levels of CRP and IL-6 during periodontitis may occur when bacteria and bacterial products, such as lipopolysaccharide, as well as locally produced pro-inflammatory cytokines enter the circulation.

However, further studies are necessary to confirm the role of oral infections in the process of atherosclerosis and cerebrovascular accidents [23, 24].

In our study, we have demonstrated high levels of serum CRP and IL-6 in patients with periodontitis, particularly in the subgroup of patients with generalized disease, supporting data from literature (Paraskevas *S et al.* [4], Fitzsimmons TR *et al.* [16], Mirrielees J *et al.* [25]). More recent evidences, however, has indicated that patients with severe periodontitis have increased serum levels of CRP, hyperfibrinogenemia, moderate leukocytosis, as well as increased serum levels IL-6 when compared with unaffected control populations.

Interestingly, we have also demonstrated that patients with either chronic generalized or chronic localized periodontitis may be at higher risk for cardiovascular disease, since CRP level was higher in these subgroups. However, only serum CRP was statistically significant increased as compared to controls, while no statistical significant difference has been reported in serum IL-6 between generalized and localized periodontal disease in our study ($p>0.05$).

The highest levels of both systemic (serum) and locally (GCF) assessed CRP have been reported in the group of patients diagnosed with periodontal disease and TIAs; however, the difference was statistically significant ($p<0.05$) only for serum CRP when comparing with either controls population. Moreover, increased serum and GCF CRP levels have been demonstrated in both periodontitis and TIA patients respectively (up to 10 times as compared to healthy group), without statistical significance.

Highest IL-6 levels have been demonstrated in patients with periodontitis, when assessed locally, in gingival crevicular fluid. In addition, an increase of about four times in serum IL-6 from patients with cerebrovascular involvement and about 2.5 times increase in GCF IL-6 in have also been detected in our study.

We have demonstrated a statistically significant correlation between CRP and IL-6 serum levels in patients with localized periodontitis ($p<0.05$); conversely, other studies have reported such correlation only in chronic generalized periodontitis [4].

We have also established a statistical significant correlation between IL-6 levels in GCF and serum in both groups with periodontal disease and periodontal involvement with TIAs, respectively. In addition, local IL-6 (gingival crevicular fluid) and systemic CRP levels correlates in all patients enrolled in the second and the third group. On the other hand, we have not observed any relation between the systemic and locally CRP as the second inflammatory biomarker evaluated in our study.

There is few data concerning the normal values of CRP and pro-inflammatory cytokines in the gingival crevicular fluid. While CRP is primarily synthesized in the liver, it was assumed that human gingivae may produce CRP and its expression could be associated with IL-6 [3, 26, 27]. Besides, CRP in GCF depends on its serum level (systemic inflammatory sites), showing the necessity to correlate serum and GCF levels [2, 8, 28, 29].

Furthermore, we have applied a score function in order to assess the possibility to predict the development of cerebrovascular disease in patients with periodontitis. Several parameters seem to be of statistical significance, mainly those related to periodontal status. Surprisingly, only the serum level of IL-6 could predict the development of TIAs in periodontitis; according to our results, local inflammatory biomarker activity is not related to the risk of TIAs.

The proposed score for patients with periodontitis could be used as a common tool for the assessment of periodontal disease in daily dental practice. Moreover, several steps should be considered in order to obtain an optimal score function: (i) to include certain variables, (ii) to enhance the volume of the research group up to 1000 subjects, so that the score function includes more variables that are statistically correlated with both periodontal disease degree and transient ischemic accidents and also with serum and gingival crevicular fluid levels of IL-6 and CRP, and (iii) to use identical assessment kits. Despite the relative small size of patient groups included in the current work, our results could be the basis of further research.

☞ Conclusions

Periodontal disease as the paradigm of chronic infection in dental pathology shares several pathogenic pathways with cardiovascular diseases, based on pro-inflammatory mediators such as PGE₂, IL-1, IL-6, and TNF- α . In contrast, these mediators are known to promote systemic acute phase response. Cerebrovascular disease represents a heterogeneous group of conditions associated with significant morbidity and mortality. During the last decade, poor dental health has been found to be significantly correlated with cardiovascular disease, particularly with coronary heart disease. Interdisciplinary assessment (periodontist–neurologist–neuroradiologist–cardiologist) should be promoted in order to implement the stratification of periodontal patients according to the risk for TIA as suggested by the proposed algorithm.

Acknowledgments

The study was supported by the Ministry of Education, Research, Youth and Sport (CNCSIS Grant No. 2034/2008–2011) and “Grigore T. Popa” University of Medicine and Pharmacy.

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Received: September 15th, 2011

Accepted: December 14th, 2011