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Interleukin-1α and -1β assessment in the gingival crevicular fluid of periodontal patients with chronic hepatitis C

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Abstract. The study assessed whether the increased production of interleukin-1α (IL-1α) and interleukin-1β (IL-1β), as a result of chronic hepatic inflammation, could be the expression of the negative impact on periodontal disease. The study included chronic periodontitis patients who were systemically healthy, chronic periodontitis patients suffering from chronic hepatitis C, as well as control patients, being systemically and periodontally healthy. After periodontal examination and the assessment of certain periodontal parameters, gingival crevicular fluid was collected from all participating patients. By using the enzyme-linked immunosorbent assay method, a quantitative assessment of IL-1α and IL-1β levels was possible. The immunologic results were correlated to the clinical periodontal data. The gingival fluid levels of cytokines were higher for periodontitis patients with chronic hepatitis C than for the systemically healthy periodontitis patients (1.8-fold higher for IL-1α and 2.1-fold higher for IL-1β). In addition, the gingival fluid cytokine levels were significantly higher for the periodontal patients (with/without chronic hepatitis C) than for the control group. Positive correlations were found between gingival fluid IL-1α and IL-1β levels and certain clinical periodontal parameters or the age of the viral hepatitis C diagnosis, in periodontitis patients with chronic hepatitis C. The chronic hepatic inflammation may have an important additional negative impact on the periodontal status, as both inflammatory reactions seem to be promoted by common pro-inflammatory cytokines.

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Introduction

Periodontal disease (PD) is a chronic inflammatory condition that leads to the gradual destruction of the tissues supporting the teeth (1). The disease occurs in patients who have subgingival bacterial biofilm and an inadequate type and intensity of host immune response (2). Although the bacterial subgingival biofilm can be found in numerous patients, the disease is triggered only in part of them, suggesting an important impact of the interaction between the bacterial challenge and the host immune response on the pathological mechanisms that govern this condition (3).

During periodontal inflammation, host immune cells, such as polymorphonuclear neutrophils (PMNs), macrophages and lymphocytes, will produce increased levels of pro-inflammatory mediators: cytokines, mainly interleukin-1 family (IL-1), interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α), prostaglandins, prostaglandin E_2 (PGE_2) and enzymes, matrix-metalloproteinases (MMPs), collagenases or gelatinases (4-6). These pro-inflammatory mediators are intended to improve the quality and intensity of the host immune response, but due to the particular characteristics of periodontal

inflammation, they lack efficacy (7). Consequently, their increased levels inside the gingival connective tissue or epithelium result in an exacerbated chronic inflammatory reaction, which will contribute to periodontal damage (8).

The levels of pro-inflammatory cytokines can be a useful tool for the assessment of periodontal disease activity and evolution (9). The inflammatory infiltrate, found inside the gingival connective tissue, creates an augmented osmotic pressure which increases the production of gingival crevicular fluid (GCF) (10). This fluid contains important inflammation indicators, such as cytokines, leucocytes, bacterial cells and degraded periodontal tissue components (11). The cytokines from the gingival fluid can be assessed qualitatively and quantitatively, being a useful indicator of the periodontal inflammatory status (12). Important risk factors for the PD's aetiology are systemic diseases and conditions (13). These can have a major impact on the host immune response and can alter the inflammatory reaction, which occurs during periodontal bacterial challenge, causing important tissue damage (5,13). A large number of systemic conditions can have oral or periodontal manifestations (13). The correlation between PD and various systemic conditions such as diabetes mellitus (DB), cardiovascular disease (CAD), rheumatoid arthritis (RA) and others has been intensely studied and relevant results have been united under the concept of 'periodontal medicine', which offers practitioners a comprehensive perspective of PD pathology, diagnosis and therapeutic strategies (14).

Chronic viral hepatitis C (CHC) occurs as a consequence of the infection with the hepatitis C virus (HCV) that uses hepatic or peripheral blood cells for replication (15). The spread of the infection is considered a global health issue, as it interests more than 200 million people worldwide, and is particularly difficult to combat, because during its initial stages, the disease has no symptoms (15). As a result, infected persons may be unaware and prone to infect others (15). After the acute stage of infection, most patients will develop a chronic inflammation of the hepatic tissue. Gradually, the hepatic function is impaired, as the hepatic tissue is replaced with fibrotic tissue (16) and liver cirrhosis occurs. Chronic hepatitis C patients can also develop other life-threathening complications, such as hepatocellular carcinoma (17,18). Being a chronic inflammatory condition, CHC could exhibit some correlations and interactions with PD, possibly via the pro-inflammatory mediators that are discharged into the blood flow of HCV patients (19). Natural killer cells (NK), which have TNF production capabilities, play an important role in the immunological pathogenesis of CHC (20). Blood levels of certain cytokines, such as IL-18 and IL-33, are used as an indicator of CHC disease activity and severity, as these patients have been shown to exhibit increased serological levels of these interleukins (21,22). Elevated levels of interleukin-1α have also been found in serum samples of chronic hepatitis C patients, and have been directly associated with the severity of the disease (23,24). Furthermore, some pro-inflammatory mediators, such as interleukin-1β, have been shown to promote liver inflammatory processes in chronic hepatitis C patients (25), expressing increased serum levels in these patients (26).

The study objective was to analyse the impact that chronic hepatitis C could have on the inflammatory status of patients with chronic periodontal disease, who also suffer from HCV infection, by assessing the GCF levels of interleukin-1α (IL-1α) and interleukin-1β (IL-1β) in these patients, and comparing the results to those of systemically healthy periodontal patients. Higher levels of these particular interleukins, which have considerable implications in the pathogenic processes of both PD and CHC, could imply that chronic hepatitis C acts as an additional risk factor for PD and that it has a negative impact on the inflammatory status of periodontal patients, assessed by interleukin detection.

Patients and methods

Patient selection. The patients were selected among those who addressed the Department of Periodontology of the University of Medicine and Pharmacy of Craiova (Craiova, Romania), for periodontal complaints or pre-orthodontic treatment periodontal evaluation. Upon approval from the University's Ethical Scientific Committee, the patients who expressed their informed consent for participating in the study were subjected to an oral and periodontal examination (27). The patients were also requested to fill a medical questionnaire regarding their medical history and existing systemic diagnosis or medication.

For each patient the following periodontal parameters were recorded: The number of teeth with periodontal pockets deeper than 4 mm (AT), the maximum periodontal probing depth (MD) and the number of existing/remnant teeth (RT). Only patients having at least two periodontal pockets in two distinct teeth, with minimum 5 mm probing depth, were included in the study.

Patients suffering from asymptomatic chronic hepatitis C were included in the study, the diagnosis being confirmed in the Gastroenterology Clinic of the University of Medicine and Pharmacy of Craiova (Craiova, Romania) based on blood tests results regarding the aspartate transaminase (AST) and alanine transaminase (ALT) serum levels. The age of the HCV infection diagnosis was also recorded for the chronic hepatitis C patients.

Patients not suffering from systemic diseases, and who were diagnosed with chronic periodontal disease, through the periodontal examination, were included in the study, as part of the periodontally affected, but systemically healthy group.

The study protocol and patient informed consent form for participation in the study and collection of gingival crevicular samples were approved by the University Ethics Scientific Committee (University of Medicine and Pharmacy of Craiova, Romania, no. 17/2016). Written informed consent was given by all participating patients.

The study also comprised a group of control patients, periodontally and systemically healthy, who were referred to the Department of Periodontology for pre-orthodontic periodontal evaluation.

For the unbiased evaluation of their inflammatory status, patients were excluded from the study if they met at least one of the following exclusion criteria: i) Anti-inflammatory treatment in the last month; ii) antibiotic treatment in the last 3 months; iii) pregnancy; iv) decompensated or symptomatic chronic hepatitis C; v) current smoker and vi) any diagnosed systemic condition other than chronic hepatitis C.

After applying the inclusion and exclusion criteria, three groups of patients were set up for the study: i) P group,

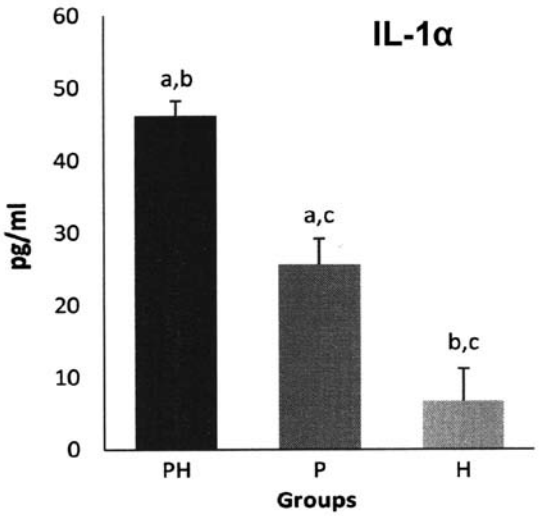

Figure 1. GCF levels of IL-1α for each group (pg/ml). [a]Statistically significant difference between PH and P groups for IL-1α GCF levels; [b]statistically significant difference between PH and H groups for IL-1α GCF levels; [c]statistically significant difference between P and H groups for IL-1α GCF levels. GCF, gingival crevicular fluid; IL-1α, interleukin-1α.

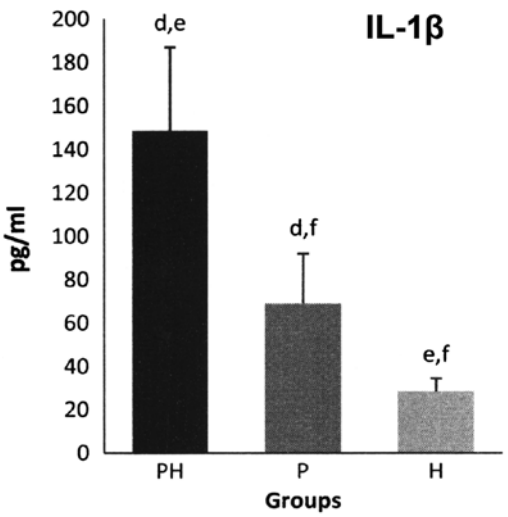

Figure 2. GCF levels of IL-1β for each group (pg/ml). [d]Statistically significant difference between PH and P groups for IL-1β GCF levels; [e]statistically significant difference between PH and H groups for IL-1β GCF levels; [f]statistically significant difference between P and H groups for IL-1β GCF levels. GCF, gingival crevicular fluid; IL-1β, interleukin-1β.

13 patients with chronic periodontal disease and no systemic disease (6 male and 7 female, aged between 40-58 years); ii) PH group, 11 patients with chronic periodontal disease and asymptomatic chronic hepatitis C (8 male and 3 female, aged between 36-62 years); iii) H group, 11 control patients with no systemic and periodontal disease (7 male and 4 female, aged between 31-43 years).

GCF sampling. Gingival crevicular fluid samples were collected from all participating patients by using absorbent paper strips (PerioPaper; Oraflow Inc.). The paper strips were inserted into the gingival sulcus or periodontal pockets and kept for 30 sec. For each participating patient two samples of gingival fluid were collected, from two distinct teeth and placed into polypropylene containers with 200 μl of phosphate-buffered saline (PBS) solution. The sampling was repeated at 1 min interval from the same two teeth and the paper strips were pooled into another container. For the periodontitis patients, the teeth selected for gingival fluid sampling were the two teeth with the deepest periodontal pockets, while the same analogue teeth were used for the control group patients. Preventive measures to avoid sample contamination were taken. To prevent saliva contamination, cotton rolls were placed on oral vestibule and saliva suction was used underneath the tongue, and to prevent blood contamination, the paper strips were only inserted into the sulcus after no clearly visible signs of gingival bleeding were observed. Only the paper strips with no visible stains of blood on their surface were used. To assess the quantity of GCF absorbed by the paper strips, these were inserted into a special volume analyzer, Periotron 8000 (Oraflow Inc.). Afterwards, the two containers from each patient were refrigerated at -20˚C, until completion of sample collection.

Assessment of GCF IL-1α and IL-1β level. The enzyme-linked immunosorbent assay (ELISA) was used for qualitative and quantitative assessment of the two pro-inflammatory cytokines in the GCF samples. Commercial detection kits designed for the micro-detection of the two cytokines were used (Quantikine; R&D Systems) in accordance with the manufacturer's indications and prescribed method. In order to decrease optical imperfections on the reading plate, reading was performed at 450 with 540 nm corrections. The obtained results were expressed in pg/ml.

Statistical analysis. Data was statistically analyzed with Microsoft Office Excel Data Analysis tool kit software (Microsoft Corporation), the resulted mean values and standard deviations being used as primary data (mean ± SD). The statistical significance of the results was assessed using the Mann-Whitney U test ($P<0.05$) for comparison between the different study groups. Correlations between statistical and clinical data were made using Pearsons's test.

Results

The GCF levels of pro-inflammatory cytokines, for both IL-1α and IL-1β, were the highest for the PH group, followed by the P group, while the lowest were those of the H group (Figs. 1 and 2).

Statistically significant differences ($P<0.05$) were recorded for GCF IL-1α levels, between the PH and P groups (the levels for the PH group being 1.8-fold higher than that for P group), between the PH and H groups (the levels for the PH group being 6.9-fold higher than that for the H group) and between P and H groups (the levels for the P group being 3.8-fold higher than that for the H group) (Fig. 1).

The average GCF levels of IL-1β expressed statistically significant differences ($P<0.05$) between the PH and P groups (the levels for the PH group being 2.1-fold higher than that of the P group), between the PH and H groups (the levels for the PH group being 5.1-fold higher than that for the H group) and between the P and H groups (the levels for the P group being 2.41-fold higher than that for the H group) (Fig. 2).

Table I. Correlations between the assessed clinical and immunologic parameters.

	PH Group		P Group		H Group	
Index	IL-1α	IL-1β	IL-1α	IL-1β	IL-1α	IL-1β
RT						
r	-0.44	-0.29	-0.42	-0.3	-0.37	-0.8
p	0.17	0.37	0.13	0.3	0.25	0.02
MD						
r	0.23	0.35	0.21	0.21		
p	0.48	0.26	0.42	0.41		
AT						
r	0.32	0.25	0.28	0.68		
p	0.33	0.37	0.31	0.01		
DG						
r	0.23	0.29				
p	0.49	0.38				

RT, number of existing/remnant teeth; MD, maximum probing depth; AT, number of teeth with periodontal pockets >4 mm; DG, age of HCV infection diagnosis; r, Pearson's r for correlation strength; p, statistical significance index; IL, interleukin.

In P and PH patients, the levels of GCF cytokines expressed correlations with certain parameters of periodontal status (Table I). The results of the periodontal and hepatic evaluation have been previously published (27), expressing statistically significant differences between the P and PH groups, regarding the average number of teeth, the maximum periodontal probing depth and the AST/ALT serum levels.

For the PH group, the MD was positively correlated to the GCF levels of both IL-1α and IL-1β. For the P group, a moderate positive correlation was found between the MD parameter and the level of GCF cytokines.

As previously shown (27), the AT did not express significant statistical difference between the PH and P groups. No significant correlations were found between this periodontal parameter and the GCF levels of IL-1α or IL-1β. As it was shown (27), the RT was significantly different between the PH and P groups. This parameter expressed negative correlations with the GCF levels of both IL-1α and IL-1β.

For the PH group, the age of the HCV infection diagnosis expressed a slight positive correlation with the GCF levels of IL-1α and IL-1β.

Discussion

In PD patients, an important imbalance exists between the subgingival bacterial plaque aggression and the host immune response, a situation that can lead to extensive damage of the periodontal tissues, such as the periodontal ligament and alveolar bone. Consequently, the teeth lose their supporting structures and gain increasing mobility (28).

PD usually has a chronic evolution, when episodes of disease activity and high tissue destruction alternate with periods of inactivity and slight improvement of the clinical features of periodontal inflammation (29). This is caused either by fluctuations in bacterial aggressiveness, or by improvements of the host immune response. Unlike other gingival changes that do not involve deep tissue destruction, as those occurring during orthodontic treatment (30), these events will eventually lead to tooth loss, if left untreated or if continuous periodontal treatment is disrupted (5).

Periodontal infection is caused by the subgingival bacterial biofilm, with important influences from local and general risk factors (31,32). The bacterial biofilm develops gradually and can survive for an undetermined period of time, if left undisrupted by mechanical and chemical means. This causes the periodontal tissues to be exposed for a long period to bacterial toxins [lipopolysaccharide (LPS)], enzymes (proteases) and other noxious metabolic products (ammonia, hydrogen sulphide and butyric acid) (33). Moreover, bacterial cells invade the periodontal connective tissues via the internal and junctional epithelium. All these factors act as aggressors and cause chemical, physical and biological damage to cells. Consequently, the inflammatory reaction is triggered and cytokine production is elevated, in order to boost the immune response and to uphold the bacterial challenge (5).

Elevated gingival fluid levels for IL-1, found in periodontal patients, compared to healthy controls, have been shown to decrease after periodontal treatment (34), a fact which further supports their important role in periodontal disease development. The results of the present study expressed a statistically significant difference between the GCF cytokine levels of the PH and P groups. Since patients in both groups were diagnosed with periodontal disease, in similar degrees of severity and evolution, the higher GCF cytokines levels in periodontitis patients with chronic hepatitis C, could be explained by the additional chronic hepatic inflammation that these patients manifest. This fact can impact the intensity of the inflammatory periodontal reaction as well. Moreover, the GCF cytokine level was significantly different between P and H groups and between PH and H groups, suggesting the reliability of cytokines as indicators of the inflammatory status.

In chronic periodontitis patients, the levels of GCF cytokines expressed a positive correlation to the degree of periodontal inflammation. In the present study, the clinical indicators used for the assessment of the periodontal status (MD, AT, RT) were correlated to the GCF levels of IL-1α and IL-1β in periodontal patients with chronic hepatitis C. There was a moderate positive correlation also between these clinical parameters in periodontal patients with no systemic condition and the GCF cytokine levels, suggesting the additional effect that systemic chronic inflammation can have on the periodontal status of such patients. The number of remnant teeth was statistically significantly different between the three groups and in a negative correlation with the GCF cytokine levels, emphasizing the important impact that severe chronic inflammation has on the dental and periodontal history of the patient, frequently resulting in the loss of teeth, as a consequence of periodontal disease. Due to the reduced number of participating patients in the study, consequent to the numerous exclusion criteria, required in order to follow a precise and reliable scientific method, the identified correlations generally lacked statistical significance, despite their moderate

correlation strength. This issue motivates the extension of the study design on a broader cohort of participating patients.

The number of teeth with periodontal pockets deeper than 4 mm (AT) was not statistically different between the PH and P groups and it did not correlate with the GCF cytokine levels. However, the average maximum periodontal pocket depth (MD) was statistically different between the PH and P groups and expressed a positive correlation to the GCF levels of both IL-1α and IL-1β. This fact could imply that hepatic chronic inflammation could have a significant impact on the severity of periodontal disease and a less important one on its extent, as in the number of affected teeth.

In this study, there was a moderate positive correlation between the GCF levels of cytokines of the PH group patients and the age of their HCV infection diagnosis. This correlation suggests that, as the chronic hepatic inflammatory reaction progresses, it has an increasingly important negative impact on the inflammatory periodontal status of CHC patients, who also suffer from periodontal disease. Thus, periodontal and hepatic chronic inflammatory reactions could influence one another, as they may be fuelled by the same pro-inflammatory cytokines, i.e. IL-1α and IL-1β. These correlations suggest the important negative impact that hepatic chronic inflammation has on the periodontal status regarding the intensity of the periodontal inflammatory reaction.

In CHC patients, elevated levels of IL-1α and IL-1β were showed in serum samples, in previous studies (23,24). Levels of certain cytokines were significantly elevated when the CHC patients were also suffering from diabetes (35). Furthermore, the incidence of type II diabetes among CHC patients is higher than that of the general population (36). One explanation for this correlation can be found in the impact of chronically elevated systemic IL-1β levels on general homeostasis (37). It appears that, cellular insulin resistance is closely linked to elevated levels of cytokines, which are common in CHC patients (38-40). In the hepatic tissue of affected patients, insulin receptor substrate has been reported to be impaired, together with the disruption of normal insulin signalling pathways (40). Increased cytokine expression (such as TNF-α) could also contribute to the onset of insulin resistance in CHC patients (40). Consequently, the cells become insensitive to insulin action and glucose is unable to enter inside them, in order to be metabolised. Thus, chronic hyperglycemia occurs, the most important pathologic manifestation of diabetes mellitus. Interestingly, insulin resistance has also been associated with PD, while elevated levels of IL-1β have often been recorded in chronic periodontitis patients (41,42). The interactions between PD and liver diseases, including CHC, could be mediated by either bacterial elements, pro-inflammatory cytokines or oxidative stress (43). Elevated GCF levels of IL-1α and IL-1β could explain the pathogenic mechanism that connects PD and CHC. By changing the general homeostasis, the chronic inflammatory status caused by PD and CHC, inflicts a modified cellular response. Patients with CHC and PD exhibit elevated cytokine profiles, suggesting the bi-directional impact that chronic hepatic inflammation and periodontal disease could manifest on each other. Insulin resistance could be one of the probable pathogenic mechanisms to mediate the PD-CHC relationship (19,43), motivating extended future research on the matter.

In conclusion, elevated levels of IL-1α and IL-1β, which have considerable implications in the pathogenic processes of both PD and CHC, could imply that chronic hepatitis C has a negative impact on the inflammatory status of periodontal patients, as assessed by interleukin detection.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

PS, DNG, AR, LL, SIS, MVB and IR contributed to the study design. DMP and DNG performed the clinical periodontal assessment and collection of gingival crevicular fluid samples. MD and IR interpreted the hepatic status of chronic hepatitis C patients. MVB and LB carried out the immunological analysis and acquisition of laboratory data. AMM, SS and DNG performed the statistical data analysis. DR and DNG drafted the manuscript, which was critically revised by PS, AR, LL, SS, SIS and LF. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The protocol and patient informed consent form for participating and collection of gingival crevicular samples were approved by the University Ethics Scientific Committee (University of Medicine and Pharmacy of Craiova, Romania, no. 17/2016). Written informed consent was given by all participating patients.

Patients consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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