 DOI: https://doi.org/10.5114/ceji.2019.89601

# Salivary interleukin 6, interleukin 8, interleukin 17A, and tumour necrosis factor α levels in patients with periodontitis and rheumatoid arthritis

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***Abstract***

***Introduction:*** *Rheumatoid arthritis (RA) and periodontitis share risk factors and inflammatory pathways that could be related to cytokines, such as interleukin (IL)-6, IL-8, IL-17A, and tumour necrosis factor-α (TNF-α). The aim of this study was to compare periodontal status and salivary levels of selected cytokines between patients diagnosed with RA and periodontitis. RA patients were assessed for the potential influence of anti-rheumatic therapy.*

***Material and methods:*** *One hundred and six patients were enrolled in a cross-sectional study. Medical assessment and periodontal examination were performed in 35 patients with chronic periodontitis, in 35 patients with RA and chronic periodontitis, and in 36 controls. Unstimulated whole saliva samples were analysed for IL-6, IL-8, IL-17A, and TNF-α.*

***Results:*** *Significant differences in biomarkers and periodontal parameters were found among groups. Study groups exhibited higher mean pocket depth (PD), number of PD > 4 mm, and mean clinical attachment loss, when compared with controls. The RA group had lower bleeding on probing index and PD, but higher values of plaque indices than the periodontitis group. Concentration of evaluated cytokines were higher in the RA and periodontitis groups, compared with controls. The periodontitis group showed also higher levels of IL-6, IL-17A, and TNF-α in comparison to RA. RA patients were treated with disease-modifying anti-rheumatic drugs (DMARDs) and glucocorticosteroids.*

***Conclusions:*** *Salivary levels of IL-6, IL-8, IL-17A, and TNF-α can be affected by periodontitis, RA, and presumably DMARDs. DMARD therapy appears to reduce destructive and inflammatory processes in periodontal tissues because lower values of PD, BOP, and salivary levels of IL-6, IL-17A, and TNF-α were found in RA.*

***Key words:*** *periodontitis, rheumatoid arthritis, salivary diagnostics, interleukin 6, interleukin 8, interleukin 17A, tumour necrosis factor α.*

*(Centr Eur J Immunol 2019; 44 (3): 269-276)*

## Introduction

Periodontitis is a chronic destructive inflammatory condition that affects soft and hard tissues surrounding and supporting the teeth. According to recent epidemiological studies, periodontitis is a social disease with high occurrence among the middle-aged and elderly population [1]. Prevalence of periodontitis has been associated with other systemic conditions such as type II diabetes, cardiovascular diseases, osteoporosis, premature birth, and low birth weight [2]. Recent studies indicate a possible link between periodontitis and rheumatoid arthritis (RA), which is supported by case-control [3, 4], prospective cohort [5, 6], and cross-sectional studies [7, 8]. Individuals with RA were found to be at increased risk of developing periodontitis in

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Submitted: 11.03.2019; Accepted: 30.03.2019

two independent meta-analyses [9, 10]. Even though this association might be based on shared environmental and genetic risk factors, such as smoking [11, 12] and genetic factors [13-15], a possible causal relation was advocated by interventional studies [16-19].

The diagnosis of periodontitis is currently based on clinical and radiological findings. This methodology is relatively time-consuming and may be subject to error due to lack of inter- and intra-examiner reproducibility [20]. This led clinicians to search for additional chair side measures to aid in early diagnosis and monitoring. Studies showed that oral fluids, such as gingival crevicular fluid (GCF) and saliva, can be used for this purpose [21-23]. The use of saliva is appealing because it has the advantage of being non-invasive and easy to collect, with potential use for point-of-care analysis [24].

Systemic inflammatory conditions may influence levels of certain salivary biomarkers because saliva contains serum-derived components, which may tamper with periodontal diagnostics. The imbalance between pro-inflammatory and anti-inflammatory cytokines is a common feature of both RA and periodontitis, which might result in the inflammatory destruction of specific tissues. Studies have shown that certain salivary cytokines levels or biomarkers might be elevated in individuals with both periodontitis and RA [25-27].

Interleukin (IL)-6 is the most abundantly expressed cytokine in synovium in individuals with RA, and together with IL-8 might play a key role in development of the disease [28, 29]. It has been demonstrated that by inducing endothelial cells to produce IL-8 and to activate expression of adhesion molecules and recruit leukocytes to involved joints, IL-6 was involved in local joint inflammation [30]. Both IL-6 and IL-8 significantly contributed to periodontitis; IL-6 has shown several biological activities such as B-lymphocyte differentiation, T-lymphocyte proliferation, and the stimulation of immunoglobulin secretion [31].

IL-8 is a potent chemoattractant cytokine and activator of neutrophils in inflammatory regions, which can be released from endothelial cells, gingival fibroblasts, neutrophils, monocytes, and phagocytes in the gingival crevice [32]. IL-6 and IL-8 levels seem to correlate with the status of periodontium and react to periodontal treatment [33, 34].

Tumour necrosis factor α (TNF-α) is a potent macrophage-derived cytokine that presents in inflamed synovium and gingiva during the course of RA and periodontitis [35, 36]. TNF-α induces expression of matrix-degrading proteases, prostanoids, IL-1, IL-6, IL-8, and granulocyte-macrophage colony stimulating factor [37]. Concentration of TNF-α in saliva correlated with periodontal status [38, 39].

IL-17A secreting helper CD4 T cells (Th17 cells) acts on neutrophils, macrophages, fibroblasts, and osteoclasts to induce an inflammatory reaction in bone and cartilage. IL-17A provokes an inflammatory reaction by itself, but it is equally capable of synergising with TNF-α in the expression of IL-1, IL-6, and IL-8 [40]. It was suggested that Th17 cells and IL-17A could play a crucial role in the progression of both RA and periodontitis [41, 42].

As found in previous reports, both periodontitis and RA might be associated with the elevation of certain biomarkers in saliva, the aim of this study was to evaluate the influence of rheumatoid arthritis and periodontitis on salivary concentrations of selected cytokines: IL-6, IL-8, IL-17A, and TNF-α. Furthermore, all cytokines as well as periodontal and rheumatic parameters were analysed to determine significant correlations. RA patients were assessed for any potential influence of anti-rheumatic therapy on salivary cytokines or periodontal status.

## Material and methods

### Qualification of patients

One hundred and six patients were enrolled in this cross-sectional study performed at the Department of Periodontology and Oral Mucosa Diseases, Medical University of Warsaw and the Department of Rheumatology, National Institute of Geriatrics, Rheumatology and Rehabilitation. Thirty-five randomly selected patients with diagnosed severe chronic periodontitis (periodontitis group – PG) based on the criteria defined by the American Academy of Periodontology [43], 35 randomly selected individuals with diagnosis of severe chronic periodontitis and seropositive RA (rheumatoid arthritis group – RAG) according to the ACR/EULAR criteria [44], and 36 randomly selected non-periodontitis systemically healthy control patients (control group – CG) were introduced to the study. However, it should be underlined that taking into consideration the new classification system of periodontal diseases, all of the periodontitis cases involved in this study would be categorised as periodontitis Stage III or IV Grade B [45]. The study received the positive approval by the institutional review board. All clinical procedures were carried out in accordance with the Helsinki Declaration of 1975, as revised in Tokyo in 2004. Written, informed consent forms were signed by every patient.

The inclusion criteria for the study were: 1) the presence of at least 15 teeth (excluding third molars) and 2) age between 18 and 75 years. Additional criteria for PG were: 1) the presence of at least two teeth with pocket depth (PD) ≥ 5 mm and interproximal clinical attachment loss (CAL) ≥ 5 mm in each quadrant, 2) bleeding on probing (BOP) ≥ 30%, 3) evidence of radiographic alveolar bone loss ≥ 5 mm at ≥ 30%; and for RAG: 1) mild or severe disease activity according to disease activity score (DAS28) [46], 2) treatment with classic disease-modifying anti-rheumatic drugs (DMARDs). CG patients had: 1) no sites with PD ≥ 5 mm, 2) no sites with CAL ≥ 2 mm, and 3) no evident radiographic alveolar bone loss. The exclusion criteria

were as follows: 1) no periodontal treatment during the 12 months prior to the study, 2) taking local or systemic antibiotics in the previous three months, 3) active smoking or smoking within the previous five years, 4) diagnosis of any systemic disease that may modulate the course of periodontal disease or affect the systemic inflammatory response (excluding RA), and 5) pregnancy and lactation.

### Clinical evaluation

The clinical examination included measurements at six points within each tooth (three lingual/palatal: mesial, central, and distal and three buccal: mesial, central, and distal). The clinical examination was performed by one calibrated examiner after collection of saliva and included evaluation of bleeding on probing (BOP) according to Ainamo and Bay protocol [47], plaque index (PI) according to O'Leary *et al.* [48], approximal plaque index (API) according to Lange *et al.* [49], PD, and CAL. Measurements were carried out with a manual periodontal probe (PCPUNC 15, Hu-Friedy, USA), and the reference point for evaluation of clinical attachment loss was the cementoenamel junction (CEJ). If it was not possible to locate the CEJ, the apical margin of the restoration or prosthetic crown was assumed as the reference point.

The examination of the joints in patients in the RAG was carried out by one rheumatologist and included an assessment of the number of painful and swollen joints, as well as a global assessment of the patient's disease. The patient assessed global disease activity on the visual analogue scale. Data on current treatment with DMARDs and steroids (their type and dose) were collected. In addition, the levels of C-reactive protein, erythrocyte sedimentation rate, rheumatic factor, and cyclic citrullinated peptide antibodies were measured.

### Saliva collection

Five millilitres of whole unstimulated saliva was collected from all patients. The material was collected in the morning (between 08.00 and 12.00), at least two hours after the last meal, according to the technique described by Navazesh [50]. The collected samples were frozen at –80°C, and their analysis was performed within a period not exceeding six months from the moment of freezing.

### Cytokine analysis

Luminex xMAP technology for multiplexed quantification of cytokines in the saliva samples was used. The multiplexing analysis was performed using the Bio-Plex Luminex™ 200 system (Bio-Rad Laboratories, Hercules, CA, USA). Cytokines were simultaneously measured using a Bio-Plex Pro Human Cytokine Assay (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's protocol.

### Statistical analysis

Statistical analysis was performed using Statistica 13.1 software (StatSoft Inc., Tulsa, USA). Compatibility with the normal distribution of data obtained was assessed with the Shapiro-Wilk test. For normally distributed groups one-way analysis of variance (ANOVA) was used to compare differences among groups. The Tukey test was used in conjunction with ANOVA to find which means were significantly different from one another. Kruskal-Wallis test was used to compare differences among groups that were not normally distributed, with the Bonferroni test used to find which ranks were significantly different from one another. Correlations were based on Spearman's and Pearson's rank correlation coefficient. Values of $p < 0.05$ were considered statistically significant.

## Results

A total of 106 patients (35 in the PG, 35 in the RAG, and 36 in the CG) were included in the study. The demographics and clinical parameters of all groups are shown in Table 1. The mean ages for the three groups were similar, but they differed in terms of clinical parameters. In a comparison between PG and RAG significantly higher values of BOP and PD were observed in the former, but PI and API values were higher in RAG. The mean CAL values were similar. The RAG in contrast to the CG had significantly higher values of PI and API as well as PD, PD ≥ 4 mm and CAL. When compared with controls, PG had similar values of PI and API, but significantly higher values of BOP, PD, PD ≥ 4 mm and CAL. The CG had a significantly higher mean number of teeth than the other groups.

The characteristics of RAG patients are presented in Table 2. The most commonly used DMARD was methotrexate (MTX), followed by sulfasalazine, leflunomide, and hydroxychloroquine. Seventeen of the RA patients, in addition to DMARDs, were on glucocorticoids with a mean dose of 6.35 ±4.14 mg of methylprednisolone.

Salivary levels of IL-6, IL-8, IL-17A, and TNF-α in the three groups are presented as box plots in Figure 1. Mean levels of all cytokines were significantly elevated in periodontitis and RA when compared with controls ($p < 0.0001$). Concentrations of IL-6, IL-17A, and TNF-α were statistically higher in PG than in RAG ($p < 0.0001$). In RAG no differences between patients receiving different treatment modalities were found.

In RAG the levels of IL-6 significantly correlated with DAS28 and SDAI (simple disease activity index) scores ($r = 0.40$ and 0.36, respectively; $p < 0.05$). In the same group BOP and PD ≥ 4 mm positively correlated with IL-17A ($r = 0.38$ and 0.36, respectively; $p < 0.05$). Serum RF levels correlated significantly with PI ($r = 0.34$, $p < 0.05$) and the number of teeth ($r = –0.39$, $p < 0.05$).

**Table 1.** Comparison of study groups

| Characteristic | PG ($n$ = 35) | RAG ($n$ = 35) | CG ($n$ = 36) | $p$-value |
|---|---|---|---|---|
| Age (years, mean ±SD) | 59.31 ±9.75 | 55.66 ±10.56 | **53.92 ±10.96** | NS |
| Females (%) | 48.57 | 85.71 | 75.00 | 0.00023* |
| Number of teeth (mean ±SD) | 21.94 ±4.07 | 20.69 ±5.93 | 25.51 ±2.20 | 0.0001** |
| BOP (%, mean ±SD) | 63.59 ±19.52 | 53.55 ±17.32 | 50.29 ±15.65 | 0.0054* |
| PI (%, mean ±SD) | 50.00 ±20.50 | 74.28 ±18.69 | 48.49 ±16.74 | < 0.0001*** |
| API (%, mean ±SD) | 64.84 ±25.07 | 90.03 ±15.33 | 52.15 ±19.97 | < 0.0001*** |
| PD (mm, mean ±SD) | 3.87 ±0.80 | 3.14 ±0.54 | 2.46 ±0.35 | < 0.0001**** |
| PD ≥ 4 ($n$, mean ±SD) | 45.65 ±32.50 | 31.37 ±19.35 | 4.25 ±2.52 | < 0.0001** |
| CAL (mm, mean ±SD) | 3.68 ±1.15 | 3.12 ±1.25 | 1.81 ±0.61 | < 0.0001** |

*API – approximal plaque index, BOP – bleeding on probing, CAL – clinical attachment loss, CG – control group, NS – not statistically significant, PD – pocket depth, PG – periodontitis group, PI – plaque index, RAG – rheumatoid arthritis group, * applies to comparisons of periodontitis vs. RA or control groups, ** applies to comparisons of periodontitis or RA vs. control group, *** applies to comparisons of RA vs. periodontitis or control groups, **** applies to comparisons between all groups*

**Table 2.** Characteristics of rheumatoid arthritis patients

| Characteristic | Value |
|---|---|
| DAS28 (mean ±SD) | 5.74 ±1.42 |
| SDAI (mean ±SD) | 32.52 ±15.88 |
| severe disease activity ($n$, %) | 23 (71.87) |
| Positive for RF ($n$, %) | 28 (80) |
| Positive for ACPA ($n$, %) | 32 (91.43) |
| DMARDs (mean, $n$ ±SD) | 1.19 ±0.60 |
| methotrexate ($n$, %) | 22 (62.86) |
| sulfasalazine ($n$, %) | 5 (17.5) |
| leflunomide ($n$, %) | 5 (17.5) |
| hydroxychloroquine ($n$, %) | 5 (17.5) |
| Glucocorticoids (n, %) | 17 (48.57) |
| dose (mg of methylprednisolone, mean ±SD) | 6.35 ±4.14 |

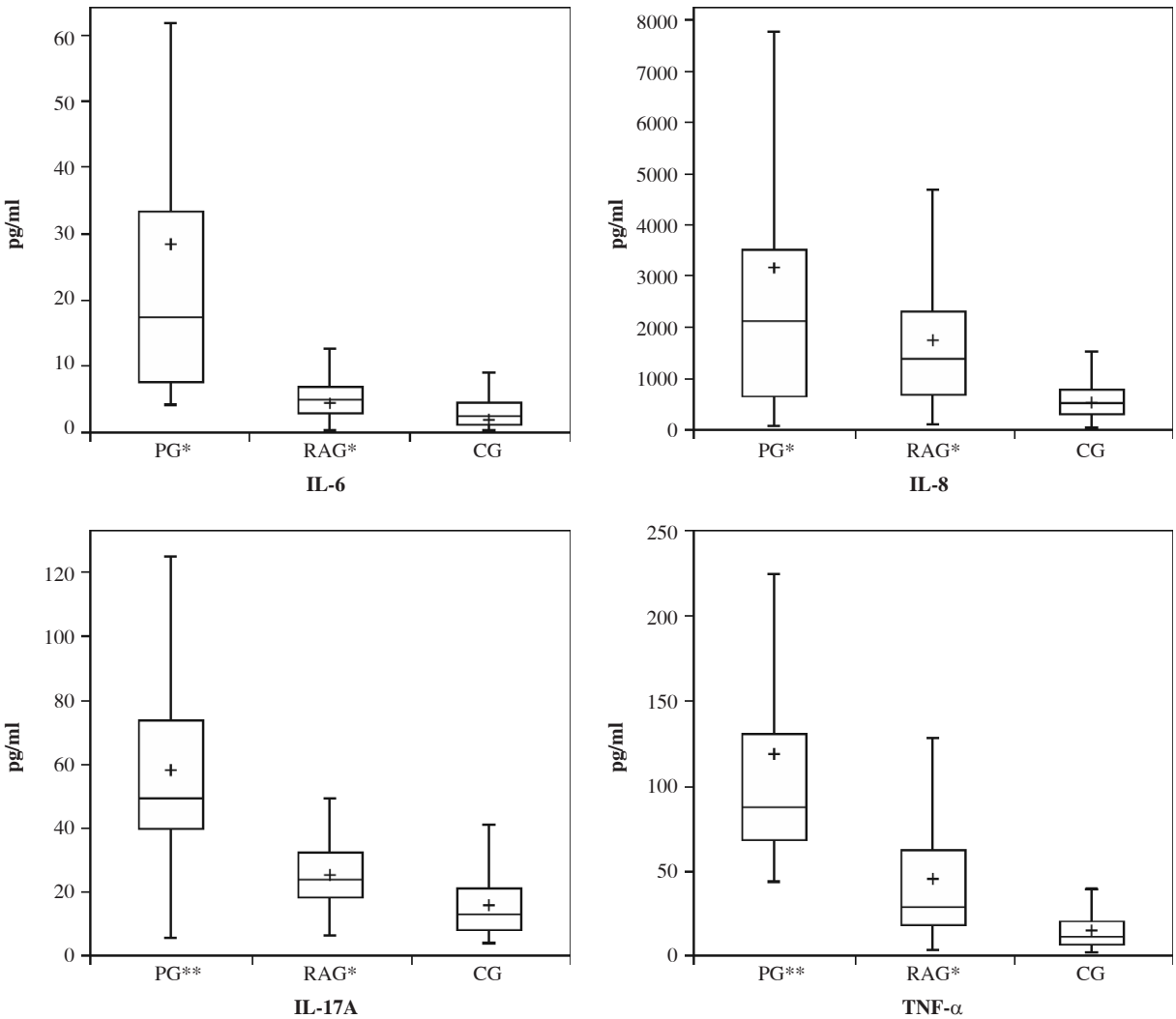
*ACPA – antibodies to citrullinated peptides, DAS28 – Disease Activity Score, DMARDs – disease-modifying antirheumatic drugs, RF – rheumatoid factor, SDAI – Simple Disease Activity Index*

The number of swollen joints positively correlated with the number of teeth ($r$ = 0.39, $p < 0.05$). There were no correlations that were significant in PG.

## Discussion

This study found that salivary levels of IL-6, IL-8, IL-17A, and TNF-α were elevated in both study groups (PG and RAG) when compared with controls (CG). However, lower values were found in RAG patients than in PG. There are only a few studies that have dealt with this topic. Mirrielees *et al.* [25] evaluated the influence of RA and periodontitis on salivary biomarkers and observed elevated TNF-α levels in the PG. This is in agreement with our findings of salivary TNF-α levels. The same study found significantly lower salivary TNF-α levels in RA patients treated with anti-TNF therapy. In our report patients were treated with classical DMARDs, but no differences between patients treated with various DMARDs were noted. Another research conducted by Gamel *et al.* [26] assessed salivary TNF-α levels and reported no differences between periodontitis and RA. Our result demonstrates that RAG had elevated salivary IL-6 levels when compared with the non-periodontitis CG, which is in accordance with results obtained by Silvestre-Rangil *et al.* [51]. To the best of our knowledge, no other studies evaluated salivary cytokines between periodontitis and RA. Our study examined the influence of RA on salivary IL-8 and IL-17A levels for the first time. Another report that assessed IL-6 levels in GCF found no differences in IL-6 levels between periodontitis and RA, despite significantly higher PI values in the latter group [52]. Cetinkaya *et al.* [53] investigated differences in clinical status and GCF levels of IL-1b, IL-4, IL-10, and TNF-α among periodontitis, RA, and controls, quite similarly to our research. The authors recorded no significant differences in terms of TNF-α levels, but RA subjects exhibited significantly higher PI despite lower BOP, PD, and CAL. This is in agreement with our findings. We demonstrated higher PD and BOP in PG, even though RAG showed worse oral hygiene as assessed with API and PI. The similar attachment loss found in both groups could indicate a comparable rate of progression of periodontitis in the past.

We can only speculate that less severe periodontal disease and lower levels of IL-6, IL-8, IL-17A, and TNF-α in RAG might be related to DMARD or glucocorticosteroid therapy. This opinion is supported by results obtained by Ziebolz *et al.* [54], which suggest that RA therapy with different DMARDs can affect periodontal inflammation and levels of periodontal pathogenic bacteria. MTX, which was the most commonly used DMARD, inhibits the enzyme dihydrofolate reductase and hence thymidine synthesis, transmethylation of RNA, DNA, proteins, phospholipids,




*PG – periodontitis group, RAG – rheumatoid arthritis group, CG – control group, horizontal lines – the 25th, 50th, and 75th percentiles; + the mean, * statistically significant difference compared to CG, ** statistically significant difference compared to RAG and CG*

**Fig. 1.** Box plots of salivary concentrations of IL-6, IL-8, IL-17A, and TNF-α in all three groups

and de novo purine synthesis. As a result, MTX is able to suppress secretion of IL-6, IL-8, IL-17A, and TNF-α [55-58]. The use of DMARDs combined with glucocorticoids have also been found to reduce levels of IL-6 [59]. Leflunomide is a DMARD that inhibits dihydroorotase dehydrogenase and thereby pyrimidine synthesis and has been reported to potentially reduce levels of IL-6, IL-8, IL-17A, and TNF-α [60-62]. Another DMARD, sulfasalazine, has several mechanisms of action that include inhibition of chemotaxis of inflammatory cells, inhibition of cytokine expression in mononuclear cells, inhibition of lymphocyte proliferation and activation, as well as inhibition of angiogenesis. Various studies have reported that it has the potential to inhibit expression of IL-8 and TNF-α [63, 64]. Hydroxychloroquine is an antimalarial agent that has been used in RA treatment for many years. Despite the lack of complete understanding of its anti-inflammatory mechanism, it is proposed that the inhibition of Toll-like receptor signalling was responsible for this phenomenon [65]. Evidence from an in vitro study suggests that hydroxychloroquine might inhibit the production of IL-6 and IL-17A [66].

Salivary IL-6 correlations with disease activity displayed by DAS28 and SDAI scores are consistent with observations found in other fluids. Several studies exhibited elevated IL-6 concentration in serum and synovium in RA individuals, which was associated with disease activity [28, 67]. Kobayashi *et al.* [68] found that serum IL-6 and TNF-α significantly correlated with DAS28 in RA patients.

Current knowledge about salivary cytokine levels in patients with both RA and PD is limited. Our study ex-

pands knowledge on this subject. The strength of our study is the clinical evaluation of both the PD and RA status of all individuals recruited. The biggest limitation is the cross-sectional character of the study and the relatively small sample size. Large, prospective studies would be the most appropriate, especially for studying the impact of RA therapy on salivary cytokines levels. Furthermore, the studied group were not sex matched; in the RA group there were significantly more females compared to the PD and CG, which reflects the higher prevalence of females in the RA population [69]. However, this could affect our results only to a small extent because several previous studies showed that salivary levels of evaluated cytokines are not gender dependent [70-75].

## Conclusions

The present study underscores evidence that salivary levels of IL-6, IL-8, IL-17A, and TNF-α can be influenced by local and systemic inflammatory diseases, as well as by anti-inflammatory drugs like DMARD and glucocorticoids. Our findings provide further support for the use of salivary diagnostics in monitoring periodontal status, especially in otherwise healthy individuals.

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*The authors declare no conflict of interest.*

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