Salivary and Serum Concentrations of Monocyte Chemoattractant Protein-1, Macrophage Inhibitory Factor and Fractalkine in Relation to Rheumatoid Arthritis and Periodontitis

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Abstract

Background: Monocyte chemoattractant protein-1 (MCP-1), macrophage migration inhibitory factor (MIF), and fractalkine are chemokines that are expressed by a variety of cell types to regulate macrophage inflammatory response. The aim of the study was to examine the effects of periodontitis and rheumatoid arthritis (RA) on their serum and salivary concentrations.

Methods: Adults with either periodontitis (P, n=21), or with rheumatoid arthritis (RA, n=23), or with both diseases (RA+P, n=23) were included in the study. Systemically and periodontally healthy individuals (n=22) were served as controls. Saliva and serum samples were collected from all participants before the medical and periodontal examinations. Salivary and serum MCP-1, MIF, and fractalkine concentrations were measured by the Luminex technique. Total salivary protein levels were determined by the Bradford assay.

Results: Salivary MCP-1, MIF, and fractalkine concentrations were elevated in both RA groups (RA+P and RA) in comparison to systemically healthy controls. As related to total salivary protein levels, higher MCP-1 (P=0.003) and fractalkine (P=0.045) concentrations were found in controls compared to the P group. In serum, MCP-1 concentrations in the RA+P group were higher (P=0.003) than those of group P. Elevated serum fractalkine concentrations were observed in both periodontitis groups (RA+P, P=0.014 and P, P=0.013) compared to controls.

Conclusion: In RA, MCP-1, MIF, and fractalkine concentrations are elevated in saliva. These chemokines may disrupt oral macrophage responses and potentially take part in the interaction between periodontitis and RA.

Keywords: Saliva, periodontitis, inflammation, innate immunity.

1. INTRODUCTION

Periodontitis, a multifactorial disease of tooth-supporting tissues, is induced by dysbiotic bacterial biofilms, which result in ineffective, uncontrolled, and destructive host inflammatory response.[1] Indeed, being a chronic infection-induced inflammatory condition, periodontitis is associated with various systemic diseases, such as atherosclerosis, diabetes, and rheumatoid arthritis (RA).[2] RA is an autoimmune disease characterized by synovitis and irreversible joint destruction.[3] Its etiology remains unclear but it is presumed to be triggered by mucosal inflammation in combination with genetic and environmental risk factors.[3] Over the years, RA and periodontitis have been thought to be interrelated and to share common risk factors.[4-6] Chronic and uncontrolled inflammatory response against dysbiotic subgingival biofilms has systemic consequences, which manifest as an increased production of proinflammatory cytokines and chemokines.[4] Periodontitis and RA exhibit similar cytokine profiles; high levels of proinflammatory and osteoclastogenesis-related cytokines, including interleukin-1beta (IL-1β) and tumor necrosis factor-alpha (TNF-α), are detected in both diseases.[7] While the dysregulation of the cytokine network can be one explanation for the clinical and epidemiological link between between these diseases,[4-5] the exact mechanims have not been elucidated.

The most common leukocytes present in the gingival crevice and in periodontal pockets are neutrophils.[8] Periodontopathogens can interfere with neutrophil-mediated killing in a persisting inflammatory environment.[8] Although macrophages suppress bacterial infection by fagocytosing apoptotic neutrophils, their accummulation to the site of persisting infection and dysfunction in inflammatory responses can disrupt tissue homeostasis, contributing to the pathogenesis of inflammatory diseases, such as periodontitis and RA.[9] Macrophage behavior is regulated by various intrinsic and extrinsic cytokine networks.[10] The monocyte chemo-attractant protein-1 (MCP-1), also known as CC chemokine ligand-2 (CCL-2), is a potent

chemoattractant for monocytes.[11] It stimulates lytic enzymes and enhances phagocytic activity and osteoclast formation.[11] In endothelial cells, MCP-1 expression is induced by macrophage migration inhibitory factor (MIF), which is a pleiotropic inflammatory chemokine.[12] It is constitutively expressed by monocytes and macrophages and acts as a regulator of responses to inflammation and stress.[13] MCP-1 and MIF have been implicated in several inflammatory diseases; for example, increased MCP-1 and MIF levels in synovial fluid and serum have been reported from RA patients.[14-15] However, no consensus exists regarding the salivary levels of these chemokines in relation to periodontitis.[16-18]

Fractalkine is a unique chemokine with CX3CL structure, differing from other chemokines; it is membrane-anchored and exists in soluble glycoprotein forms.[19] It is mainly procuded by endothelial cells, monocytes, and macrophages.[20] Fractalkine has multifunctional properties, including angiogenic capacity by induction of endothelial tube formation,[21] and it also plays an important role in bone destruction by dual functions as a chemotactic factor and an adhesion molecule for osteoclast precursors.[22] In serum and synovial fluid, fractalkine levels are significantly higher in RA patients than in patients with osteoarthritis (OA) and healthy controls.[23-24] Serum fractalkine levels correlate with disease activity in RA.[24] However, the information on fractalkine in periodontitis is limited.[25-27]

MCP-1, MIF, and fractalkine partipate in monocyte recruitment to the site of infection and in macrophage activation.[11,13,20] These three cytokines are also involved in RA-associated synovial angiogenesis and their concentrations are elevated in RA patients even in the absence of infection.[3] To the best of authors' knowledge, salivary and serum concentrations of MCP-1, MIF, and fractalkine in respect to RA and periodontitis have not been studied before. Here, we hypothesized that MCP-1, MIF, and fractalkine concentrations in saliva and serum of RA patients are elevated regardless of periodontal status. Therefore, the aim of the study was to

examine the independent and combined effects of periodontitis and RA on serum and salivary concentrations of these chemokines.

2. MATERIALS AND METHODS

2.1 Study participants

This study was approved by the human subjects ethics board of Sakarya University, Faculty of Medicine, Turkey (Protocol Number:16214662/050.01.04/114) and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013.

Overall, 89 participants were recruited to the study, among those who applied for regular dental and/or rheumatology check-ups to Department of Periodontology, Faculty of Dentistry and Department of Rheumatology (Internal Medicine), Faculty of Medicine at Sakarya University during June 2018 to September 2019. They were informed about the study protocol verbally and written informed consents were obtained. Demographic variables, including age, gender, medical and dental treatment history, alcohol consumption, and disease duration, if diagnosed with RA, were obtained by interviews. Exclusion criteria were: having less than 16 teeth, reporting the intake of antibiotics and/or having received periodontal treatment during the preceding 6 months prior to the initiation of the study, having chronic disease or related medication use with known effect on the periodontium, having diagnosed with other forms of arthritis, being pregnant or in lactating period, carrying genetic, renal, and hepatic disorders or HIV, or having history of transplantation.

Smoking status was received by self-reports. Participants, who never smoked or quitted smoking at least 2 years prior to the initiation of the study, were determined as non-smokers. Recent quitters (<2 years) and occasional smokers were excluded from the study.

2.2 Medical examination

The diagnosis of RA was based on the American College of Rheumatology criteria.[28] RA related biochemical analyses, including serum erythrocyte sedimentation rate (ESR) (♀: ≤ (age+10)/2, ♂: ≤ age/2 mm/hour), rheumatoid factor (RF) (0-15.9 IU/mL), and C-reactive protein levels (CRP) (0-5 mg/L) were performed. RA status was assessed by evaluating joints and was based on the numeric disease activity score index (DAS28).[29] According to this index, the following values determined the RA status: ≤2.6 in remission, >2.6 but ≤3.2 inactive, >3.2 but ≤5.1 moderate and >5.1 very active phase of RA.[29] The medication regimen of the participants with RA was also recorded; the use of methotrexate, sulfasalazine, leflunomide and hydroxychloroquine was defined as the conventional disease-modifying antirheumatic drug group (cDMARD)[30] (n=43) and the use of anti-tumor necrosis factor (TNF)-α, abatacept, rituximab, and tocilizumab as the biological DMARD group (bDMARD)[30] (n=6). All rheumatology assessments and recordings were performed by a single rheumatologist (EG).

2.3 Periodontal examination

Periodontal examination was performed by a single calibrated (Kappa: 0.89) periodontist (DY). Periodontitis was diagnosed according to the 2017 Classification of Periodontal and Peri-Implant Diseases and Conditions.[31] Clinical periodontal data, including plaque index (PI)[32], gingival index (GI)[33], probing pocket depth (PPD), and clinical attachment level (CAL), were recorded by using a manual periodontal probe‖ from six sites per tooth except the third molars. Alveolar bone loss was estimated by using an optimal quality panoramic radiograph. In case of unclear images, intra-oral radiological techniques were applied to correctly identify the presence and type of alveolar bone loss. Participants were diagnosed of having periodontitis, if they had bleeding on probing (BOP) ≥10% of the surfaces and interdental clinical attachment loss was detectable at ≥2 non-adjacent teeth with PPD ≥4 mm, while periodontal health was defined as BOP <10% of the surfaces and no sites with PPD >3 mm besides no loss of clinical

attachment or alveolar bone. Periodontitis patients were classified by using the stage (I-IV) and grade (A-C) system.[31] Briefly, in stages III and IV, radiographic bone loss extends to mid-third of the root or beyond with interdental CAL ≥5 mm at site of the greatest loss, and tooth loss due to periodontitis (PTL) ≤4 or ≥5 teeth, respectively. Complexity of disease management was also referred during the stage determination. For each stage, the extent was described as localized (<30% of teeth involved) or generalized (≥30% of teeth involved). The grade of periodontitis was identified by indirect evidence of progression, which was the bone level of the worst affected tooth and age (BL/A) ratio and case phenotype.[31] Grade B was defined as 0.25 to 1.0 BL/A.

Based on the systemic and periodontal clinical status, the participants were divided into four study groups: patients with both RA and periodontitis (RA+P) (n=23, male %=26.1), periodontally healthy RA patients (RA) (n=23, male %=21.7), systemically healthy periodontitis patients (P) (n=21, male %=52.4), and systemically and periodontally healthy controls (control) (n=22, male %=40.9). Of the 44 patients with periodontitis, 34 (77%) were identified with stage III/grade B and 10 (23%) with stage IV/grade B. The extent of disease was generalized in all patients diagnosed with periodontitis.

2.4 Sample collection

Saliva and serum samples were collected from all participants. An unstimulated saliva collection technique was applied. Briefly, the procedure was performed before periodontal and medical examinations, between at 9 to 11 AM. The participants were required to attend the procedure at least one hour after the last food intake.[34] They were kindly asked to spit and fill the calibrated, plastic 2 mL tubes for 5 minutes. After collection, the samples were centrifuged at 6000 g for 5 minutes and immediately stored at -80°C until analyses. Venous blood samples were obtained from antecubital veins by the standard venipuncture method. A 10 mL of venous

blood was collected into blood collection tubes without anticoagulant, and the samples were centrifuged at 1500 rpm for 15 minutes and stored at -80°C. All saliva and serum samples were sent to the laboratories of Institute of Dentistry at the University of Turku for chemokine analyses.

2.5 Chemokine analyses

Saliva and serum samples were thawed and centrifuged at 10 000 rpm at room temperature. MCP-1, MIF, and fractalkine levels were identified by a flow cytometry-based technique[¶] with commercial kits according to manufacturer's instructions[#]. The sensitivity (limit of detection) value for the assay, which means the minimum concentration of analyte for the detection of fluorescence intensity signal, was 0.1 pg/ml for MCP-1, 15.4 pg/ml for MIF, and 0.9 pg/ml for fractalkine. Total salivary protein levels were determined by the Bradford assay, as described by Hammond & Kruger.[35]

2.6 Statistical analyses

The primary outcome variables of the study were MCP-1, MIF, and fractalkine concentrations. In the post-hoc power analysis, alpha error was accepted as 0.05 in order to control type I error. The power ranged from 76.9 to 99.1%. Post-hoc power analysis was performed by the G* Power 3.0.10.[**]

Distributions of the continuous data were tested with Kolmogorov-Smirnov test. Data distributions of age, PI, GI, PPD, CAL, age, RP, ESH, CRP, disease duration, DAS28, cDMARD, bDMARD, MCP-1, MIF, and fractalkine were skewed, thus Kruskal-Wallis (for multiple comparisons) and Mann-Whitney U tests (post-hoc between group comparisons) were used in intragroup comparisons. A P value of < 0.05 was considered statistically significant. To compare the percentage of smokers, medication users, and male gender between the groups,

Chi-Square test was used. For correlation analyses, Spearman's correlation test was applied. All statistical analyses were performed using a statistical program.[††]

3. RESULTS

Clinical periodontal data are presented in Table 1. As expected, PI, GI, PPD, and CAL were significantly higher in the periodontitis groups (RA+P and P) in comparison to periodontally healthy controls. The gender distribution did not significantly differ between the study groups (P=0.126). Table 2 presents the demographic and medical data of the study participants. RA-related biochemical indicators and disease activity scores were similar in both RA groups (RA+P and RA).

In the RA+P group, significantly higher concentrations of salivary MCP-1 (P=0.006), MIF (P=0.009) and fractalkine (P=0.002) were detected in comparison to the P group (Figure 1). In periodontally healthy RA patients, these chemokine concentrations were higher than those in systemically and periodontally healthy controls (MCP-1, P=0.008; MIF, P <0.01; fractalkine, P <0.01; respectively). In addition to the differences seen in Figure 1, lower concentration of salivary MCP-1 (P=0.003) and fractalkine (P=0.045) were observed in the P group than in controls when presented in proportion to total the salivary protein levels (Figure 2A, 2C). Serum concentration of the tested chemokines are illustrated in Figure 3. Fractalkine concentrations were elevated in the RA (P=0.014) and P groups (P=0.013) compared to controls, and MCP-1 concentrations in the RA+P group (P=0.002) compared to the P group.

In correlation analyses, significant correlations were found between salivary concentrations as follows: MCP-1 vs. MIF r: 0.388, P <0.001, MCP-1 vs. fractalkine r: 0.561, P <0.001, and fractalkine vs. MIF r: 0.505, P <0.001. The corresponding values in serum were: MCP-1 vs. MIF r: 0.472, P <0.001, MCP-1 vs. fractalkine r: 0.316, P=0.003, and fractalkine vs. MIF r: 0.436, P <0.001. There were also significant correlations between ESR, RF, and

CRP levels and chemokine concentrations in saliva: ESH vs. MCP-1 r: 0.348, P <0.001, ESH vs. MIF r: 0.438, P <0.001, ESH vs. fractalkine r: 0.416, P <0.001, RF vs. MCP-1 r: 0.38, P <0.001, RF vs. MIF r: 0.443, P <0.001, RF vs. fractalkine r: 0.512, P <0.001; CRP vs. MCP-1 r: 0.314, P=0.003, CRP vs. MIF r: 0.439, P <0.001, and CRP vs. fractalkine r: 0.537, P <0.001.

4. DISCUSSION

Here we demonstrated that RA patients have elevated concentrations of MCP-1, MIF, and fractalkine in their saliva. Furthermore, positive correlations were found in salivary and serum concentration of these chemokines.

Saliva is a predictable diagnostic tool, which is considered to reflect periodontal and systemic status. To the best of the authors' knowledge, this is the first study, which investigated salivary concentrations of MCP-1, MIF, and fractalkine in relation to RA and periodontitis. The sample size could not be calculated before the initiation of the study due to lack of comparable data in the literature. Therefore, achieved power was appointed. Variations in the salivary flow rate may affect chemokine concentrations to be detected in saliva. To overcome this shortcoming, salivary chemokine concentrations were also given in proportion to the total amount of salivary proteins. One limitation of the study is its cross-sectional study design, which did not allow us to monitor possible fluctuations of the tested chemokines in respect to active and inactive phases of inflammatory states. Both RA and periodontitis are chronic inflammatory diseases with different phases in their clinical pictures. In the present study, the phase of RA was determined by DAS28 scores[29], while for periodontitis, the recent classification system was appointed.[31] According to these classifications, RA patients were in regression or inactive phase, while periodontitis patients suffered from severe form of disease. The age and sex-matched study groups, consisting of patients with a homogeneous disease phase, formed one of the strengths of our study. However, future studies, including all different

phases of RA and stages of periodontitis are warranted to clarify the relationship of chemokines to these inflammatory diseases. In the treatment of RA, different medication regimens are utilized; among them, bDMARDs have a direct effect on cytokine and chemokine levels.[36] In the present study, the RA patients were treated with either conventional or biological DMARDs. Future studies including RA patients with different DMARDs status are warranted.

Here we demonstrated that salivary concentrations of MCP-1, MIF, and fractalkine are increased in RA patients compared to systemically healthy participants. Notably, periodontal status did not have an influence on this. In saliva, elevated MCP-1 levels have been found in patients with autoimmune diseases.[37] Indeed, there is an association between circulating MCP-1 and joint infiltration by immune cells, especially by macrophages, in RA patients.[38] Only one study has presented data on salivary MCP-1 levels in RA patients with periodontitis.[39] Using a longitudinal study design, Üstün and coworkers[39] focused on the impact of TNF blockers on salivary markers without comparing the salivary MCP-1 levels between individuals with and without periodontitis. In the present study, decreased concentrations of salivary MCP-1 and fractalkine in proportion to total salivary protein were found in periodontitis. In inflamed gingival tissues and gingival crevicular fluid (GCF), MCP-1 and MIF have been detected in increased concentrations.[16, 40-41] However, conflicting data exist on salivary levels of MCP-1 and MIF in relation to periodontitis. In the literature, elevated[16], steady[17-18] or suppressed[39,42] salivary levels of MCP-1 and MIF have been reported from saliva of periodontitis compared to periodontally healthy individuals. Discrepancies between these study results may be explained by the variations in presenting the data, i.e., whether concentrations, concentrations per sample collection time, or concentrations per mg of salivary protein were used. In our study, presenting the chemokine concentrations in proportion to total protein levels demonstrated significant differences between the periodontitis and control groups in terms of their salivary MCP-1 and fractalkine concentrations. These disappeared when chemokine concentrations

were directly compared between the groups. Thus, our results are given both as concentrations and as concentrations per mg protein to allow other researchers to compare their results easily.

In spite of distinct effects of fractalkine in RA and immunity[38], little is known about its expression in periodontal tissues and oral fluids.[25-27] Hosokawa et al.[25] demonstrated increased fractalkine and CXCR1R mRNA levels in inflamed gingival tissues; however, in saliva of periodontitis patients, we did not observe any increase in fractalkine levels. It is possible that fractalkine expression is diluted in saliva, explaining the difference between these studies. Despite its decreased salivary concentration, the high serum level of fractalkine strengthens this hypothesis. In-vitro results by Peyyala et al.[27] indicated that multispecies biofilms inhibit fractalkine expression from oral epithelial cells. In the present study, where periodontitis patients suffered from severe forms of disease, elevated proteolytic activity could have affected salivary chemokine levels.

According to our study results, the presence of periodontitis in RA patients has no impact on salivary MCP-1, MIF, and fractalkine concentrations. Since these RA patients were under medical treatment, it was interesting to find high chemokine levels in their saliva even in the absence of periodontal disease. Chemokines form an important target for therapeutic intervention in the treatment of RA.[43] However, both cDMARD and bDMARD medications may fail to reduce chemokine levels in serum and synovial tissue due to difficulties in targeting the chemokine pathways (an appropriate dosage and timing, structure modification etc.).[43-45]

In the present study, increased MCP-1 concentrations were found in serum of RA patients with periodontitis compared to systemically healthy periodontitis patients and to RA patients without periodontitis. It may be speculated that both RA and periodontitis are needed to affect circulating MCP-1 concentration. Our observation on elevated fractalkine concentrations in serum in RA patients and in periodontitis patients in comparison to controls is in accordance with a recent study investigating circulating inflammatory biomarkers in

patients with RA and periodontitis.[26] The fractalkine-CXCR1 axis is involved in macrophage patrolling in tissue damage.[38] High fractalkine levels in serum have been found in RA patients, especially in those with extra-articular manifestation or vasculitis.[23-24] According to our findings, serum MIF concentrations did not differ between the study groups. In the literature, elevated serum levels of MIF have been reported from RA patients and associated with disease course.[46] It was also suggested that disease activity and duration may have an impact on MIF levels. Patients with relatively low DAS28 scores in our study may premise this disparity. The existing information on circulating MIF levels in periodontitis patients is limited to one clinical study[18], where no significant difference in serum MIF levels was found between periodontally healthy individuals and aggressive periodontitis patients.

Chemokines and their cellular receptors regulate the initial recruitment of circulating leukocytes to the site of inflammation.[10] In RA, MAP kinase (MAPK) and NF-κB pathways are activated.[47] These inflammatory pathways could be responsible for increased salivary MCP-1, MIF, and fractalkine concentrations seen in RA patients in our study. Furthermore, increased chemokine concentrations were detected in RA patients regardless of their periodontal status and in saliva rather than in serum. It was previously demonstrated that chemokine receptors on monocytes/macrophages in peripheral blood, synovial fluid, and synovial tissue express differently in RA patients.[48] The observed differences in salivary and serum concentrations of tested chemokines may be explained by variations in chemokine/receptor expression profiles of monocytes/macrophages in peripheral blood and in the oral cavity. In addition, there is a continuous bacterial challenge in the oral cavity even in health, reflecting higher chemokine concentrations in saliva in comparison to those in serum. Finally, inhibitory, heat-labile factors present in serum of RA patients downregulate the activation of peripheral-blood monocytes,[49] which eventually may result in relatively low

chemokine concentrations in serum compared to saliva. These explanations, however, need to be tested in well-designed studies.

5. CONCLUSION

Within the limitations of the study, RA is related to elevated salivary MCP-1, MIF, and fractalkine concentrations. In serum, only fractalkine concentration is prone to both RA and periodontitis. Increased salivary concentrations of these chemokines may be associated with the disrupted macrophage activation, which is a shared factor in pathogenesis of RA and periodontitis.

FOOTNOTES

[‖] Hu-Friedy Mfg. Co., LLC, Chicago, IL.

[¶] Luminex Corporation, Austin, TX.

[#] Bio-Rad, Santa Rosa, CA.

[**] Franz Faul, Universitat Kiel, Kiel, Germany.

[††] SPSS 26, IBM Corporation, Armonk, NY.

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Table 1: Clinical periodontal status of the study groups.

	RA+P **n=23**	**RA** **n=23**	**P** **n=21**	**C** **n=22**	**RA+P vs. RA** ***p***	**P vs. C** ***p***	**RA+P vs. P** ***p***	**RA vs. C** ***p***
No of teeth	25 (18-28)	27 (18-28)	24 (16-28)	28 (18-28)	0.038	0.001	0.303	0.367
PI	2.5 (2.1-2.9)	0.3 (0-0.8)	2.5 (2-2.9)	0.3 (0-0.9)	**<0.001**	**<0.001**	0.451	0.689
GI	2.4 (1.6-2.8)	0.2 (0-0.8)	2.3 (1.6-2.7)	0.1 (0-0.8)	**<0.001**	**<0.001**	0.533	0.862
PPD (mm)	5.7 (4.4-7.8)	2 (1.4-2.8)	5.5 (5.1-6.9)	2.1 (1-2.7)	**<0.001**	**<0.001**	0.495	0.452
CAL (mm)	6.4 (5.2-7.8)	0 (0-1.1)	6.3 (5.7-7.5)	0 (0-1.5)	**<0.001**	**<0.001**	0.589	0.704

RA+P: Rheumatoid arthritis with periodontitis, RA: Rheumatoid arthritis with periodontal health, P: systemically healthy with periodontitis, C: systemically and periodontally healthy controls. Number of teeth, plaque index (PI), gingival index (GI), periodontal probing depth (PPD) and clinical attachment level (CAL) are expressed in medians (minumim and maximum values in parenthesis.) Statistically significant p-values are bolded.

Table 2: Demographic and medical data of the study participants.

	RA+P **n=23**	**RA** **n=23**	**P** **n=21**	**C** **n=22**	**RA+P vs. RA** ***p***	**P vs. C** ***p***	**RA+P vs. P** ***p***	**RA vs. C** ***p***
Age (years)	53 (33-68)	54 (40-68)	54 (35-63)	48 (34-66)	0.766	0.091	0.814	0.038
RF (IU/mL)	33.4 (8.9-1890)	12.5 (8.9-714)	7.8 (2.1-12.3)	7.2 (3.1-94)	0.472	0.444	**<0.001**	**<0.001**
ESH (mm/hour)	33 (9-84)	33 (14-126)	14 (9-24)	14 (10-18)	0.291	0.126	**0.001**	**<0.001**
CRP (mg/L)	6.9 (3.0-200)	10.3 (3.2-54)	2.7 (1.1-3.9)	2.9 (1.6-3.9)	0.460	0.884	**<0.001**	**<0.001**
Duration (years)	7 (1-25)	6 (1-30)	-	-	0.929	-	-	-
DAS28	2.7 (1.1-5)	2.6 (1.9-6.6)	-	-	0.621	-	-	-

Rheumatoid factor (RF), serum erythrocyte sedimentation rate (ESH), C-reactive protein level (CRP) and disease activity score index (DAS28) are expressed in medians (minumim and maximum values in parenthesis). Statistically significant p-values are bolded. For group name abbreviations, see Table 1.

FIGURE LEGENDS

FIGURE 1 Salivary MCP-1 (A), MIF (B), and fractalkine (C) concentrations in relation to medical and periodontal status. Significant differences between the groups are indicated with *P* values. (RA+P: Rheumatoid arthritis with periodontitis, RA: Rheumatoid arthritis with periodontal health, P: systemically healthy with periodontitis, Control: systemically and periodontally healthy).

FIGURE 2 Salivary concentrations of MCP-1 (A), MIF (B), and fractalkine (C) in proportion to total salivary protein levels. For name abbreviations, see Figure 1 legend.

FIGURE 3 Serum concentrations of MCP-1 (A), MIF (B), and fractalkine (C). For name abbreviations, see Figure 1 legend.

Figure 1

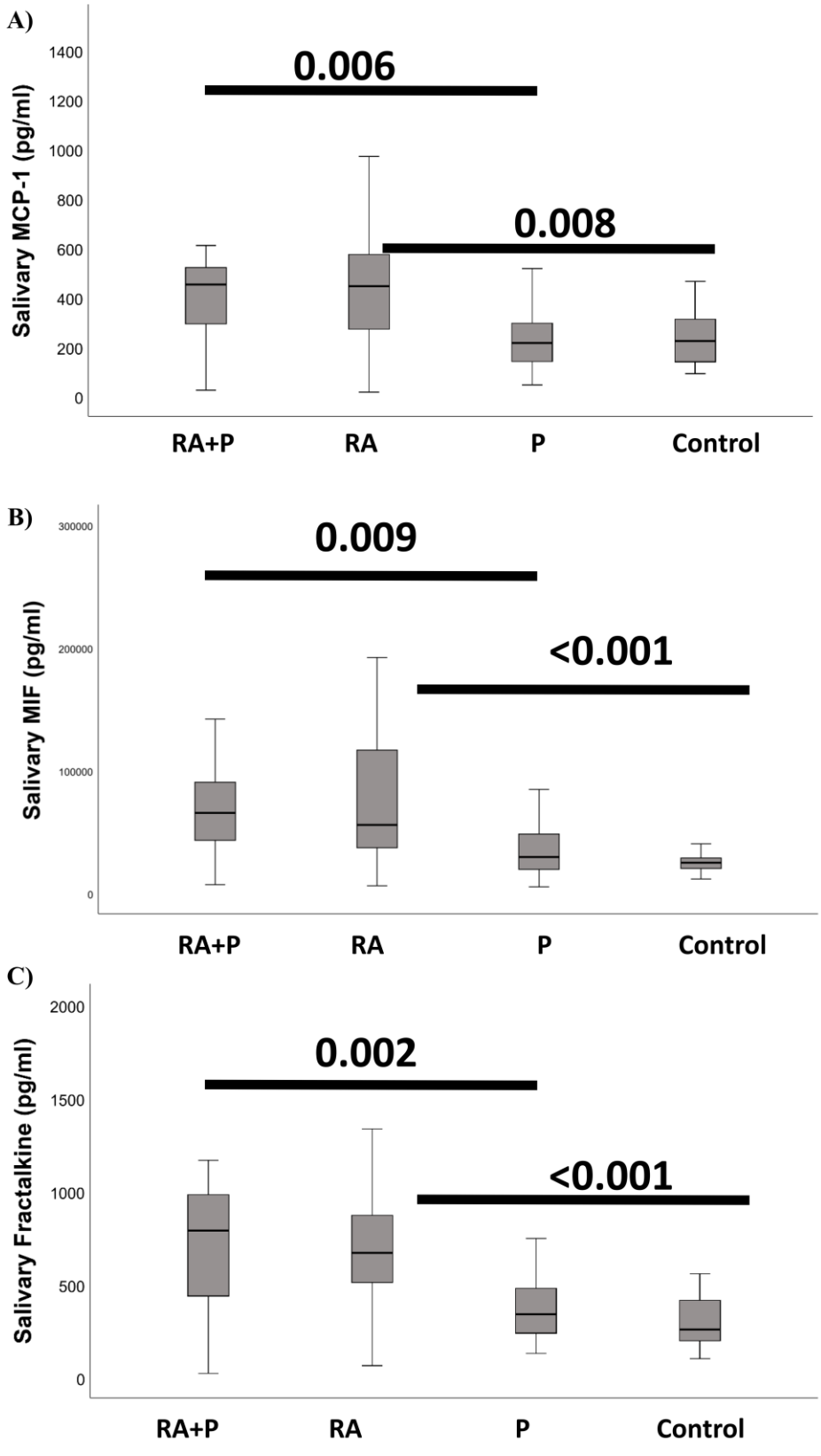

A)
Salivary MCP-1 (pg/ml)
1400
1200
1000
800
600
400
200
0
0.006
0.008
RA+P
RA
P
Control
B)
Salivary MIF (pg/ml)
300000
200000
100000
0
0.009
<0.001
RA+P
RA
P
Control
C)
Salivary Fractalkine (pg/ml)
2000
1500
1000
500
0
0.002
<0.001
RA+P
RA
P
Control

Figure 2

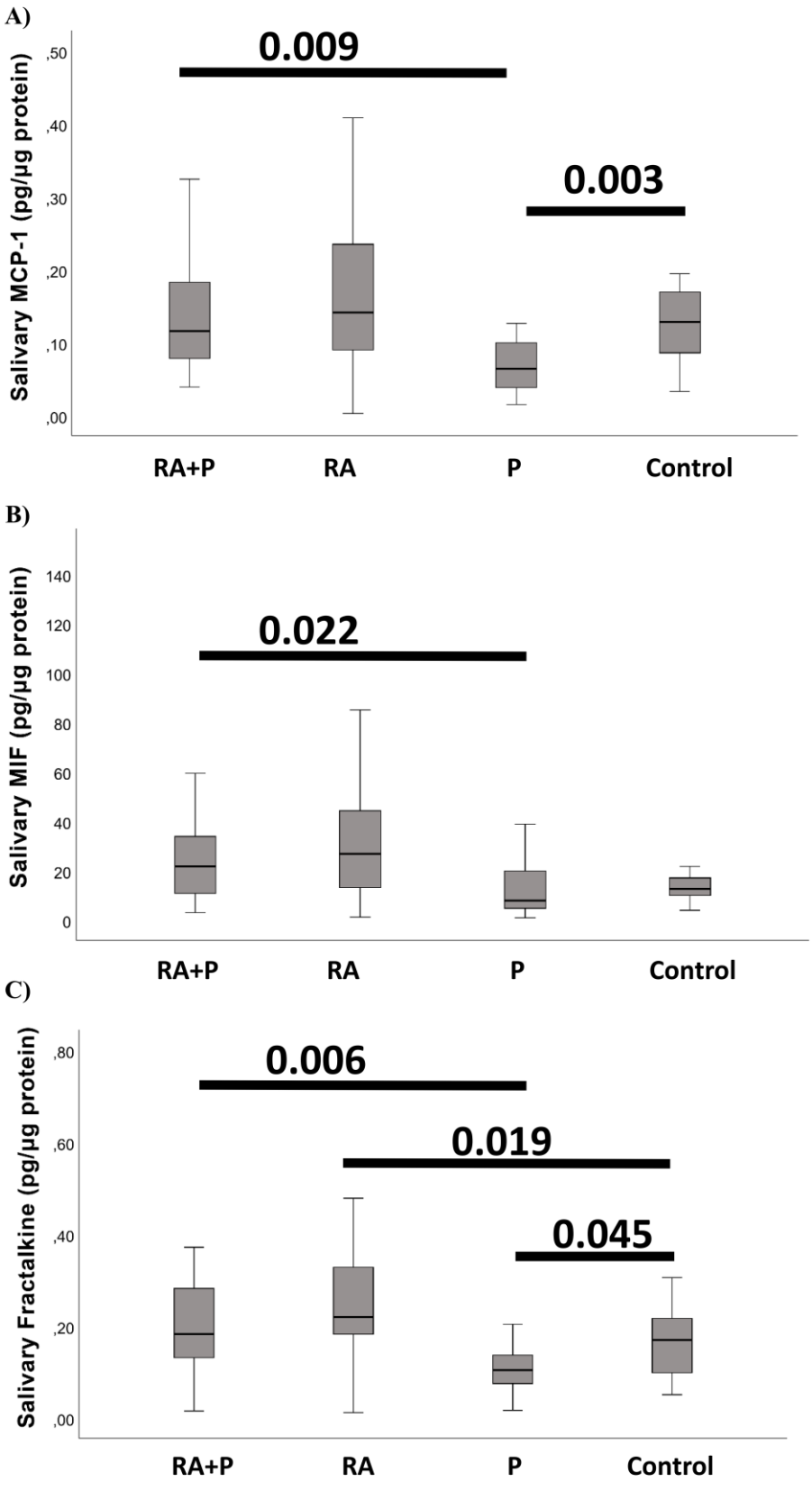
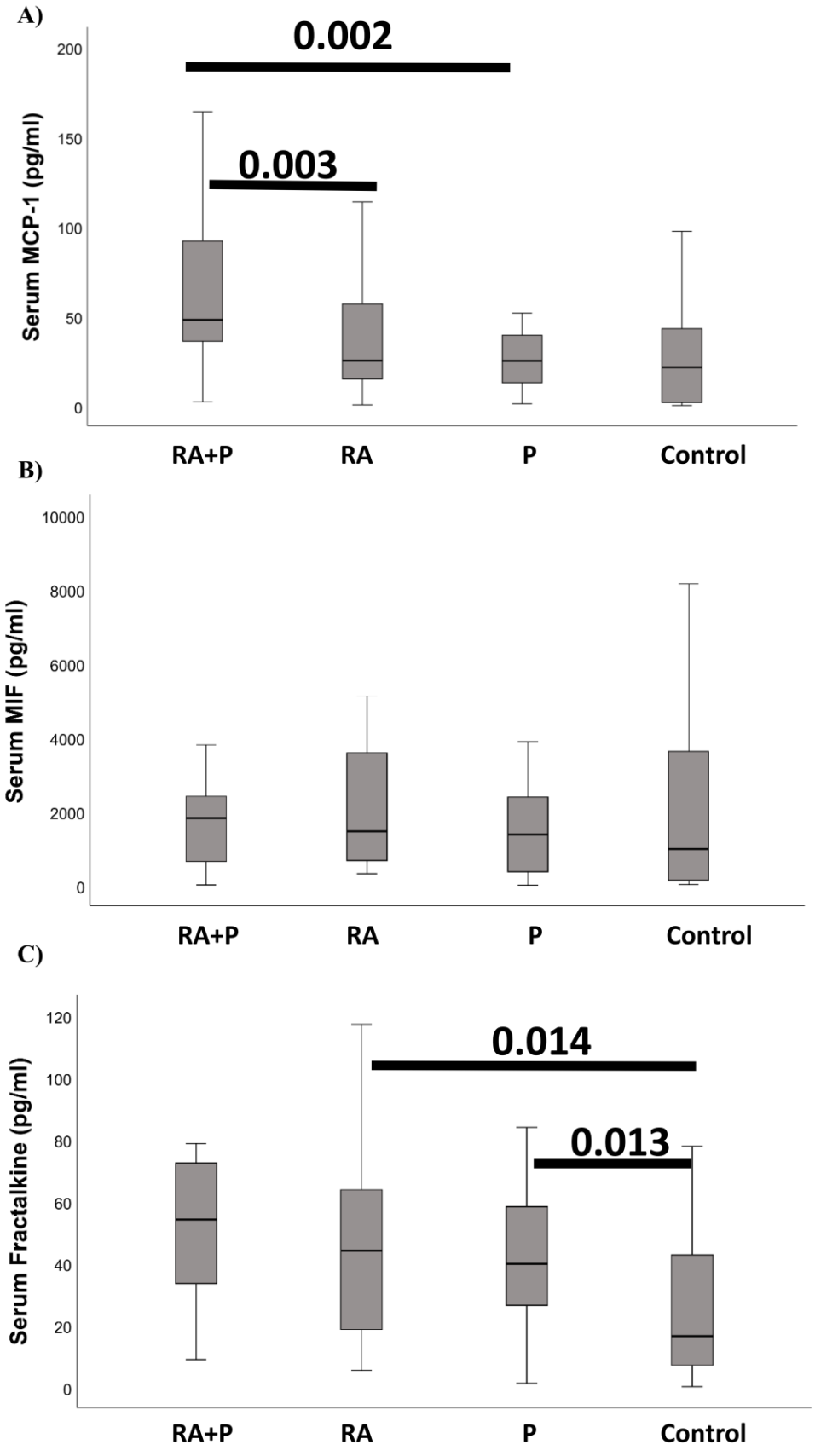

A)
Salivary MCP-1 (pg/µg protein)
,50
,40
,30
,20
,10
,00
0.009
0.003
RA+P
RA
P
Control
B)
Salivary MIF (pg/µg protein)
140
120
100
80
60
40
20
0
0.022
RA+P
RA
P
Control
C)
Salivary Fractalkine (pg/µg protein)
,80
,60
,40
,20
,00
0.006
0.019
0.045
RA+P
RA
P
Control

Figure 3


A)
0.002
0.003
Serum MCP-1 (pg/ml)
200
150
100
50
0
RA+P
RA
P
Control
B)
Serum MIF (pg/ml)
10000
8000
6000
4000
2000
0
RA+P
RA
P
Control
C)
0.014
0.013
Serum Fractalkine (pg/ml)
120
100
80
60
40
20
0
RA+P
RA
P
Control