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Salivary and serum levels of neutrophil proteases in periodontitis and rheumatoid arthritis

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Abstract

Objective: The aim was to profile serum and salivary levels of active-matrix metalloproteinase (aMMP)-8, tissue inhibitor MMP (TIMP)-1, aMMP-8/TIMP-1 ratio, total MMP (tMMP)-9, tMMP-9/TIMP-1 ratio, myeloperoxidase (MPO), and human neutrophil elastase (HNE) in periodontitis and rheumatoid arthritis (RA).

Materials and Methods: Rheumatoid arthritis patients with periodontitis (RA + P, $n = 26$), periodontally healthy RA patients (RA, $n = 23$), systemically healthy periodontitis patients (P, $n = 24$), and controls (C, $n = 24$) were included. aMMP-8 levels were determined by a time-resolved immunofluorescence assay (IFMA), TIMP-1, tMMP-9, MPO, and HNE levels were measured by enzyme-linked immunosorbent (ELISA) assays.

Results: Higher salivary aMMP-8 ($p < 0.001$), aMMP-8/TIMP-1 ratio ($p = 0.043$), tMMP-9 ($p = 0.011$), tMMP-9/TIMP-1 ratio ($p = 0.022$), MPO ($p = 0.026$) and HNE ($p < 0.001$) levels were detected in P relative to the controls. Salivary TIMP-1 was increased in RA patients regardless of periodontal status (RA + P vs. P: $p = 0.038$; RA vs. C: $p = 0.020$). Serum neutrophil proteases were increased in RA groups (RA + P, RA) compared to systemically healthy groups (P, C) ($p < 0.05$).

Conclusions: Serum levels of neutrophil proteases were increased in RA study groups; however rheumatologic status seemingly does not affect salivary levels of these proteins.

KEYWORDS
periodontitis, protease, rheumatoid arthritis, saliva, serum

1 | INTRODUCTION

Rheumatoid arthritis (RA) and periodontitis are two distinct chronic inflammatory diseases (Koziel & Potempa, 2022); however, they share similarities in their pathogenesis including soft tissue inflammation, bone destruction, and certain environmental risk factors, including smoking (Bartold & Lopez-Oliva, 2020; Li et al., 2017). Studies demonstrated a strong association between periodontitis and RA, with odds ratios ranging from 1.82 to 20.57 (Disale et al., 2020; Rodríguez-Lozano et al., 2019). Furthermore, evidence exists that

treating any of these diseases may improve the counterparts' clinical parameters (Ancuța et al., 2021; Monsarrat et al., 2019). However, the exact mechanisms underlying the associations between RA and periodontitis were not fully elucidated.

In chronic infection-induced inflammation, the host response is elicited to dysbiosis by proinflammatory cytokines and chemokines attracting neutrophils to the site of infection (Scott et al., 2012). In an attempt to control the external stimuli, for example, bacterial invasion, neutrophils release matrix metalloproteinases (MMP), myeloperoxidase (MPO), and elastase (HNE; Arnhold, 2020). These are

destructive enzymes, stored in specific and azurophilic granules of polymorphonuclear neutrophils with the capability of degrading almost all extracellular matrix proteins (Fousert et al., 2020). MMPs are a group of genetically distinct but structurally related proteases acting in physiological development and tissue remodeling but also in pathological tissue destruction (Franco et al., 2017). Among MMPs, MMP-8 and MMP-9 are involved in both RA and periodontitis pathogenesis (Arnhold, 2020). Furthermore, MMP-8 can efficiently degrade type I collagen in periodontium and type II collagen in damaged joint cartilage (Hasty et al., 1987; Sorsa et al., 1988) The regulation of the activity of MMPs is arranged by TIMPs (de Brouwer et al., 2022). TIMP-1 endogenously inhibits MMP-8 and MMP-9 (de Brouwer et al., 2022). MPO, after being released by degranulating neutrophils, can produce oxidants capable of activating proMMPs and inactivating TIMP-1 (Sorsa et al., 2006). Furthermore, HNE can proteolytically inactivate TIMP-1 (Jackson et al., 2010). An imbalance between MMP-8 and TIMP-1 can lead to tissue destruction and functional alterations, thereby leading to destructive inflammatory diseases (Bostanci et al., 2021; Lahdentausta et al., 2018). Enzymes among MPO and HNE can also potentiate the destructive MMP cascade (Nizam et al., 2014).

Neutrophils cause local tissue damage through the release of their cytotoxic contents directly onto host tissues (Arnhold, 2020). In RA, neutrophils are inappropriately activated by immune complexes such as rheumatoid factor (RF). This auto-immune activation may affect their function and longevity (O'Neil & Kaplan, 2019). For instance, in serum and synovial fluid, MMP-8, MMP-9, MPO, and HNE are significantly higher in RA compared to controls (Arvikar et al., 2021; Sur Chowdhury et al., 2014; Wang et al., 2018). Oral cavity is a unique environment that has its own dynamics, the interaction between the constant microbial stimulus and neutrophils that are located in junctional epithelium in gingival sulcus maintains the healthy homeostasis (Fousert et al., 2020). Nevertheless, the information regarding the intra-oral extension of disrupted neutrophilic protease activity in RA is limited (Arvikar et al., 2021; Äyräväinen, Heikkinen, Kuuliala, Ahola, Koivuniemi, Laasonen, et al., 2018; Äyräväinen, Heikkinen, Kuuliala, Ahola, Koivuniemi, Moilanen, et al., 2018; Schmalz et al., 2019). To the best of the authors' knowledge, salivary and serum levels of MPO and HNE with respect to RA and periodontitis have not been studied before. In the present study, we hypothesized that RA affected the serum levels of neutrophil proteases while periodontitis regulated the salivary levels of these proteases. Therefore, the study aimed to examine the effects of RA and periodontitis on serum and salivary levels of aMMP-8, TIMP-1, aMMP-8/TIMP-1 ratio, tMMP-9, tMMP-9/TIMP-1 ratio, MPO, and HNE.

2 | MATERIALS AND METHODS

2.1 | Study participants

Participant recruitment took place between June 2018 and September 2019. RA patients, who were undergoing treatments and regular follow-ups at the Department of Rheumatology (Internal Medicine),

Faculty of Medicine, and the individuals, who were referred to the Department of Periodontology, Faculty of Dentistry at Sakarya University for consult or periodontal treatments were invited to participate in the study. Exclusion criteria were having <16 teeth, periodontal treatment history and antibiotic use within at least 3 months or more before the initiation of the study, having inflammatory and/or mucocutaneous disease and disorders of the oral cavity, having additional general disorders or diseases such as diabetes mellitus, renal, hepatic disorders, or HIV as well as pregnancy and lactating period, having a history of transplantation, diagnosed with other forms of arthritis. Participants who never smoked or quit smoking for more than 2 years were considered non-smokers (Yilmaz et al., 2021). Recent quitters (<2 years) and occasional smokers were also excluded from the study. Ninety-seven participants were included in the study. The participants were informed verbally about the study protocol, and written consent was obtained from all participants.

2.2 | Medical and periodontal examinations

Demographic variables (age, sex, medical and dental treatment history, alcohol consumption, and smoking status) were obtained through interviews. Rheumatology assessments were performed by a single rheumatologist (EG) without any information about the participants' periodontal status. All RA patients met 2010 ACR/EULAR diagnosis criteria ($n=49$; Aletaha et al., 2010). The disease-related biochemical variables including serum erythrocyte sedimentation rate (ESR) (age <50 years, men 0–15 mm/h, women 0–20 mm/h; age >50 years, men 0–20 mm/h, women 0–30 mm/h), rheumatoid factor (RF) (0–15.9 IU/mL), and C-reactive protein levels (CRP) (0–5 mg/L) were analyzed in the institution's laboratories. The RA status of patients was determined according to the numeric disease activity score index (DAS28) (van Riel & Renskers, 2016). Within the reference of DAS28 scores, the patients were considered in remission, inactive, moderate, and very active phase of RA (≤2.6, >2.6 between ≤3.2, >3.2 between ≤5.1 and >5.1 respectively) (van Riel & Renskers, 2016). The duration of the disease and the medication regime were obtained from the patient's medical records. According to this data, 43 patients were treated with conventional disease-modifying anti-rheumatic drugs (cDMARD), and the rest was treated with biological DMARDs (bDMARD) (Singh et al., 2016; $n=6$).

Full mouth oral hard and soft tissue examinations (including oral mucosa, buccal mucosa, hard and soft palate, lips, tongue, and floor of mouth) were performed before periodontal examination. Participants with diagnosed or had suspicious lesions regarding inflammatory and/or chronic mucocutaneous diseases and disorders of the oral cavity were ruled out. The prosthetic devices, which were used by the participants, were not recorded. Periodontal clinic examinations were performed by a single calibrated (κ: 0.89) periodontist (DY). Plaque index (PI) (Silness & Loe, 1964), gingival index (GI) (Loe & Silness, 1963), probing pocket depth (PPD), and clinical attachment level (CAL) were recorded from six sites per tooth, excluding third molars, by using a manual periodontal probe. Alveolar bone status

was screened by panoramic and intra-oral radiographs. Periodontal status was diagnosed according to the 2017 Classification of Periodontal and Peri-Implant Diseases and Conditions (Papapanou et al., 2018). Periodontitis, defined as interdental CAL, is detectable at ≥2 non-adjacent teeth, or buccal or oral CAL ≥3 mm with PPD >3 mm is detectable at ≥2 teeth. While the participants with bleeding on probing (BOP) <10% of the surfaces and no sites with PD >3 mm besides no evidence of clinical attachment or alveolar bone defined as periodontally healthy. Subclassification of periodontitis patients was performed according to the staging and grading system. Briefly, Stages III and IV were defined as radiographic bone loss extending to the mid-third of the root, interdental CAL ≥5 mm at the site of greatest loss, and tooth loss due to periodontitis ≤4 teeth or ≥5 teeth, respectively. The extent of periodontitis was described as generalized (≥30%) or localized (≤30%) based on the number of teeth involved. The disease grade was identified by indirect evidence of progression. Of the 50 periodontitis patients, 36 were diagnosed as Stage III/Grade B, and 14 were Stage IV/Grade B. The extent of periodontitis was generalized in all patients with periodontitis.

The participants were divided into four study groups after medical and periodontal examinations. Patients diagnosed with both RA and periodontitis (RA + P) ($n=26$; males 30%), periodontally healthy RA patients (RA) ($n=23$; males 21%), systemically healthy periodontitis patients ($n=24$; male 50%), and controls (C), ($n=24$; males 41%).

2.3 | Sample collection

Unstimulated saliva samples were obtained between 9 AM and 11 AM. Participants were kindly required to avoid eating, drinking, and performing oral hygiene 2 h before the visit (Yilmaz et al., 2021). During the procedure, participants spat and filled the calibrated 2 mL plastic tubes for 5 min. The samples were centrifuged at 6000 g for 5 min and kept at −80°C until transferred. Venous blood samples were obtained from antecubital veins by the standard venipuncture method. The blood samples in 10 mm tubes were centrifuged at 252 g for 15 min and stored at −80°C. All biological samples were transferred to Helsinki University Hospital, the University of Helsinki, in dry ice for the analyses.

2.4 | Immunofluorometric assay

The aMMP-8 levels from saliva and serum samples were analyzed by a time-resolved immunofluorescence assay (IFMA). The aMMP-8-specific monoclonal antibodies 8708 and 8706 (Actim Oy) were used in the analysis as a catching antibody and a tracer antibody, respectively. Samples were diluted in assay buffer containing 20 mM Tris–HCl, pH 7.5, 5 mM $CaCl_2$, 0.5 M NaCl, 0.5% bovine serum albumin, 50 μM $ZnCl_2$, 0.05% sodium azide, and 20 mg/L diethylenetriaminepentaacetic acid. The diluted samples were allowed to incubate for 1 h and then again for 1 h with the europium-labeled tracer antibody. Between the incubations, the wells were washed.

After incubations, 100 μL of enhancement solution was added for 5 min. The fluorescence was measured using an EnVision 2015 multimode reader (PerkinElmer). For the calculation of aMMP-8 IFMA/TIMP-1 molar ratios, the levels were converted to mol per l and based on the capacity of MMPs to form a complex with TIMPs in a 1:1 stoichiometry relation (Nagase & Brew, 2003).

2.5 | Enzyme-linked immunosorbent assay

The levels of TIMP-1, tMMP-9, MPO, and HNE were determined by using enzyme-linked immunosorbent (ELISA) kits according to the protocols of the manufacturers (Human Biotrak, GE Healthcare, Immundiagnostik AG and Bender Med Systems mbH). Briefly, the levels of proteins were measured using pre-coated wells (96-well microplates) by adding samples and standards into wells and incubating for 1–2 h depending on each protocol, then washing and peroxidase-conjugated secondary antibodies were added. As substrate solutions, tetramethylbenzidine (TMB) was used in all kits. Depending on the instructions, the incubation time of the substrate solutions ranged from 15 to 30 min. When the substrate reactions were stopped with usually sulfuric acid solution, its color turned yellow. The intensity of the yellow color reflected the level of the analyzed protein, which was measured with a Victor X4 Multilabel Reader (PerkinElmer) using a wavelength of 450 nm. Based on standards, the calibration curve was drawn and used to calculate the levels of the measured proteins.

2.6 | Statistical analyses

Since no study existed in the literature that investigated MPO and HNE in the same study groups, the effect size of 1.596 was defined based on sprevious research reporting significantly different salivary levels of MMP-8 between periodontitis and healthy groups/individuals (Mirrielees et al., 2010). With a power of 80% and $\alpha=0.05$, a minimum number of 21 participants was required for the comparison. Power analyses were performed by the G*Power 3.1. The data are presented as medians or standard deviations. Non-parametric Kruskal–Wallis (for multiple comparisons) and Dunn–Bonferroni post hoc methods were used when comparing non-normally distributed parameters. The significance values were adjusted by the Bonferroni correction for multiple tests. The chi-square test was used to compare the percentage of sex and medication usage between the groups. Spearman correlation test was applied to determine the possible correlation between related biomarkers and clinic variables. A p value of <0.05 was considered statistically significant. The statistical analyses were performed using SPPS V.28.0.1.1 (SPPS).

3 | RESULTS

There were no significant differences within the RA groups (RA + P and RA) in terms of metabolic variables, DAS28 scores, and disease

duration, as presented in Table 1. The sex and age distribution did not significantly differ between the study groups ($p = 0.79$, $p = 0.180$, respectively).

The clinic periodontal variables are demonstrated in Table 2. As expected, periodontitis groups (RA+P and P) had higher PI, GI, PPD, and CAL scores than periodontally healthy participants. In systemically healthy periodontitis patients, significantly higher salivary levels of aMMP-8 ($p < 0.001$), aMMP-8/TIMP-1 ratio ($p = 0.043$), tMMP-9 ($p = 0.011$), tMMP-9/TIMP-1 ratio ($p = 0.022$), MPO ($p = 0.026$) and HNE ($p < 0.001$) were detected in comparison to the controls. In study group P, salivary tMMP-9 and HNE levels were significantly higher compared to RA and RA+P, respectively ($p = 0.026$, $p = 0.016$). Salivary tMMP-9/TIMP-1 ratio was also increased in P group compared to RA ($p = 0.004$). Salivary TIMP-1 level were significantly increased in RA patients regardless of their periodontal status compared to systemically healthy participants, as illustrated in Figure 1 (RA+P vs. P: $p = 0.038$; RA vs. C: $p = 0.020$). The serum levels of aMMP-8, aMMP-8/TIMP-1 ratio, tMMP-9, tMMP-9/TIMP-1 ratio, MPO, and HNE are presented in Figure 2. Briefly, systemic levels of aMMP-8, aMMP-8/TIMP-1 ratio, tMMP-9, tMMP-9/TIMP-1 ratio, MPO, and HNE were significantly increased in RA+P and RA groups compared to P and controls, respectively ($p < 0.001$), (aMMP-8/TIMP-1 ratio, RA+P vs. P, $p = 0.007$), (tMMP-9, RA vs. C, $p = 0.021$; tMMP-9/TIMP-1 ratio, RA vs. C, $p = 0.006$). TIMP-1 level was elevated in the RA ($p < 0.001$) and P ($p = 0.019$) groups compared with the controls.

In correlation analysis, the following significant correlations were found between salivary MPO and HNE levels with periodontal clinic variables: MPO with PI ($r: 0.210$, $p = 0.039$), GI ($r = 0.227$, $p = 0.025$), and PPD ($r = 0.211$, $p = 0.038$), HNE with PI ($r = 0.287$, $p = 0.005$), GI ($r = 0.284$, $p = 0.005$), PPD ($r = 0.265$, $p = 0.009$), and CAL ($r = 0.268$, $p = 0.009$). Serum MPO and HNE levels correlated with RA-related metabolic variables as follows: MPO with RF ($r: 0.309$, $p = 0.002$), ESH ($r = 0.309$, $p = 0.002$), and CRP ($r = 0.394$, $p < 0.001$), HNE with ESH ($r = 0.207$, $p = 0.041$) and CRP ($r: 0.201$, $p = 0.047$). No significant correlations were detected between the salivary and serum levels of aMMP-8, TIMP-1, aMMP-8/TIMP-1, tMMP-9, and tMMP-9/TIMP-1 ratio with periodontal and RA-related metabolic variables.

4 | DISCUSSION

In the present study, systemic (serum) levels of aMMP-8, aMMP-8/TIMP-1 ratio, TIMP-1, tMMP-9, tMMP-9/TIMP-1 ratio, MPO, and HNE were elevated in RA, however, the salivary levels of these neutrophil proteases did not differ according to participants RA status. To the best of our knowledge, salivary and serum levels of MPO and HNE and their correlations with periodontal and rheumatologic clinical parameters in patients with RA and periodontitis were investigated for the first time.

The age and gender-matched study groups and the exclusion of common risk factors for periodontitis and RA were the main strength of this study (Bartold & Lopez-Oliva, 2020; Li et al., 2017). Periodontitis and RA are chronic inflammatory diseases classified into subgroups according to disease severity (Papapanou et al., 2018; van Riel & Renskers, 2016) In our study, DAS28 scores (van Riel & Renskers, 2016) and 2017 periodontal disease classification (Papapanou et al., 2018) were appointed to determine disease stages. According to the evaluation, the RA patients were defined as having regression or inactive phase while periodontitis patients had severe form of the disease. These findings offered us an advantage in constituting homogenous study groups. However, the possible effects on moderate and very active phases of RA and mild and moderate phases of periodontitis on related proteins and enzymes should be investigated in further studies. Our study also has some limitations. The present study's cross-sectional design prevents us from monitoring possible fluctuations of related proteins and enzymes in relation to active or inactive inflammatory

TABLE 1 Demographic and medical data of study participants.

	Study groups				Multiple comparisons	Pairwise comparisons			
	RA+P (I) N=26	RA (II) N=23	P (III) N=24	C (IV) N=24	I–II–III–IV p^a	I–II p^b	III–IV p^b	I–III p^b	II–IV p^b
Age (years)	53 (33–68)	53 (40–68)	52 (35–63)	49 (34–66)	0.180	–	–	–	–
RF (IU/mL)	39.6 (8.9–1890)	12.5 (8.9–714)	7.8 (2.1–12.3)	7.3 (3.1–9.4)	**<0.001**	0.507	0.452	**<0.001**	**<0.001**
ESR (mm/h)	35.5 (9–84)	33.5 (14–126)	14.5 (9–24)	14 (9–18)	**<0.001**	0.264	0.329	**0.001**	**<0.001**
CRP (mg/L)	7.04 (3.0–200)	10.2 (3.2–54)	2.65 (1.1–3.9)	2.8 (1.6–3.9)	**<0.001**	0.814	0.943	**<0.001**	**<0.001**
Duration (year)	5.5 (1–25)	7 (1–30)	–	–	0.769	0.769	–	–	–
DAS28	2.66 (1.1–5)	2.61 (1.9–6.6)	–	–	0.741	0.741	–	–	–

Note: Statistically significant *p* values are in bolded.

Abbreviations: CRP, creactive protein; DAS28, disease activity score are expressed in median (minimum and maximum values in parenthesis); ESH, erythrocyte sedimentation rate; RF, rheumatoid factor.

[a] Kruskal–Wallis test was applied.

[b] Dunn–Bonferroni post hoc method was applied.

TABLE 2 Clinical periodontal parameters of the study participants.

	Study groups				Multiple comparisons	Pairwise comparisons			
	RA + P (I) N = 26	RA (II) N = 23	P (III) N = 24	C (IV) N = 24	I–II–III–IV p^a	I–II p^b	III–IV p^b	I–III p^b	II–IV p^b
Number of teeth	25 (18–28)	27 (18–28)	24 (16–28)	27 (18–28)	**0.003**	0.100	**0.024**	0.884	0.693
PI	2.5 (2.1–2.9)	0.2 (0–0.8)	2.5 (2–2.9)	0.3 (0–0.9)	**<0.001**	**<0.001**	**<0.001**	0.757	0.745
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**	**<0.001**	0.628	0.855
PPD (mm)	5.6 (4.4–7.8)	2 (1.4–2.8)	5.4 (5.1–6.9)	2.1 (1–2.7)	**<0.001**	**<0.001**	**<0.001**	0.792	0.666
CAL (mm)	6.4 (5.2–7.8)	0 (0–1.1)	6.2 (5.7–7.5)	0 (0–1.5)	**<0.001**	**<0.001**	**<0.001**	0.723	0.724

Note: Statistically significant *p* values are in bolded.

Abbreviations: CAL, clinical attachment level are expressed in median (minimum and maximum values in parenthesis); GI, gingival index; PI, plaque index; PPD, probing pocket depth.

[a]Kruskal–Wallis test was applied.

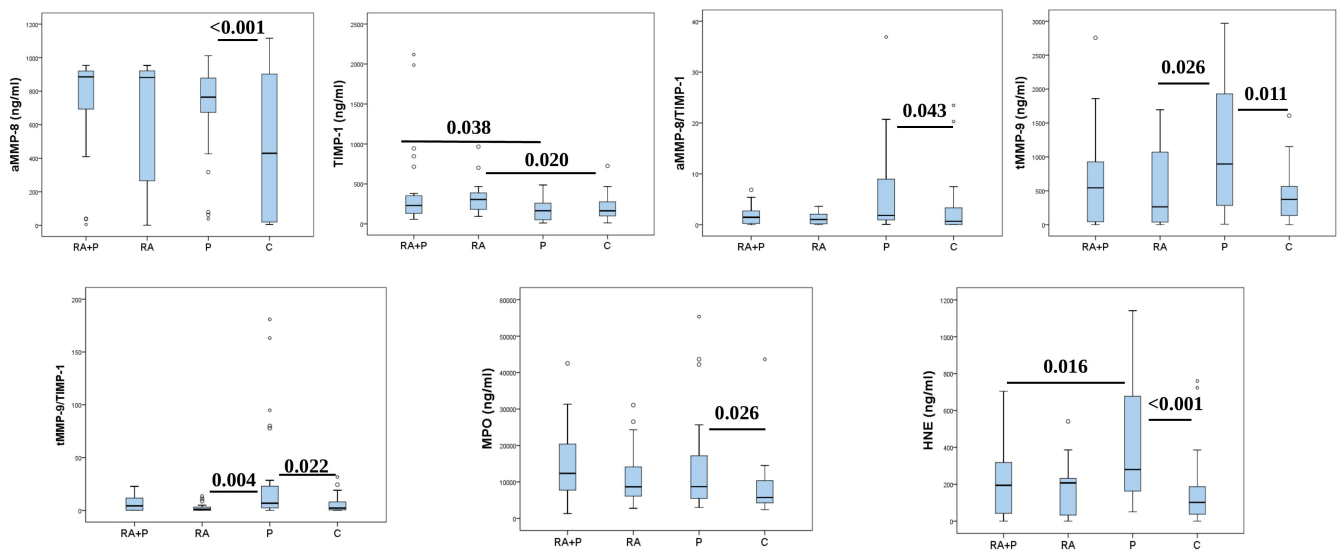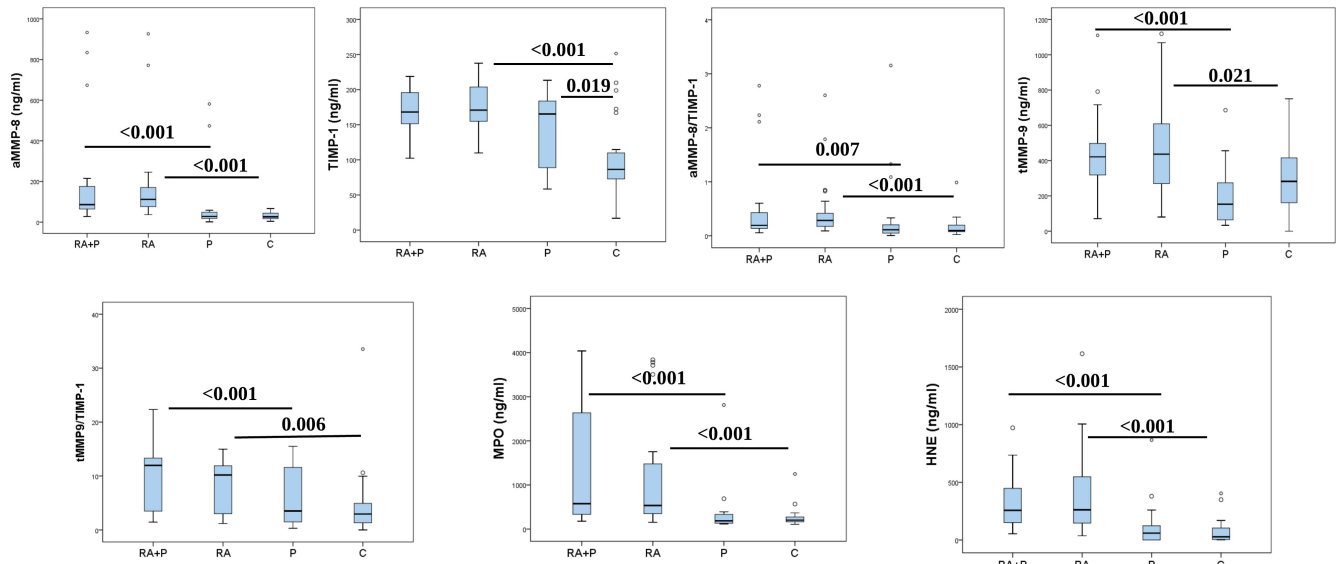[b]Dunn–Bonferroni post hoc method was applied.

FIGURE 1 Salivary aMMP-8, TIMP-1, aMMP-8/TIMP1 molar ratio, tMMP-9, tMMP-9/TIMP-1 ratio, MPO and HNE levels in relation to participants medical and periodontal status. Statistically significant *p* values are presented. C, controls; P, periodontitis with systemically healthy; RA + P, rheumatoid arthritis + periodontitis; RA, rheumatoid arthritis with periodontal healthy.

status. DMARDs are the most effectively used medication group in the treatment of RA, and they target inflammation pathways. Especially bDMARDs, a subgroup of related medication that targets specific cells or proteins in the inflammatory response (Rein & Mueller, 2017). In our study, chronic RA patients with prescription of both cDMARD (n = 44) and bDMARD (n = 6) were included. When subgroups of RA patients were compared according to their medication status, saliva and serum levels of neutrophil proteases did not differ between groups (data not shown). It can be hypothesized that the medications may have influence on salivary levels of neutrophil proteases in patients with RA. However, future studies are warranted to investigate these possible effects. In the present study, aMMP-8 and tMMP-9 were analyzed. MMP-8 and MMP-9 can be found in latent and active forms in saliva and serum (Gürsoy et al., 2018). The active forms of MMP-8 and MMP-9 indicates a stronger association with periodontal status, however in terms of

inflammation it could be more critical to monitor both pro- and active form of MMP-9 (Kim et al., 2020; Öztürk et al., 2021; Sorsa et al., 2006). According to previous information, MMP-8 is activated upon selective PMNL degranulation associated with inflammation related to both P and RA (Sorsa et al., 2006). Based on this, we preferred to measure only active form of MMP-8 with a selective IFMA-aMMP-8 immunoassay from serum and oral fluid samples (Gursoy et al., 2010; Sorsa et al., 2016).

According to our findings, RA did not have an influence on the salivary levels of aMMP8 and tMMP-9 based on the comparisons between RA + P vs. P and RA vs. C groups. In the literature, there is no consensus regarding salivary levels of aMMP-8 and tMMP-9 in patients with RA and periodontitis (Arvikar et al., 2021; Äyräväinen, Heikkinen, Kuuliala, Ahola, Koivuniemi, Laasonen, et al., 2018; Äyräväinen, Heikkinen, Kuuliala, Ahola, Koivuniemi, Moilanen, et al., 2018; Mirrielees et al., 2010). One possible explanation of the

FIGURE 2 Serum aMMP-8, TIMP-1, aMMP-8/TIMP-1 molar ratio, tMMP-9, tMMP-9/TIMP-1 ratio, MPO and HNE levels in relation to participants medical and periodontal status. Statistically significant *p* values are in presented. C, controls; P, periodontitis with systemically healthy; RA+P, rheumatoid arthritis+periodontitis; RA, rheumatoid arthritis with periodontal healthy.

discrepancy between the studies was the severity of the disease and the medication status of the participants. The production of autoantibodies is one plausible mechanism in the relationship between RA and periodontitis (Eezammuddeen et al., 2022) may explain the present findings. It is known that the autoantibodies increase the levels of neutrophil proteases in serum and synovial fluids (Koziel & Potempa, 2022). Among these autoantibodies, local production of anti-citrullinated protein antibodies in saliva was not significantly differed in patients with RA and periodontitis compared to periodontitis patients (Svärd et al., 2020). Therefore, the absence of autoantibody overexpression in saliva may explain why we did not observe an RA-associated elevation of neutrophil proteases in saliva. In the present study, the serum aMMP-8, aMMP-8/TIMP-1 ratio, tMMP-9, and tMMP-9/TIMP-1 ratio levels were significantly increased in RA study groups compared to systemically healthy participants regardless of their periodontal status. This outcome might indicate the continuous inflammatory burden and joint destruction of patients with RA even though they were classified as a regression or inactive phase of the disease. The increased serum levels of aMMP-8 and tMMP-9 in RA patients were clearly demonstrated in the literature (Arvikar et al., 2021; Äyräväinen, Heikkinen, Kuuliala, Ahola, Koivuniemi, Laasonen, et al., 2018; Äyräväinen, Heikkinen, Kuuliala, Ahola, Koivuniemi, Moilanen, et al., 2018; Tchetverikov et al., 2004). Furthermore, aMMP-8, tMMP-9 and especially the aMMP-8/TIMP-1 and MMP-9/TIMP-1 ratios were proposed to have prognostic significance in RA and connected with other systemic diseases (Rein & Mueller, 2017). Overall, according to our findings, it can be hypothesized that salivary aMMP-8 and tMMP-9 levels are affected by the local periodontal environment, and serum levels are selectively influenced by systemic inflammatory conditions such as RA.

On one hand, our findings demonstrated that RA+P and RA study groups had increased salivary levels of TIMP-1 compared

to P and C groups, respectively. On the other hand, serum levels of the RA and P groups were significantly increased compared to controls. Controversial findings exist in the literature regarding salivary and serum levels of TIMP-1 in patients with RA+P. The discrepancies can be related to different methodologies and the selection criteria of the RA patients (Äyräväinen, Heikkinen, Kuuliala, Ahola, Koivuniemi, Laasonen, et al., 2018; Äyräväinen, Heikkinen, Kuuliala, Ahola, Koivuniemi, Moilanen, et al., 2018). TIMP-1 is a specific inhibitor of MMP-8 and MMP-9 in-vivo activity and has an important role in the development of inflammation (Sorsa et al., 2006). TIMP-1 has a more restricted inhibitory range than the other TIMPs, also strongly binds to both active and zymogen of MMP-9 than to MMP-8. An imbalance between the MMP-8, MMP-9, and TIMP-1 subsequently drives the various pathologic disorders (Bostanci et al., 2021; Vandenbroucke & Libert, 2014). In literature, studies reported higher salivary MMP-8/TIMP-1 and MMP-9/TIMP-1 ratios accompanied by decreased TIMP-1 levels in periodontitis patients compared to controls (Äyräväinen, Heikkinen, Kuuliala, Ahola, Koivuniemi, Laasonen, et al., 2018; Äyräväinen, Heikkinen, Kuuliala, Ahola, Koivuniemi, Moilanen, et al., 2018; Bostanci et al., 2021; Gursoy et al., 2010; Nascimento et al., 2019). However, the imbalance between MMP-8, MMP-9, and TIMP-1 does not necessarily relate to decreased TIMP-1 level. A recent meta-analysis from de Brouwer et al. (2022) pointed out that there is no difference between TIMP-1 levels in oral fluids of periodontitis/gingivitis patients with controls, which is consistent with the present findings.

MPO is the most frequent protein in neutrophils and contributes pro-oxidatively, i.e., producing reactive oxygen species such as HOCl. This reactive oxygen species can oxidatively activate latent proMMP-8 to aMMP-8 (Sorsa et al., 2006). In this way, MPO can promote collagenolysis, immune modifications, and chronic

inflammatory process in various diseases (Arnhold, 2020; Fousert et al., 2020). HNE is stored in azurophilic granules of neutrophils, which is part of the host defense. Due to its ability to degrade surrounding tissues and proteins, it exerts strong pro-inflammatory effect. HNE can proteolytically inactive TIMP-1, likewise, MPO can do the same oxidatively (Sorsa et al., 2006). According to present outcomes, despite an increasing trend in salivary MPO and HNE levels of patients with RA + P and RA, the rheumatologic status of participants did not cause a statistical difference compared with the P and C study groups. Interestingly, participants only diagnosed with periodontitis demonstrated a higher salivary level of HNE in comparison to participants diagnosed with both RA and periodontitis. To the best of our knowledge, this is the first study investigating the salivary levels of MPO and HNE with respect to RA and periodontal status. Due to this reason, we could not compare our findings with the literature. Neutrophils are the first-line defenders in inflammation (Fousert et al., 2020). In the oral cavity, saliva, and even healthy gingiva contain neutrophils in steady condition. It may be speculated that the source of higher MPO and HNE levels in the saliva of patients with periodontitis mostly originated from surrounding inflamed periodontal tissues and oral fluids instead of circulatory neutrophils. In our study, elevated serum levels of MPO and HNE were also observed in RA + P and RA study groups when compared to the systemically healthy P and C counterparts. Data from different reports indicated increased serum levels of MPO and HNE in RA (Fernandes et al., 2012; Stamp et al., 2012; Sur Chowdhury et al., 2014; Wang et al., 2014). However, the association between serum MPO levels and RA disease variables is controversial (Stamp et al., 2012; Wang et al., 2014). Our findings detected positive correlation between salivary and serum MPO levels between periodontal (PI, GI, and PPD) and RA clinic variables (RF, ESH, and CRP) and these outcomes were in line with the Wang et al. (2014) findings. Contrary, Fernandes et al. (2012) did not find any significant correlation between serum levels of MPO and rheumatologic clinical variables. The conflicting results may have resulted from different participant selection and diagnosis criteria.

In clinical point of view, the most of biomarker studies aim to develop a rapid point of care (POC) or chairside testing model that fits well into the dental practice. Evidence clearly indicates the association between aMMP-8 levels in mouth rinse and the severity of periodontal disease by using aMMP-8-specific chairside tests (Sorsa et al., 2020). Thus, by referring present outcomes, it may be recommended to implement these POC tests into diagnose, treatment, and maintenance phases of patients with RA and periodontitis. This might be also helpful for non-dental clinicians to detect diseases and refer the patients to a specialized and enabled to provide better personalize treatment planning. The premise outcomes of MPO and HNE levels and its correlation with periodontal and RA clinical variables will let the clinicians and scientist to investigate these biomarkers as possible adjunctive diagnostic tools for detecting related diseases as well as to develop different treatment strategies targeting neutrophil inflammation and proteases.

5 | CONCLUSION

In conclusion, elevated serum levels of aMMP-8, aMMP-8/TIMP-1 ratio, tMMP-9, tMMP-9/TIMP-1 ratio, MPO, and HNE in RA study groups compared to systemically healthy counterparts do not correspond to the levels of these proteins in saliva. The present outcomes indicate that periodontitis is related to increased neutrophil protease levels of saliva while RA is only related to serum levels of neutrophil elastases.

AUTHOR CONTRIBUTIONS

Dogukan Yilmaz: Conceptualization; investigation; methodology; writing – review and editing; writing – original draft; formal analysis; visualization; validation. **Katariina Niskanen:** Writing – original draft; writing – review and editing; investigation; methodology; formal analysis. **Emel Gonullu:** Conceptualization; investigation; writing – original draft; writing – review and editing; methodology. **Taina Tervahartiala:** Writing – original draft; writing – review and editing; methodology; formal analysis; investigation. **Ulvi Kahraman Gürsoy:** Conceptualization; investigation; writing – original draft; writing – review and editing; methodology; validation; visualization; formal analysis. **Timo Sorsa:** Funding acquisition; writing – original draft; writing – review and editing; methodology; project administration; formal analysis; resources.

CONFLICT OF INTEREST STATEMENT

Timo Sorsa is an inventor of US-patents 5652223, 5736341, 5866432, 6143476, 20170023571A1 (granted 6.6.2019), WO 2018/060553 A1 (granted 31.5.2018), 10 488 415 B2, a Japanese patent 2016-554676 and patent application No. 10-2016-7025378 in South Korea (report grant notification, due 25.6.2021).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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REFERENCES

Aletaha, D., Neogi, T., Silman, A. J., Funovits, J., Felson, D. T., Bingham, C. O., 3rd, Birnbaum, N. S., Burmester, G. R., Bykerk, V. P., Cohen, M. D., Combe, B., Costenbader, K. H., Dougados, M., Emery, P., Ferraccioli, G., Hazes, J. M., Hobbs, K., Huizinga, T. W., Kavanaugh, A., … Hawker, G. (2010). 2010 rheumatoid arthritis classification criteria: An American College of Rheumatology/European league against rheumatism collaborative initiative. *Arthritis and Rheumatism*, *62*(9), 2569–2581. https://doi.org/10.1002/art.27584

Ancuța, C., Chirieac, R., Ancuța, E., Țănculescu, O., Solomon, S. M., Fătu, A. M., Doloca, A., & Iordache, C. (2021). Exploring the role of interleukin-6 receptor inhibitor tocilizumab in patients with active rheumatoid arthritis and periodontal disease. *Journal of Clinical Medicine*, 10(4), 878. https://doi.org/10.3390/jcm10040878

Arnhold, J. (2020). The dual role of myeloperoxidase in immune response. *International Journal of Molecular Sciences*, 21(21), 8057. https://doi.org/10.3390/ijms21218057

Arvikar, S. L., Hasturk, H., Strle, K., Stephens, D., Bolster, M. B., Collier, D. S., Kantarci, A., & Steere, A. C. (2021). Periodontal inflammation and distinct inflammatory profiles in saliva and gingival crevicular fluid compared with serum and joints in rheumatoid arthritis patients. *Journal of Periodontology*, 92(10), 1379–1391. https://doi.org/10.1002/JPER.20-0051

Äyräväinen, L., Heikkinen, A. M., Kuuliala, A., Ahola, K., Koivuniemi, R., Laasonen, L., Moilanen, E., Hämäläinen, M., Tervahartiala, T., Meurman, J. H., Leirisalo-Repo, M., & Sorsa, T. (2018). Inflammatory biomarkers in saliva and serum of patients with rheumatoid arthritis with respect to periodontal status. *Annals of Medicine*, 50(4), 333–344. https://doi.org/10.1080/07853890.2018.1468922

Äyräväinen, L., Heikkinen, A. M., Kuuliala, A., Ahola, K., Koivuniemi, R., Moilanen, E., Hämäläinen, M., Tervahartiala, T., Meurman, J. H., Leirisalo-Repo, M., & Sorsa, T. (2018). Anti-rheumatic medication and salivary MMP-8, a biomarker for periodontal disease. *Oral Diseases*, 24(8), 1562–1571. https://doi.org/10.1111/odi.12930

Bartold, P. M., & Lopez-Oliva, I. (2020). Periodontitis and rheumatoid arthritis: An update 2012–2017. *Periodontology 2000*, 83(1), 189–212. https://doi.org/10.1111/prd.12300

Bostanci, N., Mitsakakis, K., Afacan, B., Bao, K., Johannsen, B., Baumgartner, D., Müller, L., Kotolová, H., Emingil, G., & Karpíšek, M. (2021). Validation and verification of predictive salivary biomarkers for oral health. *Scientific Reports*, 11(1), 6406. https://doi.org/10.1038/s41598-021-85120-w

de Brouwer, P., Bikker, F. J., Brand, H. S., & Kaman, W. E. (2022). Is TIMP-1 a biomarker for periodontal disease? A systematic review and meta-analysis. *Journal of Periodontal Research*, 57(2), 235–245. https://doi.org/10.1111/jre.12957

Disale, P. R., Zope, S. A., Suragimath, G., Varma, A. S., & Pisal, A. (2020). Prevalence and severity of periodontitis in patients with established rheumatoid arthritis and osteoarthritis. *Journal of Family Medicine and Primary Care*, 9(6), 2919–2925. https://doi.org/10.4103/jfmpc.jfmpc_398_20

Eezammuddeen, N. N., Vaithilingam, R. D., & Hassan, N. H. M. (2022). Influence of periodontitis on levels of autoantibodies in rheumatoid arthritis patients: A systematic review. *Journal of Periodontal Research*, 58, 29–42. https://doi.org/10.1111/jre.13065

Fernandes, R. M., da Silva, N. P., & Sato, E. I. (2012). Increased myeloperoxidase plasma levels in rheumatoid arthritis. *Rheumatology International*, 32(6), 1605–1609. https://doi.org/10.1007/s00296-011-1810-5

Fousert, E., Toes, R., & Desai, J. (2020). Neutrophil extracellular traps (NETs) take the central stage in driving autoimmune responses. *Cell*, 9(4), 915. https://doi.org/10.3390/cells9040915

Franco, C., Patricia, H. R., Timo, S., Claudia, B., & Marcela, H. (2017). Matrix metalloproteinases as regulators of periodontal inflammation. *International Journal of Molecular Sciences*, 18(2), 440. https://doi.org/10.3390/ijms18020440

Gursoy, U. K., Könönen, E., Pradhan-Palikhe, P., Tervahartiala, T., Pussinen, P. J., Suominen-Taipale, L., & Sorsa, T. (2010). Salivary MMP-8, TIMP-1, and ICTP as markers of advanced periodontitis. *Journal of Clinical Periodontology*, 37(6), 487–493. https://doi.org/10.1111/j.1600-051X.2010.01563.x

Gürsoy, U. K., Könönen, E., Tervahartiala, T., Gürsoy, M., Pitkänen, J., Torvi, P., Suominen, A. L., Pussinen, P., & Sorsa, T. (2018). Molecular forms and fragments of salivary MMP-8 in relation to periodontitis. *Journal of Clinical Periodontology*, 45(12), 1421–1428. https://doi.org/10.1111/jcpe.13024

Hasty, K. A., Jeffrey, J. J., Hibbs, M. S., & Welgus, H. G. (1987). The collagen substrate specificity of human neutrophil collagenase. *The Journal of Biological Chemistry*, 262(21), 10048–10052.

Jackson, P. L., Xu, X., Wilson, L., Weathington, N. M., Clancy, J. P., Blalock, J. E., & Gaggar, A. (2010). Human neutrophil elastase-mediated cleavage sites of MMP-9 and TIMP-1: Implications to cystic fibrosis proteolytic dysfunction. *Molecular Medicine*, 16(5–6), 159–166. https://doi.org/10.2119/molmed.2009.00109

Kim, H. D., Lee, C. S., Cho, H. J., Jeon, S., Choi, Y. N., Kim, S., Kim, D., Jin Lee, H., Vu, H., Jeong, H. J., & Kim, B. (2020). Diagnostic ability of salivary matrix metalloproteinase-9 lateral flow test point-of-care test for periodontitis. *Journal of Clinical Periodontology*, 47(11), 1354–1361. https://doi.org/10.1111/jcpe.13360

Koziel, J., & Potempa, J. (2022). Pros and cons of causative association between periodontitis and rheumatoid arthritis. *Periodontology 2000*, 89(1), 83–98. https://doi.org/10.1111/prd.12432

Lahdentausta, L., Paju, S., Mäntylä, P., Buhlin, K., Tervahartiala, T., Pietiäinen, M., Alfthan, H., Nieminen, M. S., Sinisalo, J., Sorsa, T., & Pussinen, P. J. (2018). Saliva and serum biomarkers in periodontitis and coronary artery disease. *Journal of Clinical Periodontology*, 45(9), 1045–1055. https://doi.org/10.1111/jcpe.12976

Li, R., Tian, C., Postlethwaite, A., Jiao, Y., Garcia-Godoy, F., Pattanaik, D., Wei, D., Gu, W., & Li, J. (2017). Rheumatoid arthritis and periodontal disease: What are the similarities and differences? *International Journal of Rheumatic Diseases*, 20(12), 1887–1901. https://doi.org/10.1111/1756-185X.13240

Loe, H., & Silness, J. (1963). Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontologica Scandinavica*, 21, 533–551. https://doi.org/10.3109/00016356309011240

Mirrielees, J., Crofford, L. J., Lin, Y., Kryscio, R. J., Dawson, D. R., 3rd, Ebersole, J. L., & Miller, C. S. (2010). Rheumatoid arthritis and salivary biomarkers of periodontal disease. *Journal of Clinical Periodontology*, 37(12), 1068–1074. https://doi.org/10.1111/j.1600-051X.2010.01625.x

Monsarrat, P., Fernandez de Grado, G., Constantin, A., Willmann, C., Nabet, C., Sixou, M., Cantagrel, A., Barnetche, T., Mehsen-Cetre, N., Schaeverbeke, T., Arrivé, E., Vergnes, J. N., & ESPERA Group. (2019). The effect of periodontal treatment on patients with rheumatoid arthritis: The ESPERA randomised controlled trial. *Joint, Bone, Spine*, 86(5), 600–609. https://doi.org/10.1016/j.jbspin.2019.02.006

Nagase, H., & Brew, K. (2003). Designing TIMP (tissue inhibitor of metalloproteinases) variants that are selective metalloproteinase inhibitors. *Biochemical Society Symposium*, 70, 201–212. https://doi.org/10.1042/bss0700201

Nascimento, G. G., Baelum, V., Sorsa, T., Tervahartiala, T., Skottrup, P. D., & López, R. (2019). Salivary levels of MPO, MMP-8 and TIMP-1 are associated with gingival inflammation response patterns during experimental gingivitis. *Cytokine*, 115, 135–141. https://doi.org/10.1016/j.cyto.2018.12.002

Nizam, N., Gümüş, P., Pitkänen, J., Tervahartiala, T., Sorsa, T., & Buduneli, N. (2014). Serum and salivary matrix metalloproteinases, neutrophil elastase, myeloperoxidase in patients with chronic or aggressive periodontitis. *Inflammation*, 37(5), 1771–1778. https://doi.org/10.1007/s10753-014-9907-0

O'Neil, L. J., & Kaplan, M. J. (2019). Neutrophils in rheumatoid arthritis: Breaking immune tolerance and fueling disease. *Trends in Molecular Medicine*, 25(3), 215–227. https://doi.org/10.1016/j.molmed.2018.12.008

Öztürk, V. Ö., Emingil, G., Umeizudike, K., Tervahartiala, T., Gieselmann, D. R., Maier, K., Köse, T., Sorsa, T., & Alassiri, S. (2021). Evaluation of active matrix metalloproteinase-8 (aMMP-8) chair-side test as a diagnostic biomarker in the staging of periodontal diseases.

Archives of Oral Biology, 124, 104955. https://doi.org/10.1016/j.archoralbio.2020.10495

Papapanou, P. N., Sanz, M., Buduneli, N., Dietrich, T., Feres, M., Fine, D. H., Flemmig, T. F., Garcia, R., Giannobile, W. V., Graziani, F., Greenwell, H., Herrera, D., Kao, R. T., Kebschull, M., Kinane, D. F., Kirkwood, K. L., Kocher, T., Kornman, K. S., Kumar, P. S., … Tonetti, M. S. (2018). Periodontitis: Consensus report of workgroup 2 of the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions. *Journal of Periodontology, 89*(Suppl 1), S173–S182. https://doi.org/10.1002/JPER.17-0721

Rein, P., & Mueller, R. B. (2017). Treatment with biologicals in rheumatoid arthritis: An overview. *Rheumatology and Therapy, 4*(2), 247–261. https://doi.org/10.1007/s40744-017-0073-3

Rodríguez-Lozano, B., González-Febles, J., Garnier-Rodríguez, J. L., Dadlani, S., Bustabad-Reyes, S., Sanz, M., Sánchez-Alonso, F., Sánchez-Piedra, C., González-Dávila, E., & Díaz-González, F. (2019). Association between severity of periodontitis and clinical activity in rheumatoid arthritis patients: A case-control study. *Arthritis Research & Therapy, 21*(1), 27. https://doi.org/10.1186/s13075-019-1808-z

Schmalz, G., Davarpanah, I., Jäger, J., Mausberg, R. F., Krohn-Grimberghe, B., Schmidt, J., Haak, R., Sack, U., & Ziebolz, D. (2019). MMP-8 and TIMP-1 are associated to periodontal inflammation in patients with rheumatoid arthritis under methotrexate immuno-suppression – First results of a cross-sectional study. *Journal of Microbiology, Immunology and Infection, 52*(3), 386–394. https://doi.org/10.1016/j.jmii.2017.07.016

Scott, A. E., Milward, M., Linden, G. J., Matthews, J. B., Carlile, M. J., Lundy, F. T., Naeeni, M. A., Lorraine Martin, S., Walker, B., Kinane, D., Brock, G. R., & Chapple, I. L. (2012). Mapping biological to clinical phenotypes during the development (21 days) and resolution (21 days) of experimental gingivitis. *Journal of Clinical Periodontology, 39*(2), 123–131. https://doi.org/10.1111/j.1600-051X.2011.01825.x

Silness, J., & Loe, H. (1964). Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontologica Scandinavica, 22*, 121–135. https://doi.org/10.3109/00016356408993968

Singh, J. A., Saag, K. G., Bridges, S. L., Jr., Akl, E. A., Bannuru, R. R., Sullivan, M. C., Vaysbrot, E., McNaughton, C., Osani, M., Shmerling, R. H., & Curtis, J. R. (2016). 2015 American College of Rheumatology Guideline for the treatment of rheumatoid arthritis. *Arthritis & Rheumatology, 68*(1), 1–26. https://doi.org/10.1002/art.39480

Sorsa, T., Alassiri, S., Grigoriadis, A., Räisänen, I. T., Pärnänen, P., Nwhator, S. O., Gieselmann, D. R., & Sakellari, D. (2020). Active MMP-8 (aMMP-8) as a grading and staging biomarker in the periodontitis classification. *Diagnostics, 10*(2), 61. https://doi.org/10.3390/diagnostics10020061

Sorsa, T., Gursoy, U. K., Nwhator, S., Hernandez, M., Tervahartiala, T., Leppilahti, J., Gursoy, M., Könönen, E., Emingil, G., Pussinen, P. J., & Mäntylä, P. (2016). Analysis of matrix metalloproteinases, especially MMP-8, in gingival crevicular fluid, mouthrinse and saliva for monitoring periodontal diseases. *Periodontology 2000, 70*(1), 142–163. https://doi.org/10.1111/prd.12101

Sorsa, T., Tjäderhane, L., Konttinen, Y. T., Lauhio, A., Salo, T., Lee, H. M., Golub, L. M., Brown, D. L., & Mäntylä, P. (2006). Matrix metalloproteinases: Contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. *Annals of Medicine, 38*(5), 306–321. https://doi.org/10.1080/07853890600800103

Sorsa, T., Uitto, V. J., Suomalainen, K., Vauhkonen, M., & Lindy, S. (1988). Comparison of interstitial collagenases from human gingiva, sulcular fluid and polymorphonuclear leukocytes. *Journal of Periodontal Research, 23*(6), 386–393. https://doi.org/10.1111/j.1600-0765.1988.tb01618.x

Stamp, L. K., Khalilova, I., Tarr, J. M., Senthilmohan, R., Turner, R., Haigh, R. C., Winyard, P. G., & Kettle, A. J. (2012). Myeloperoxidase and oxidative stress in rheumatoid arthritis. *Rheumatology, 51*(10), 1796–1803. https://doi.org/10.1093/rheumatology/kes193

Sur Chowdhury, C., Giaglis, S., Walker, U. A., Buser, A., Hahn, S., & Hasler, P. (2014). Enhanced neutrophil extracellular trap generation in rheumatoid arthritis: Analysis of underlying signal transduction pathways and potential diagnostic utility. *Arthritis Research & Therapy, 16*(3), R122. https://doi.org/10.1186/ar4579

Svärd, A., Renvert, S., Sanmartin Berglund, J., Persson, R. G., & Söderlin, M. (2020). Antibodies to citrullinated peptides in serum and saliva in patients with rheumatoid arthritis and their association to periodontitis. *Clinical and Experimental Rheumatology, 38*(4), 699–704.

Tchetverikov, I., Ronday, H. K., Van El, B., Kiers, G. H., Verzijl, N., TeKoppele, J. M., Huizinga, T. W., DeGroot, J., & Hanemaaijer, R. (2004). MMP profile in paired serum and synovial fluid samples of patients with rheumatoid arthritis. *Annals of the Rheumatic Diseases, 63*(7), 881–883. https://doi.org/10.1136/ard.2003.013243

van Riel, P. L., & Renskers, L. (2016). The disease activity score (DAS) and the disease activity score using 28 joint counts (DAS28) in the management of rheumatoid arthritis. *Clinical and Experimental Rheumatology, 34*(5 Suppl 101), S40–S44.

Vandenbroucke, R. E., & Libert, C. (2014). Is there new hope for therapeutic matrix metalloproteinase inhibition? *Nature Reviews Drug Discovery, 13*(12), 904–927. https://doi.org/10.1038/nrd4390

Wang, W., Jian, Z., Guo, J., & Ning, X. (2014). Increased levels of serum myeloperoxidase in patients with active rheumatoid arthritis. *Life Sciences, 117*(1), 19–23. https://doi.org/10.1016/j.lfs.2014.09.012

Wang, W., Peng, W., & Ning, X. (2018). Increased levels of neutrophil extracellular trap remnants in the serum of patients with rheumatoid arthritis. *International Journal of Rheumatic Diseases, 21*(2), 415–421. https://doi.org/10.1111/1756-185X.13226

Yilmaz, D., Gönüllü, E., Gürsoy, M., Könönen, E., & Gürsoy, U. K. (2021). Salivary and serum concentrations of monocyte chemoattractant protein-1, macrophage inhibitory factor, and fractalkine in relation to rheumatoid arthritis and periodontitis. *Journal of Periodontology, 92*(9), 1295–1305. https://doi.org/10.1002/JPER.20-0632