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Original Article PERIODONTOLOGY

Assessment of *Epstein–Barr virus*, *Candida albicans*, and some periodontal pathogens in rheumatoid arthritis patients with periodontitis

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ABSTRACT

OBJECTIVE: To comparatively investigate the periodontal results and microbial load in subgingival biofilm samples (SBS) in rheumatoid arthritis subjects and healthy volunteers.

METHODS: One hundred twenty subjects were classified into different cohorts: healthy (H-C); periodontitis with good systemic health (H-P); rheumatoid arthritis (RA) and good periodontal health (RA-C); and periodontitis with RA (RA-P). The periodontal parameters were recorded, and SBS were collected to determine periodontal pathogens including *Epstein–Barr Virus* (*EBV*) and *Candida albicans* using reverse transcription-polymerase chain reaction (RT-PCR).

RESULTS: Subjects that had greater disease course, determined by moderate or high disease activity scores 28 (DAS28), suffered from worse oral health conditions (higher plaque index, gingival index, bleeding on probing, probing depth, and excessive clinical attachment loss) than those with low DAS28 scores. A higher prevalence of *Treponema denticola* (*T. denticola*) was observed in the RA-P group. Cyclic citrullinated peptide was associated with the occurrence of *T. denticola* and *Campylobacter rectus*. DAS28 using C-reactive protein (DAS28-CRP) had a significant association with *Capnocytophaga gingivalis* and *EBV*. The duration of the RA disease was associated with the presence of *T. denticola*.

CONCLUSION: Subgingival microbial difference could reliably discriminate RA from healthy individuals. Especially, *T. denticola* and *EBV* may play a key role in periodontitis associated with RA.

Keywords: DAS28; microbiota; periodontitis; rheumatoid arthritis.

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Periodontitis is a gum infection caused by various bacteria that result from an imbalance in the host's inflammatory response. This disease results in damage to the attachment apparatus and eventually, the alveolar bone [1, 2]. Even though the disease is a response to inflammation in the surrounding dental tissues, it might be a risk factor for declining systemic health as suggested by recent studies on this bidirectional relationship [3].

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Rheumatoid arthritis (RA) is caused by inflammation of the joint synovium, leading to joint damage and limited joint function. The disease has increased the involvement of inflammatory infiltrates, such as macrophages and T cells, within the synovial membrane [4]. The patient's habits, such as smoking, and the presence of periodontitis may contribute to RA pathogenesis. RA and periodontitis, while affecting different parts of the body, are both chronic inflammatory diseases and have similar pathological features. In these cases, chronic inflammation happens within the bone and surrounding connective tissue, and the host response driven by immunogenetic factors determines the inflammatory events. In addition, pathological processes that determine the extent of tissue damage, such as cells, cytokines, and enzymes, are similar in RA and periodontitis [5].

These two disease processes can be traced back to overlapping inflammatory causes. Microbiologically, chronic lipopolysaccharide (LPS) release from periodontopathogens in a biofilm can serve as the source of superantigens that increase the inflammatory secretion characteristic of RA. In addition, *Porphyromonas gingivalis* (*P. gingivalis*), which is a major periodontopathogen, is the only bacterium that can secrete peptidyl-arginine-deiminase (PAD), an enzyme capable of converting arginine into citrulline ("citrullinating") [6, 7], which is a key event in RA pathogenesis [8]. Several studies have suggested that *P. gingivalis* may negatively impact the creation of anti-citrullinated protein antibodies (ACPAs), which are highly specific in RA diagnosis [9–11].

Previous reports about RA-specific periodontal pathogens have examined only a limited number of prespecified microbes because of the difficulties in culturing these organisms [12, 13]. In RA patients, the extent of dysbiosis in disease- and health-associated subgingival sites and the influence that the severity of RA has on microbial diversity remain unclear.

Epstein-Barr virus (*EBV*) has several characteristics that suggest that it is a likely factor in RA pathophysiology, including its ability to modify host responses and adapt to its environment by producing an interleukin (IL) 10–like cytokine and modulating IL-6 production [14]. One such inflammatory cytokine, IL-17, augments tumor necrosis factor (TNF)-α, IL-1β, and IL-6, which are being investigated for therapy in RA treatment. *Candida albicans* (*C. albicans*), a strongly Th17-dependent pathogen, resides in healthy gastrointestinal (GI) tracts with resident microbiota and is benign in the immunocompetent host [15]. Bishu et al. [16] concluded that RA patients exhibited dysfunctional oral immune responses to *C. albicans* despite an elevated IL-17A baseline. However, similar studies demonstrated mixed results; therefore, the contribution of RA on *C. albicans* is inconclusive [17, 18].

Highlight key points

- The oral microbiota may play a pathogenetic role in rheumatoid arthritis.
- Rheumatoid arthritis may affect subgingival biofilm and increase exposure to periodontitis.
- *Treponema denticola* had a significant association with cyclic citrullinated peptide and the duration of the disease.
- Increased *Treponema denticola* was found in rheumatoid arthritis patients with periodontitis.
- A significant correlation was shown for DAS28-CRP in RA and the presence of *Epstein–Barr Virus*.
- Dentists must understand the importance of ongoing periodontal screening in RA patients to be able to educate them about periodontal disease prevention and progression.

The aim of this study is to analyze the levels of *EBV* and *C. albicans* in the subgingival flora in RA subjects. In addition, the goal was to also define the periodontopathogenetic bacterial, *EBV*, and *C. albicans* components in the RA subgingival microbiome and to reveal their relationship to RA DAS and periodontal status.

MATERIALS AND METHODS

The Clinical Research Ethics Committee of Bolu Abant Izzet Baysal University (IRB: 2019/153), approved this study, performed in accordance with the Declaration of Helsinki. Power analysis was done as previously described [19], and the sample size was determined to be 20 subjects for each group with a significant difference in the prevalence of *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) (power = 80% and α = 5%) based on G*Power 3.1 (Dusseldorf, Germany).

All participants in this case-control study provided written informed consent. The study was performed from November 2019 through April 2020. The 120 study participants (34 males and 86 females; ages 22 to 67 years) were divided into groups: healthy (H-C); periodontitis with good systemic health (H-P); rheumatoid arthritis (RA) and good periodontal health (RA-C); and periodontitis with RA (RA-P).

Patient Selection

Sixty RA subjects (15 men, 45 women) from the Bolu Abant Izzet Baysal University Department of Rheumatology and the Department of Physical Medicine and Rehabilitation were recruited for the study. RA diagnosis for each volunteer was confirmed by a rheumatologist (MT) based on the 2010 criteria of the American College of Rheumatology (ACR) [20]. RA status in each patient was assessed by accounting for tender and swollen joints using the disease activity score 28 with C-reactive protein (DAS28–CRP). RA duration, current drug treatment using glucocorticoids (GC), synthetic disease-modifying antirheumatic drugs (sDMARDs) (i.e., methotrexate or sulfasalazine), and/or biologic disease-modifying antirheumatic drugs (bDMARDs) (i.e., TNF-α inhibitors, IL-6 receptor antagonists, and anti-CD20 monoclonal antibodies) were recorded from the patient's medical records. Seropositivity of RA (positive rheumatoid factor (RF) and/or ACPAs), cyclic citrullinated peptide (CCP), CRP positive, sedimentation, and comorbidities were recorded from each patient's medical records.

Systemically healthy participants from the Bolu Abant Izzet Baysal University Faculty of Dentistry, Department of Periodontology, were grouped based on age and sex for the study group. Each volunteer was given a standard/detailed examination and was then categorized according to periodontal status: periodontitis and good periodontal health. A periodontologist, blinded to the study groups, conducted the measurements needed throughout the study. Cases within different groups were also age- and sex-matched.

The Classification of Periodontal and Peri-Implant Diseases and Conditions (2017) [21, 22] was used to determine the periodontal status of the 120 participants. Periodontal health was defined as healthy gingiva on an intact periodontium with bleeding on probing (BOP) 10% at a probing depth (PD) of 3 mm, no attachment loss, no alveolar bone damage, and no previous periodontitis history.

Periodontitis was diagnosed when interdental clinical attachment loss (CAL) was determined at greater than or equal to 2 non-adjacent teeth and/or buccal or CAL greater than or equal to 3 mm with PD greater than 3 mm detectable at greater than or equal to 2 teeth [21, 22].

Inclusion criteria: 1) fulfilling the 2010 ACR classification criteria for RA; 2) no changes in medication and RA treatment dose over the last 3 months; and 3) 18 years or older.

Exclusion criteria were: 1) any inflammatory state including diabetes, cardiovascular disease (CVD), 2) neurological symptoms, psychiatric issues; 3) hepatitis, renal disease (creatinine clearance less than 60 mL/min), or liver disease; 4) a history of smoking or tobacco use; 5) inflammatory or autoimmune skin diseases; 6) history of malignancy; 7) pregnancy, lactation, or planning to be pregnant during the time of the study; 8) antibiotic use within 3 months of study; 9) periodontal therapy or mouth rinse use in previous 3 months; 10) edentulism.

Clinical Examination

The plaque index (PI) [23], gingival index (GI) [24], BOP, PD, and the distance between the bottom of the tooth pocket and the cementoenamel junction, CAL, were assessed by a single professional periodontist (GU). A Williams probe was used to conduct a periodontal exam (Hu-Friedy, Chicago, IL, USA).

Results were written to the whole millimeter and the average score for PD, CAL, GI, PI, and BOP was divided by the total number of sites and multiplied by 100.

Subgingival Biofilm Sampling

Each tooth was sequestered with cotton swabs and air drying. Subgingival biofilm samples (SBS) were collected for each subject. Teeth that had the deepest probing depths were selected following supragingival biofilm sample removal. With sterile paper points, SBS was collected in 1.5-mL tubes, where they were kept at -80 °C prior to analysis.

DNA Isolation From Samples

DNA was isolated from each sample with a High Pure PCR Template Prep kit (Roche Diagnostics, Germany) using the manufacturer's instructions. All DNA was maintained at -80 °C until analysis.

Detection of Bacterial Pathogens Using Real-Time Polymerase Chain Reaction (qPCR)

The periodontal pathogens *P. gingivalis*, *T. denticola*, *Tannerella forsythia*, *Prevotella intermedia*, *F. nucleatum*, *Campylobacter rectus*, *Actinomyces naeslundii*, *Streptococcus oralis*, *Eikenella corrodens*, C. *gingivalis*, and *A. actinomycetemcomitans* were assessed using qPCR. This qPCR used oligonucleotide primers (Sentromer Ltd., Istanbul, Turkiye) and plasmid standards to quantitate the data (Bioeksen Ltd., Istanbul, Turkiye) [25, 26].

Table 1. Demographic/clinical data

	RA-C (n=30)	RA-P (n=30)	H-C (n=30)	H-P (n=30)	p
Age (mean±SD)	45.36±9.78	45.48±10.23	41.56±5.51	42.48±6.06	0.159*
Sex [female, n (%)]	24 (80)	21 (70)	18 (60)	23 (76.7)	0.376
PI	0.52±0.32[a]	1.87±0.34[c]	0.30±0.19[a]	2.34±0.28[b]	**<0.001**
GI	0.58±0.37[b]	1.83±0.36[a]	0.49±0.20[b]	2.34±0.43[a]	**<0.001**
BOP (%)	4.23±3.18[b]	77.9±12.62[a]	2.46±1.86[b]	87.85±7.36[a]	**<0.001**
PD (mean±SD) (mm)	1.36±0.27[b]	3.20±0.56[a]	1.44±0.15[b]	3.48±0.43[a]	**<0.001**
CAL (mean±SD) (mm)	1.36±0.27[b]	3.67±0.77[a]	1.44±0.15[b]	3.67±0.38[a]	**<0.001**

*: Anova test; n: Number; SD: Standard deviation; PI: Plaque index; GI: Gingival index; BOP: Bleeding on probing; PD: Probing depth; CAL: Clinical attachment loss. Healthy (H-C); periodontitis with good systemic health (H-P); rheumatoid arthritis (RA) and good periodontal health (RA-C); and periodontitis with RA (RA-P). Bold values indicate statistical significance. a, b, c: Lettering indicates differences between groups. Differences showed with letters.

Table 2. Patient characteristics with rheumatoid arthritis (RA)

Variable	RA (n=60)		
	RA-C (n=30)	RA-P (n=30)	p
RF positivity, n (%)	10 (33.3)	11 (36.67)	0.729
CCP, n (%)	17 (56.67)	15 (50)	0.571
CRP positive (mean±SD)	3.42±3.50	19.18±19.67	**0.001**
Sedimentation rate (mean±SD)	25.68±14.0	34.36±16.99	**0.015**
DAS28–CRP (mean±SD)	2.45±0.88	3.79±1.46	**0.001***
Swollen joint count (mean±SD)	0.56±0.82	2.79±3.89	0.026
Tender joint count (mean±SD)	2.32±3.74	5.12±6.22	0.191
Duration of diseases (years) (mean±SD)	4.41±4.59	5.40±4.64	0.320
Current treatment			
DMARD (%)	83.33	73.33	0.308
Biologic or Jak inhibitors (%)	13.33	16.67	0.718**
Glucocorticoids (%)	56.67	36.67	0.121
Comorbidities			
Diabetes mellitus (%)	3.33	0	0.431**
Hypertension (%)	3.33	10	0.627**
Secondary Sjogren's syndrome (%)	0	3.33	0.999**

*: Student T test; **: Fisher's Exact test; n: Number; SD: Standard deviation; RF: Positive rheumatoid factor; CCP: Cyclic citrullinated peptide; CRP: C-reactive protein; DMARD: Disease-modifying anti-rheumatic drugs; DAS28: Disease activity score 28; RA-C: Rheumatoid arthritis (RA) and good periodontal health (RA-C); RA-P: Periodontitis with RA (RA-P). Bold values indicate statistical significance.

qPCR was conducted using a LightCycler 480 II system and 5 µL DNA and 15 µL master mix. The qPCR protocols were as follows: activation for 10 min at 95 °C, 40 cycles of PCR were observed at 95 °C for 20 sec, 60 °C for 20 sec, and 72 °C for 20 sec (single reading), and 95 °C for 10 sec and 65 °C for 1 min for melting curve analysis at 95 °C (continuous reading).

Detection and Quantitation of *C. albicans* and *EBV* Using Commercial qPCR Kits

A commercial Genesig *EBV* DNA kit (Primerdesign, UK, cat. no: Path-*EBV*-standard) and a commercial Genesig *Candida albicans* kit (Primerdesign, UK, cat. no: Path-*C. albicans*-standard) were used to detect

EBV and C. *albicans*, respectively. qPCR was conducted as described above using the same system. The sample was added to the template DNA (5 µL) from the standard, positive, or negative controls, with a total volume of 20 µL. The qPCR protocols were: after denaturation for enzyme activation for 2 min at 95 °C, 50 cycles of PCR were observed at 95 °C for 15 sec and 60 °C for 1 min (single reading).

Statistical Analyses

Student's t-test was utilized for the comparison of two dependent groups with normal distribution. The Mann-Whitney U test was used for two dependent groups that did not have a normal distribution. An ANOVA was conducted for comparisons of three or more dependent groups with normal distribution, and a Kruskal Wallis test was done to compare three or more dependent groups without normal distribution. A post hoc Bonferroni analysis was conducted to determine the group or groups that created the difference in each aforementioned case. In the categorical data, the Pearson chi-square test was applied when the sample size assumption was met (expected value >5 for each cell), and Fisher's exact test was applied when it was not met. Binary logistic regression analysis was used to model categorical variables with two groups with other variables. Analyses were carried out in IBM SPSS 25 program (IBM Corp., Armonk, NY, USA).

RESULTS

The 120 participants were divided as follows: 1) healthy (H-C) (mean age: 41.56±5.51 years; 12 males, 18 females); 2) periodontitis with good systemic health (H-P) (mean age: 42.48±6.06 years; 7 males, 23 females); 3) rheumatoid arthritis (RA) and good periodontal health (RA-C) (mean age 45.36±9.78 years; 6 males, 24 females); and 4) periodontitis with RA (RA-P) (mean age: 45.48±10.23 years; 9 males, 21 females) (Table 1).

Descriptive study population characteristics and the groups are provided in Table 1. No remarkable discrepancies were found for age and gender between groups (p>0.05). The H-P group had a higher PI than the RA-P group (p<0.001); however, there were no differences in CAL, PD, GI, and BOP were detected between these two groups (p>0.05).

Clinical characteristics of the RA patients are provided in Table 2. There was no statistical difference between the seropositive in RA groups (p=0.729). RA activity (DAS28–CRP score) in RA-P was increased compared to RA-C (p<0.001). A higher CRP-positive value (p<0.001), higher sedimentation rate (p=0.015), and a higher count of swollen joints (p=0.026) were also observed in the RA-P group as compared to the RA-C group.

Patients with moderate and higher DAS28 suffered from worse oral health conditions (higher PI, GI, BOP%, PD, CAL) than those with low DAS28 scores (Table 3).

The prevalence of T. *forsythia*, C. *rectus*, *F. nucleatum*, *P. intermedia*, *A. naeslundii*, *S. oralis*, *E. corrodens*, *A. actinomycetemcomitans*, C. *albicans*, and *EBV* were similar among all groups. *P. gingivalis* was detected with the same frequency in RA-P (70%) and H-P (%72.4). While *P. gingivalis* and C. *gingivalis* were seen less in H-C compared to other groups (p=0.001, p=0.048, respectively),

Table 3. Comparison of the periodontal findings and subgingival microbiota according to activity of illness

Variable	Activity of illness		p
	Low (DAS28 ≤3.2) (n=30) (mean±SD)	Moderate/high (DAS >3.2) (n=30) (mean±SD)	
P. gingivalis	3.68±0.33	3.69±0.27	0.969
T. denticola	4.41±0.22	4.43±0.22	0.793
T. forsythia	2.26±0.29	2.43±0.13	0.080
C. rectus	3.61±0.34	3.64±0.37	0.775
F. nucleatum	3.30±0.20	3.42±0.29	0.157
P. intermedia	3.71±0.18	3.85±0.05	0.187
A. naeslundii	4.62±0.21	4.46±0.22	**0.032**
S. oralis	4.54±0.27	4.66±0.22	0.075
E. corrodens	4.20±0.37	4.24±0.43	0.753
C. gingivalis	3.48±0.26	3.33±0.27	0.239
A. actinomycetemcomitans	3±0.49	2.92±0.38	0.628
C. albicans	2.18±0.18	2.04±0.18	0.267
Epstein-Barr virus	3.02±0.47	3.54±0.53	0.252
PI	1.03±0.75	1.63±0.61	**0.002**
GI	1.09±0.76	1.57±0.58	**0.012**
BOP (%)	31.58±36.13	65.22±31.92	**0.001**
PD	2.15±1.00	2.74±0.97	**0.028**
CAL	2.34±1.27	3.07±1.20	**0.029**

SD: Standard deviation; DAS: Disease activity score; PI: Plaque index; GI: Gingival index; BOP: Bleeding on probing; PD: Probing depth; CAL: Clinical attachment loss. Bold values indicate statistical significance.

TABLE 4. Frequencies of detection (%) and microbial loads (log10 copy/µl) in subgingival plaque from study groups

n=30	Frequency of detection		p	*log10 microbial load	p
	n	%		Mean±SD	
Porphyromonas gingivalis			**0.001**		**<0.001**
RA-C	18	54.5		3.68±0.31[a]	
RA-P	21	70		4.61±0.3[b]	
H-C	6	24		3.7±0.26[a]	
H-P	21	72.4		4.55±0.31[b]	
Treponema denticola			**0.003**		0.495
RA-C	11	44		4.43±0.17	
RA-P	27	81.8		4.41±0.24	
H-C	12	40		4.51±0.12	
H-P	17	58.6		4.39±0.17	
Tannerella forsythia			0.720		0.273
RA-C	9	36		2.23±0.34	
RA-P	16	48.5		2.38±0.17	
H-C	15	50		2.36±0.3	
H-P	14	48.3		2.15±0.32	
Campylobacter rectus			0.099		0.910
RA-C	22	88		3.63±0.37	
RA-P	27	81.8		3.62±0.34	
H-C	20	66.7		3.59±0.43	
H-P	26	89.7		3.63±0.36	
Fusobacterium nucleatum			0.690		0.309
RA-C	15	60		3.37±0.27	
RA-P	16	48.5		3.34±0.24	
H-C	14	46.7		3.2±0.3	
H-P	13	44.8		3.32±0.4	
Prevotella intermedia			0.297		0.511
RA-C	6	24		3.77±0.03	
RA-P	9	27.3		3.71±0.22	
H-C	4	13.3		3.78±0.07	
H-P	10	34.5		3.64±0.27	
Actinomyces naeslundii			0.782		**0.023**
RA-C	16	64		4.56±0.25[a,b]	
RA-P	17	51.5		4.54±0.19[a,b]	
H-C	16	53.3		4.67±0.25[a]	
H-P	17	58.6		4.39±0.21[b]	
Streptococcus oralis			–		0.719
RA-C	30	100		4.56±0.26	
RA-P	30	100		4.61±0.26	
H-C	30	100		4.63±0.24	
H-P	30	100		4.59±0.31	
Eikenella corrodens			0.626		0.830
RA-C	14	56		4.18±0.36	
RA-P	21	63.6		4.24±0.42	
H-C	18	60		4.27±0.36	
H-P	21	72.4		4.25±0.29	

Table 4 (cont). Frequencies of detection (%) and microbial loads (log10 copy/µl) in subgingival plaque from study groups

n=30	Frequency of detection		p	*log10 microbial load	p
	n	%		Mean±SD	
Capnocytophaga gingivalis			**0.048**		0.362
RA-C	10	34.5		3.32±0.34	
RA-P	17	51.5		3.34±0.26	
H-C	4	16		3.59±0.23	
H-P	12	40		3.45±0.11	
Aggregatibacter actinomycetecomitans			0.066		**0.031**
RA-C	14	56		2.99±0.46[a]	
RA-P	21	63.6		2.94±0.44[a]	
H-C	10	33.3		2.58±0.38[b]	
H-P	18	62.1		2.92±0.43[a,b]	
Candida albicans			0.865**		0.219
RA-C	3	12		2.26±0.13	
RA-P	6	18.2		2.02±0.16	
H-C	4	13.3		2.1±0.2	
H-P	3	10.3		2.13±0.08	
Epstein-Barr virus			0.157**		0.274
RA-C	2	8		2.73±0.06	
RA-P	7	21.2		3.62±0.43	
H-C	2	6.7		3.68±0.03	
H-P	7	24.1		3.52±0.52	

*: The negative results are not included; **: Fisher's Exact test; n: Number; SD: Standard deviation; healthy (H-C); periodontitis with good systemic health (H-P); rheumatoid arthritis (RA) and good periodontal health (RA-C); and periodontitis with RA (RA-P). 'a, b' from the other group means demonstrates a statistically significant difference. Bold values indicate statistical significance. Differences showed with letters.

a higher prevalence of *T. denticola* was seen for RA-P compared to the other groups (p=0.003). The concentrations of *P. gingivalis* were greater in RA-P and H-P compared to RA-C and H-C (p<0.001). The concentrations of *A. actinomycetecomitans* were greater in RA compared to H-C (p=0.031) (Table 4).

CCP had a significant association with the presence of *T. denticola* (OR 6.114; 95% CI: 1.475–25.349; p=0.013) and *C. rectus* (OR 7.097; 95% CI: 1.004–50.18 p=0.05). A significant relationship was found for DAS28–CRP and the presence of *C. gingivalis* (OR 7.221; 95% CI: 1.266–41.179; p=0.026), *EBV* (OR 10.310; 95% CI: 1.387–76.612; p=0.023), and the absence of *P. intermedia* (OR 0.089; 95% CI: 0.011–0.740; p=0.025). Furthermore, the duration of the RA disease was related to *T. denticola* (OR 9.238; 95% CI: 1.729–49.354; p=0.009) and the absence of *F. nucleatum* (OR 0.231; 95% CI: 0.056–0.962; p=0.044) (Table 5).

DISCUSSION

Previous studies have reported an association with RA and periodontitis, but the effect of RA on the subgingival microbiota from periodontitis remains controversial [5, 19, 27]. In the present study, we evaluated the microbiome in RA subgingival with periodontal conditions and compared these to DAS and periodontal health. The prevalence of *T. denticola* was found to be highest in individuals with RA-P. While the prevalence of P. *gingivalis* and microbial load of *A. actinomycetecomitans* were found to be higher in RA-C individuals compared to H-C individuals, they did not differ between individuals with RA-P and H-P.

An association between periodontitis and RA [28] has been well established, which suggests that patients with RA have more tooth loss and significant CAL than healthy individuals [29–31]. Ziebolz et al. [32] concluded that most patients with RA show

TABLE 5. Association between the investigated subgingival microbiota and the rheumatological parameters

Subgingival microbiota (yes)	CCP		Duration of RA		DAS28 /CRP		RF		DMARD		Biologic or Jak inhibitors	
	p	OR	p	OR	p	OR	p	OR	p	OR	p	OR
Porphyromonas gingivalis	0.491	1.748	0.564	1.671	0.393	0.473	0.254	0.397	0.447	2.011	0.321	4.569
Treponema denticola	**0.013**	**6.114**	**0.009**	**9.238**	0.959	0.962	0.465	1.645	0.655	1.441	0.177	10.736
Tannerella forsythia	0.467	0.616	0.972	1.026	0.896	0.912	0.775	0.834	0.466	0.578	0.308	3.289
Campylobacter rectus	**0.050**	**7.097**	0.757	0.713	0.660	0.616	0.171	6.091	0.723	1.509	0.697	2.039
Fusobacterium nucleatum	0.782	1.215	**0.044**	**0.231**	0.501	1.650	0.704	0.769	0.731	1.315	0.162	0.189
Prevotella intermedia	0.722	0.757	0.105	3.945	**0.025**	**0.089**	0.373	0.495	0.980	0.978	0.467	2.788
Actinomyces naeslundii	0.391	0.560	0.154	2.982	0.744	0.790	0.417	0.573	0.105	0.256	0.176	7.772
Eikenella corrodens	0.356	0.538	0.476	1.693	0.677	0.742	0.840	1.142	0.300	2.211	0.261	0.268
Capnocytophaga gingivalis	0.610	0.673	0.946	0.944	**0.026**	**7.221**	0.775	1.240	0.149	0.295	0.829	1.276
Aggregatibacter actinomycetecomitans	0.207	2.388	0.702	0.745	0.394	1.852	0.797	0.845	0.627	1.456	0.671	0.615
Candida albicans	0.88	1.148	0.393	0.420	0.509	2.006	0.305	2.753	0.644	0.632	0.703	0.572
Epstein-Barr virus	0.895	0.895	0.925	0.914	**0.023**	**10.310**	0.536	1.682	0.762	1.358	0.433	2.637

RA: Rheumatoid arthritis; OR: Odds ratio; CCP: Cyclic citrullinated peptide; DAS28–CRP: Disease activity score 28–C-reactive protein; RF: Rheumatoid factor; DMARD: Disease-modifying anti-rheumatic drugs.

moderate-to-severe periodontitis and the presence of periodontal pathogens. The goal of this report was to comparatively examine clinical data and microbial load on SBS in RA patients and healthy volunteers with or without periodontitis.

In the present study, there were no significant changes in CAL, GI, BOP, and PD were detected for H-P and RA-P groups. Various pharmacological approaches are used to treat RA, the most common of which are non-steroidal anti-inflammatory drugs (NSAIDs), GC, as well as synthetic and biological DMARDs [7]. These results can be explained by the protective effect of drugs by reducing bleeding and inflammation [33]. Also, in the present study, the H-P group had higher PI than the RA-P group. However, PI is low in RA-P, the similarity in other periodontal parameters shows that RA disease may affect the periodontal status, independent of the PI index. In a study evaluating the role of poor oral care in the possible association with RA and periodontitis, it was determined that the role of only plaque index was 12.4%, the role of the gingival index was 11.1%, and the role of plaque index and gingival index together was 13.4% [31].

The DAS 28-CRP has been used to determine RA activity. A DAS 28 score of <2.6 implies RA remission, <3.2 implies low disease activity, while >5.1 implies very active disease [34]. In this study, patients with moderate or high DAS 28-CRP scores suffered from worse periodontal conditions (higher PI, GI, BOP%, PD, CAL) than those with low DAS 28- CRP scores. Similarly, Mikuls et al. [35] found that periodontal diseases correlated with higher DAS28-CRP. Taken together, our results indicate that the severity of the existing periodontal condition and RA disease severity increase concurrently. Also, according to our results, the RA-P group showed higher CRP, sedimentation rate, DAS28-CRP, and count of swollen joints values than the RA-H group. The higher DAS28-CRP score, especially in individuals with RA-P despite the drugs used, supports the hypothesis that the existing periodontal inflammation and periodontal pathogens in these individuals may have an effect on rheumatoid arthritis activity. Contrary to our results, Zhao [36] found no significant difference between RA groups in terms of swollen joint count, DAS28, and CRP values.

The association between *P. gingivalis* and RA is convoluted and concerning. Past clinical reports have determined that *P. gingivalis* is linked to RA [37, 38]. According to our results, *P. gingivalis* frequency of detection was increased in RA-C compared to H-C whereas there was no difference observed between RA-P and H-P. This data suggests that *P. gingivalis* contributes to RA similar to another study [39]. Also, *T. denticola*'s frequency of detection was increased in RA-P compared to RA-C. *Similar* to

the results of our study, Ziebolz et al. [32] and Schmickler et al. [40] have shown the prevalence of *T. denticola* increases in RA patients, which may indicate that *T. denticola* plays a key role in RA pathogenesis with periodontitis. On the other hand, the prevalence of *T. forsythia*, *C. rectus*, *F. nucleatum*, *P. intermedia*, *A. naeslundii*, *S. oralis*, *E. corrodens*, *A. actinomycetemcomitans*, *C. albicans*, and *EBV* were similar among all four study groups, which is consistent with previous research [40]. *A actinomycetemcomitans* has gained momentum as a likely candidate in RA since it induces citrullinated antigen production [41]. *A. actinomycetemcomitans* has been recognized as a bacterial trigger for RA, providing a link between autoimmunity and periodontal diseases [42]. In our report, the concentrations of *A. actinomycetecomitans* were increased in RA groups compared to H-C. However, some studies did not find a relationship with *A. actinomycetecomitans* and RA presence. [32, 40]. The discrepancy might be due to multiple factors including subject health, size of the population tested, and RA therapies used.

The oral pathogens may trigger the production of disease-specific autoantibodies and arthritis in susceptible individuals [42]. In our study, the probability of having CCP in the presence of *T. denticola* is 6.114 times higher than in the absence of *T. denticola* and the probability of having CCP in the presence of *C. rectus* is 7.097 times higher than in the absence of *T. denticola*. By emphasizing the importance of *P. gingivalis* in previous studies, it has been suggested that CCP may be linked to *P. gingivalis*, suggesting a possible role in RA [18, 43]. In this study, we determined that there may be a relationship between different bacteria and CCP, not only *P. gingivalis*. Also, we found a significant association between DAS28–CRP and the prevalence of *EBV*. Periodontitis was believed to be a viral–bacterial infection [44], and a previous report has suggested a link with *EBV* and different autoimmune diseases including RA [45]. According to Sakkas, Daoussis, Liossis, & Bogdanos [46], it is clear that *EBV* is an autoimmune inducer in RA. *EBV* can infect B and epithelial cells and remain latent in the resting B cells. *EBV* may also elicit a response that may result in a self-antigens attack [46]. In the patient group we investigated, we found that DAS28–CRP, disease duration, and CCP appeared to have a limited effect on the bacterial composition, and other studies comparing these factors have not been available. Reichert et al. [47] have examined both healthy subjects and periodontitis patients and analyzed SBS and serum. Similar to our report, they did not find an association for *P. gingivalis* and serum CCP. Previous research has been unable to determine any association between the investigated bacteria and CCP, RF, and DAS28–ESR in RA patients [48]. Results of our study, however, indicate that *T. denticola* had a significant association with CCP and the duration of the disease, although we suggest that this tendency could be established even though the RA patients were receiving treatment.

Epidemiologic studies have consistently demonstrated that RA patients have an increased frequency of infection compared to the general population, even when the effects of medications are considered [49, 50]. Although mucosal candidiasis is not a common side effect of RA, Bishu et al. [16] suggested that drugs that target IL-23/IL-17 might augment infection risk in a patient with RA and that those patients have an impaired oral immune response to *C. albicans*. *C. albicans* may also induce bacterial infiltration of anaerobic bacteria, *P. gingivalis*, and cause infection [51]. Reports have shown a correlation between subgingival colonization with *C. albicans*, and severe periodontitis [52, 53]. In this report, we were not able to confirm an association between *C. albicans* and RA and/or periodontitis. These may be associated with geography since plays a role in fungal infections.

Study Limitations

This study was limited because it was cross-sectional with single measurements. The relationship between the microbiota load in the oral cavity and RA activity parameters is uncertain due to the cross-sectional nature of the study design. Thus, it was difficult to determine microbiota changes as cause or effect. Furthermore, RA medications might have affected the findings. Further research should consider directional causal relationships and adjust for confounders such as medications. Separately from diagnostics, the microbiological analyses of the subgingival biofilm in this report benefit from rheumatological data. Nonetheless, our data suggested that RA may modulate the subgingival microbiome and we encourage further investigation using a larger cohort to confirm these results.

Conclusion

Using data from a case-control study and known factors that influence oral health and RA disease activity, these findings may be important to steadfastly differentiate RA from healthy individuals. Especially, *T. denticola* and *EBV* might have an important role in RA pathogenesis with periodontitis.

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