Periodontal microbiota in a cohort of Egyptian patients with rheumatoid arthritis and their relation to serum and gingival anticitrullinated peptide protein antibodies and different disease parameters

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Objective
The possibility of infectious trigger at the gingival site in rheumatoid arthritis (RA) was reported in previous studies. The aim of our study is to determine the organisms causing periodontitis (PD) in a cohort of Egyptian patients with RA and their relation to serum and gingival anticitrullinated peptide protein antibodies (ACPA) level and other disease parameters.

Patients and methods
A prospective cohort study was conducted on 100 consecutive Egyptian patients with RA. Disease activity was assessed by applying disease activity score-28, and functional status was measured using health assessment questionnaire. Dental examination, serum rheumatoid factor, ACPA in serum and gingival crevicular fluid (GCF), and radiograph of the hands were done for all patients. GCF culture was performed for all cases with PD for *Porphyromonas gingivalis* (*Pg*), *Aggregatibacter actinomycetemcomitans* (*Aa*), and *Prevotella intermedia* (*Pi*).

Results
Of the 100 patients, 66 patients had PD; of them, GCF culture was performed, and *Pg*, *Aa*, and *Pi* were found in 60.6, 15.2, and 30.3% of patients with RA with PD, respectively. Gingival ACPA showed significant higher level with *Pg* than *Pi* cases (P=0.047). No statistically significant difference was detected on comparing *Pg* with *Aa* or *Aa* with *Pi*. *Aa*-positive cases were associated with significantly higher level of C-reactive protein than *Pi*-positive cases (P=0.029), whereas no statistical significant difference was detected between *Pg*- and *Aa*-positive or *Pi*-positive cases.

Conclusion
Our findings support the relationship between PD and infectious trigger at the gingival site and RA. *Pg* is the most prevalent periodontal microbiota in our cohort of patients with RA with PD that is associated with significant higher level of gingival ACPA. None of the detected organisms correlated with the degree of RA activity or other disease parameters, apart from significantly higher C-reactive protein level with *Aa.*

Keywords:
aggregatibacter, microbiota, periodontitis, porphyromonas, rheumatoid arthritis

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Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease that is characterized by synovial inflammation and erosive arthritis [1]. An infectious cause of RA has been implicated; however, solid evidences are lacking. Microbiome is a term that describes certain bacterial colonies that populate skin and mucosal surfaces [2]. Several data demonstrated the role of microbiome in the pathogenesis of RA. Mucosal sites exposed to bacterial antigens, such as the gingiva and gut, may represent the initial site of immune autoreactivation [3]. Periodontitis (PD) is a chronic inflammatory gingival disease that serves as a reservoir for many organisms and inflammatory mediators [4]. A large surface area of the ulcerated pocket epithelium allows exchange between bacterial and host products. It has been noted that periodontal pathogens are able to invade gingival tissues, apart from the local destruction of the periodontal tissues, and gain access to the systemic circulation [5,6]. Several mechanisms may participate in these interactions including those induced by oral organisms, and those associated with host response factors such as proinflammatory cytokines [7,8].

Both PD and RA have cytokine profiles thought to be involved in the inflammatory processes, including high tumor necrosis factor-α production. Several studies

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reported significantly higher prevalence of gingival disease in patients with RA compared with the general population. They also reported that systemic inflammation induced by periodontal infection might worsen the local inflammatory reactions in the synovium of patients with RA [9]. This effect is supposed to be mediated through systemic inflammatory response such as acute-phase reactants and immune mediators [10].

Many bacteria have been identified in PD, and the most commonly implicated ones are *Porphyromonas gingivalis* (*Pg*), *Prevotella intermedia* (*Pi*), *Tannerella forsythia*, and *Aggregatibacter actinomycetemcomitans* [11]. Although many pathogens have been linked to pathogenesis of RA, *Pg* has been the major focus of investigations in RA [12,13]. Moreover, some studies identified *Aa* as the sole pathogen with the ability to produce the citrullinated antigens found in the RA [14]. These associations, if validated, could lead to the detection of potential risk, early preventive, and therapeutic approaches in the preclinical and early clinical phases of RA.

Objective

The aim of our study was to determine the organisms causing PD in a cohort of Egyptian patients with RA and their relation to serum and gingival anticitrullinated peptide protein antibodies (ACPA) level and other disease parameters.

Patients and methods

This study was carried out on a cohort of 100 consecutive Egyptian patients with RA fulfilling the 2010 American college of rheumatology/European league against rheumatism classification criteria for RA (ACR/EULAR) [15]. All patients were recruited from Rheumatology Outpatient Clinic and Rheumatology Inpatient Unit of Alexandria Main University Hospital (AMUH). Smokers, pregnant and lactating females, patients with diabetes mellitus, individuals who had received antibiotic therapy over the past 4 months as well as faulty prosthesis and fillings all were excluded from the study. Informed consent was obtained, and all patients were subjected to history taking, musculoskeletal examination with assessment of disease activity by applying the disease activity score (DAS-28/ESR version) [16], and functional state by applying health assessment questionnaire (HAQ) [17].

Dental examination was performed for all participants for the presence of PD according to American Academy of Periodontology 1999 [18]. Laboratory investigations included complete blood count, blood urea, serum creatinine, aspartate aminotransferase, alanine aminotransferase, erythrocyte sedimentation rate, and C-reactive protein (CRP). Radiograph of hands and wrists was performed, assessed, and globally scored for erosions and joint space narrowing by using the modified-Larsen scoring system [19]. Immunological profile included rheumatoid factor (RF) titer (Rose Waaler test) [20], serum, and gingival crevicular fluid (GCF) ACPA (using ELISA/Demeditec Diagnostics GmbH, Germany) [21]. For those with proven PD, GCF culture was performed for *Pg*, *Aa*, *and Pi*.

Sampling of periodontal fluid

GCF collection was performed as described by Goutoudi *et al.* [22]. For complete isolation and to prevent salivary contamination, cotton isolation and a suction were used mandatory. Each tooth was then isolated using cotton placed in its buccal vestibule. The tooth was then dried gently with air on the marginal gingival areas from all aspects. GCF samples were collected by filter paper strips which were cut into standardized size of 2×10 mm. A twiser was used to insert each paper strip into the gingival crevice until slight resistance was felt. The paper strip was left in place for about 60 s, then it was removed and examined.

Molecular detection of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Prevotella intermedia* [23]

A 16 s rRNA-based PCR detection method was used to determine the presence of *Aa*, *Pg*, and *Pi* in periodontal fluid taken from patients with PD. The samples in PBS were warmed to 37°C for 10 min and mixed well on a Vortex mixer; 0.2 ml of the microbial suspension was washed three times with distilled water. The bacterial pellets were resuspended in 0.1 ml of distilled water, boiled for 10 min and placed on ice. After centrifugation to remove cell debris, the supernatant was used for PCR analysis. In a 0.2 ml sterile eppendorf tube, 5 µl of bacterial DNA was mixed with 0.5 µl of each primer (forward and reverse) and 12.5 µl DreamTaq^{TM} Green PCR master mix (Fermentas – Thermo, USA), and completed to a final reaction volume of 25 µl with nuclease-free sterile water. PCR amplification was done on Sensoquest thermal cycler according to the following protocol:

(1) *Pg* and ubiquitous primers included an initial denaturation step at 95°C for 2 min, followed by 36 cycles of a denaturation step at 95°C for 30 s, a primer annealing step at 60°C for 1 min, an

extension step at 72°C for 1 min, and a final step of 72°C for 2 min.

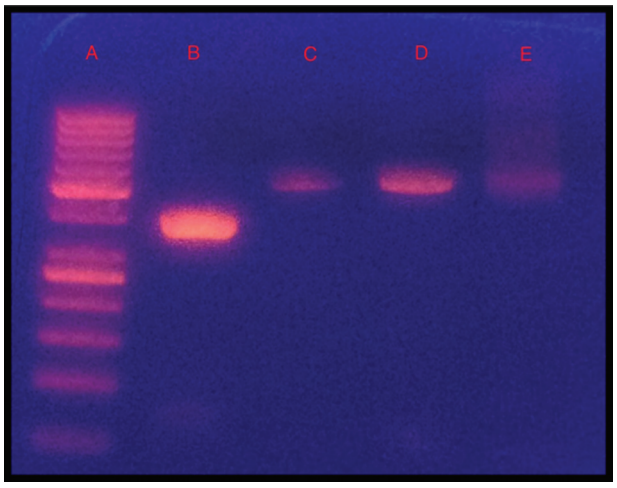
(2) *Aa* and *Pi* included an initial step of 95°C/2 min, followed by 36 cycles of 94°C/30 s, 55°C/1 min and 72°C/2 min, and a final step of 72°C/10 min.

Overall, 12 µl of PCR product was applied on a 2% agarose gel along with a 100–3000 base pair DNA ladder (Axygen, USA). Electrophoresis was done at 100 Volt for a 45 min period. Bands were detected using ethidium bromide in ultraviolet light (Fig. 1).

Statistical analysis

Data were analyzed using IBM SPSS version 20 (Armonk, NY: IBM Corp.) [24]. Qualitative data were described using number and percentage. Quantitative data were described using range (minimum and maximum), mean, SD, and median. The tests used were Spearman's correlation coefficient, χ^2-test, Mann–Whitney test, Kruskal–Wallis test, Fisher's exact or Monte-Carlo test, Student's *t*-test, and *F*-test (analysis of variance). *P* less than 0.05 was accepted as statistically significant. The diagnostic ability of GCF ACPA was identified using the receiver operating characteristic curve (ROC curve). ROC curves with the area under the curve were used to assess the diagnostic performance of GCF ACPA, 'an area=1.00 (100%) denoting (a gold standard–like) performance, whereas an area=0.5 (50%) denoting (a chance-like) performance'. Significant areas ($P<0.05$) indicate that the diagnostic performance is significantly better than chance. Cut-off values were chosen as the points that maximizes the sensitivity and specificity.

Figure 1



Results on 2% agarose gel electrophoresis of the PCR amplification products. A single DNA band of the predicted size was obtained by PCR using specific primer pair against the target organism. Lane A: 50 base pair DNA ladder. Lane B: *Porphyromonas gingivalis* (404 bp). Lane C: *Aggregatibacter actinomycetemcomitans* (557 bp). Lane D: *Prevotella intermedia* (575 bp). Lane E: Ubiquitous primers (602 bp).

Results

In this study, a cohort of 100 consecutive Egyptian patients with RA was enrolled. The mean age of cases was 44.80±9.88 years, with 81 females and 19 males. Twenty-four (24%) patients presented with early RA, and 76 (76%) with established RA with mean disease duration of 21.36±20.65 months. The mean ACR/EULAR criteria at the time of enrollment were 8.0 ±1.22 (Table 1). The mean DAS was 5.76±1.47, with 3% of patients were in remission, 3% had low disease activity, 31% had moderate disease activity, and 63% had high disease activity. However, the mean HAQ score was 1.59±0.91, with 34% of patients showed no disability, 25% had mild disability, 8% had moderate disability, and 33% had severe disability. Laboratory findings in patients with RA (*n*=100) are shown in Table 2.

PD was found in 66 (66%) patients; of them, GCF culture was performed, and *Pg*, *Aa*, and *Pi* were found in 40 (60.6%) patients, 10 (15.2%) patients, and 20 (30.3%) patients, respectively. Twenty-two (55%) of *Pg*-positive cases (*n*=40) had positive RF, whereas 20 (76.9%) of *Pg*-negative cases (*n*=26) had positive RF. Comparing the two groups revealed no statistical significant difference (*P*=0.070; Table 3). Eight (80%) of *Aa*-positive cases (*n*=10) had positive RF. On the contrary, 34 (60.7%) of *Aa*-negative cases (*n*=56) had positive RF. Comparing the two groups revealed no statistical significant difference (*P*=0.306;

Table 1 Descriptive analysis of patients with rheumatoid arthritis (*n*=100)

Variables	*n* (%)
Sex	
Male	19 (19.0)
Female	81 (81.0)
Age (years)	
Minimum–maximum	21.0–65.0
Mean±SD	44.80±9.88
Median	46.0
Duration of disease (months)	
Early	24 (24.0)
Established	76 (76.0)
Minimum–maximum	0.03–60.0
Mean±SD	21.36±20.65
Median	12.0
ACR/EULAR criteria at enrollment (6–10)	
Minimum–maximum	6.0–10.0
Mean±SD	8.0±1.22
Median	7.50

ACR/EULAR, American college of rheumatology/European league against rheumatism classification criteria for RA,

Table 2 Laboratory findings in patients with rheumatoid arthritis (*n*=100)

Variables	Min. – Max.	Mean±SD.	Median
Hb			
Male (13.5–17 g/dl)	12.50–14.40	13.45±1.10	13.45
Female (11.5–16 g/dl)	7.0–14.20	10.90±1.43	11.05
Total	7.0–14.40	11.0±1.50	11.15
WBC (4–11×10^3/mm^3)	2.0–11.10	6.58±1.94	6.40
Platelet (150–500 ×10^3/mm^3)	137.0–419.0	277.42±70.49	284.0
SGOT (15–37 µl/l)	11.0–173.0	27.32±22.45	22.0
SGPT (30–65 µl/l)	24.0–251.0	40.62±33.09	33.50
Urea (15–45 mg/dl)	15.0–60.0	25.76±8.76	25.0
Serum creatinine (0.6–1.3 mg/dl)	0.30–1.20	0.77±0.18	0.80
ESR (first hour male: <10/mm, female: <12/mm)	7.50–135.0	51.55±31.38	42.50
CRP (<6 IU/ml)	0.13–120.0	25.09±26.90	16.0
RF (<6 IU/ml)	4.0–192.0	35.10±40.95	18.06

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; RF, rheumatoid factor; SGOT, aspartate aminotransferase; SGPT, alanine aminotransferase; WBC, white blood cells.

Table 3 Prevalence of rheumatoid factor in periodontitis cases with *Porphyromonas gingivalis* (*Pg*) (*n*=66)

RF	Negative (*n*=26) [*n* (%)]	Positive (*n*=40) [*n* (%)]	χ^2	^{FE}P
Negative	6 (23.1)	18 (45.0)	3.273	0.070
Positive	20 (76.9)	22 (55.0)		

χ^2, *P*: χ^2 and *P* values for χ^2-test for comparing between the two groups. FE, Fisher exact for χ^2-test; RF, rheumatoid factor.

Table 4 Prevalence of rheumatoid factor in periodontitis cases with *Aggregatibacter actinomycetemcomitans* (*Aa*) (*n*=66)

RF	Negative (*n*=56) [*n* (%)]	Positive (*n*=10) [*n* (%)]	χ^2	^{FE}P
Negative	22 (39.3)	2 (20.0)	1.364	0.306
Positive	34 (60.7)	8 (80.0)		

χ^2, *P*: χ^2 and *P* values for χ^2-test for comparing between the two groups. FE, Fisher exact for χ^2-test; RF, rheumatoid factor.

Table 5 Prevalence of rheumatoid factor in periodontitis cases with *Prevotella intermedia* (*Pi*) (*n*=66)

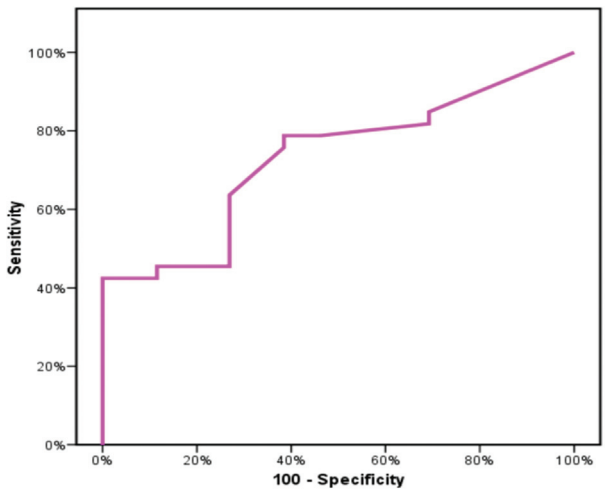
RF	Negative (*n*=46) [*n* (%)]	Positive (*n*=20) [*n* (%)]	χ^2	^{FE}P
Negative	18 (39.1)	6 (30.0)	0.502	0.479
Positive	28 (60.9)	14 (70.0)		

χ^2, *P*: χ^2 and P values for χ^2-test for comparing between the two groups. FE, Fisher exact for χ^2-test; RF, rheumatoid factor.

Table 4). Fourteen (70%) of *Pi*-positive cases (*n*=20) had positive RF, whereas 28 (60.9%) of *Pi*-negative cases (*n*=46) had positive RF. Comparing the two groups revealed no statistical significant difference (*P*=0.479; Table 5).

Eighty-two (82%) patients had positive serum ACPA (≥20 U/ml) with mean level of 296.01±500.02 U/ml. However, the mean gingival fluid ACPA level was 5.95 ±13.40 U/ml. PD was higher in ACPA-positive cases, as 54 (81.8%) of PD cases had positive serum ACPA with mean level of 254.66±458.15 U/ml in PD cases. ROC curve of GCF ACPA (Fig. 2) shows that GCF ACPA can significantly diagnose PD (area under the curve=0.730, *P*=0.001) at the cut-off level greater than 2.11 U/ml.

Figure 2



Receiver operating characteristic curve of gingival crevicular fluid anticitrullinated peptide protein antibodies (area under the curve=0.730*, *P*=0.001*). Periodontitis is diagnosed at the cut-off level greater than 2.11 U/ml.

Comparison between positive cases for the studied organisms regarding different disease parameters

In *Pg*-positive cases, 32 (80%) cases were positive for serum ACPA and 34 (85%) had positive CRP with mean GCF ACPA of 8.61±16.58 U/ml. In *Aa*-positive cases, 10 (100%) cases were positive for serum ACPA and had positive CRP, with mean GCF ACPA of 4.77 ±6.10 U/ml. In *Pi*-positive cases, 14 (70%) cases were positive to serum ACPA and 12 (60%) had positive CRP, with mean GCF ACPA of 1.38±1.29 U/ml. There was no statistical significant difference among

Table 6 Comparison between positive cases for the studied organisms regarding different disease parameters

	Pg (*n*=40) [*n* (%)]	*Aa* (*n*=10) [*n* (%)]	*Pi* (*n*=20) [*n* (%)]
Serum ACPA			
Negative	8 (20.0)	0 (0.0)	6 (30.0)
Positive	32 (80.0)	10 (100.0)	14 (70.0)
Significance between groups		P_1=0.184; P_2=0.519; P_3=0.074	
GCF ACPA (U/ml)			
Minimum–maximum	0.26–67.11	0.26–16.05	0.26–3.42
Mean±SD	8.61±16.58	4.77±6.10	1.38±1.29
Median	2.97	3.42	0.65
Significance between groups		P_1=0.807; P_2=0.047*; P_3=0.090	
DAS-28			
Low (<3.2)	2 (5.0)	0 (0.0)	0 (0.0)
Moderate (≥3.2–<5.2)	18 (45.0)	4 (40.0)	8 (40.0)
High (≥5.2)	20 (50.0)	6 (60.0)	12 (60.0)
Significance between groups		P_1=0.831; P_2=0.721; P_3=1.000	
HAQ score (0–3)			
No disability (0–1.25)	14 (35.0)	0 (0.0)	2 (10.0)
Mild disability (>1.25–1.75)	10 (25.0)	4 (40.0)	8 (40.0)
Moderate disability(>1.75–2)	4 (10.0)	0 (0.0)	0 (0.0)
Severe disability (>2)	12 (30.0)	6 (60.0)	10 (50.0)
Significance between groups		P_1=0.056; P_2=0.055; P_3=0.854	
CRP (<6 IU/ml)			
Negative	6 (15.0)	0 (0.0)	8 (40.0)
Positive	34 (85.0)	10 (100.0)	12 (60.0)
Significance between groups		P_1=0.327; P_2=0.051; P_3=0.029*	

Significance between organisms was done using Fisher exact or Monte-Carlo test. Aa, *Aggregatibacter actinomycetemcomitans*; ACPA, anticitrullinated protein antibodies; CRP, C-reactive protein; DAS, disease activity score; GCF, gingival crevicular fluid; HAQ score, health assessment questionnaire score; P_1, *P* value for comparing between *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*; P_2: *P* value for comparing between *Porphyromonas gingivalis* and *Prevotella intermedia*; P_3: *P* value for comparing between Aggregatibacter actinomycetemcomitans and Prevotella intermedia; Pg, *Porphyromonas gingivalis*; Pi, *Prevotella intermedia*. *$P \leq 0.05$, statistically significant.

the three studied organisms regarding serum ACPA level. *Aa*-positive cases were associated with positive CRP significantly higher than *Pi*-positive cases (*P*=0.029). No statistical significant difference was detected between *Pg*-positive and *Aa*-positive or *Pi*-positive cases. GCF ACPA was significantly higher in *Pg*-positive cases than *Pi*-positive cases (*P*=0.047). No significant difference was detected regarding GCF ACPA between *Pg*-positive cases and *Aa*-positive cases or between *Aa*-positive and *Pi*-positive cases (Table 6).

On comparing the DAS, in *Pg*-positive cases, 20 (50%) cases had high disease activity, 18 (45%) had moderate disease activity, and two (5%) had low disease activity. In *Aa*-positive cases, six (60%) cases had high disease activity, four (40%) had moderate disease activity, and none of them had low disease activity. In *Pi*-positive cases, 12 (60%) cases had high disease activity, eight (40%) had moderate disease activity, and none of them had low disease activity.

Regarding HAQ, in *Pg*-positive cases, 12 (30%) had severe functional disability, four (10%) had moderate disability, 10 (25%) had mild disability, and 14 (35%) had no disability. In *Aa*-positive cases, six (60%) had severe functional disability and four (40%) had mild disability. In *Pi*-positive cases, 10 (50%) had severe functional disability, eight (40%) had mild disability, and two (10%) had no disability. No statistical significant difference was detected between the cases of the three studied organisms regarding DAS-28 or HAQ.

According to modified-Larsen radiographic scoring systems used, 12 (12%) patients with RA showed either erosions or joint space narrowing by radiography, whereas 88 (88%) patients had normal radiography of the hands and wrists, with no evidence of erosion or joint space narrowing. In the group of patients with joint damage, eight (66.7%) patients had PD [of them, two (25%) patients had severe generalized PD] and four (33.3%) patients had chronic gingivitis. However, in the group of cases with normal radiography, 58 (65.9%) patients had PD [of them, 26 (44.8%) patients had severe generalized PD] and four (33.3%) patients had chronic gingivitis. No statistical significant difference was detected between the two groups regarding number of patients with PD (*P*=1.000) or number of severe generalized PD cases (*P*=0.451). Six (75%) patients with joint damage were positive for *Pg*,

Table 7 Radiographic findings with regard to periodontitis and periodontal microbiota

	Radiography findings		Test of significance	*P*
	Normal (*n*=58) [*n* (%)]	Combined erosion and JSN (*n*=8) [*n* (%)]		
Periodontitis (*n*=66)	58 (65.9)	8 (66.7)	χ^2=0.003	^{FE}P=1.000
Porphyromonas gingivalis				
Negative	24 (41.4)	2 (25.0)	χ^2=0.790	^{FE}P=0.464
Positive	34 (58.6)	6 (75.0)		
Aggregatibacter actinomycetemcomitans				
Negative	50 (86.2)	6 (75.0)	χ^2=0.687	^{FE}P=0.596
Positive	8 (13.8)	2 (25.0)		
Prevotella intermedia				
Negative	40 (69.0)	6 (75.0)	χ^2=0.121	^{FE}P=1.000
Positive	18 (31.0)	2 (25.0)		

FE, Fisher exact for χ^2-test; JSN, joint space narrowing.

two (25%) patients were positive to *Aa*, and two (25%) patients were positive to *Pi*. On the contrary, in the normal radiography group, 34 (58.6%) patients were positive for *Pg*, eight (13.8%) patients were positive for *Aa*, and 18 (31%) patients were positive for *Pi*. No statistical significant difference was detected between the two groups (normal and abnormal radiography groups) regarding the oral microbiota (Table 7).

Discussion

Many studies showed an increased prevalence of RA in patients with PD [25,26]. Others demonstrated the presence of RF in the gingiva, saliva, and serum of patients having PD [27,28].

Pg as an important periodontopathic bacterium has been implicated as a main factor in the immunopathogenesis of RA; it has been proposed that individuals with PD owing to *Pg* are exposed to antigens that will lead to the production of RF and local inflammation of both gingiva and synovium [29].

In the present study, *Pg* was more prevalent (60.6%) than *Aa* (15.2%) and *Pi* (30.3%) in PD cases. Presence of either *Pg*, *Aa*, or *Pi* was not associated with more prevalence of RF over its absence. No statistical significant difference between the three organisms was reflected on RA disease activity, functional disability, or serum ACPA positivity. *Aa* was associated with CRP positivity significantly higher than *Pi*. Moreover, no statistical significant difference was detected regarding the radiography findings. A possible explanation for the absence of *Pg* in the remaining 39.4% of patients with RA with PD is that periodontopathic bacteria could have been present before but were affected by treatments the patients were exposed to all through the course of their disease, that is why at the time of culture this bacterium was not detected.

Scher *et al.* [30] did not show any relationship between anti-Pg or *Pg* abundance and the presence of RA autoantibodies, including RF and ACPA, and concluded that overall exposure to *Pg* was not associated with high serum ACPA level. Furthermore, they found two other organisms, *Prevotella* spp. and *Leptotrichia* spp., were prevalent in new-onset RA. In the study by Ziebolz *et al.* [31], 11 periodontal pathogens in subgingival plaque profile from patients with RA were examined. There was no association between the presence or abundance of *Pg* with RF. On the contrary, Mikuls *et al.* [32] studied the anti-*Pg* antibodies and they demonstrated that anti-Pg titers were higher in RA than controls. They also showed that *Pg* level was associated with ACPA. However, they did not show any association between prior infection with *Pg* and established RA. However, in another study by Mikuls *et al.* [33], who examined serum ACPA and antibodies against three different periodontopathic bacteria, they showed that anti-*Pg* was associated with ACPA and RF. This relationship was seen with anti-*Pg* but not with the other oral bacteria tested, suggesting that *Pg* exposure was the main susceptibility factor in the development of RA antibodies, even in the preclinical arthritis.In our study, ACPA was detected in all examined GCF samples from PD cases, being of significant higher levels with *Pg* than *Pi*. No statistically significant difference was detected on comparing *Pg* with *Aa* or *Aa* with *Pi*. Interestingly, GCF ACPA can significantly diagnose PD (ROC curve) at the cut-off level greater than 2.11 U/ml. This may point to the origin of ACPA from the gingiva. This might indicate that citrullination of proteins occurs in gingival tissues during periodontal inflammation and raises the possibility that the periodontium may be an extra-articular site of citrullinated proteins. Furthermore, autoantibodies to these citrullinated proteins may be formed in the periodontium, eventually contributing to the RA disease process.

Okada *et al.* [13] studied relationships between anti-Pg and RA antibodies in Japanese patients with RA and they found that anti-*Pg* was associated with RF, but not with ACPA. In another study conducted by De Smit *et al.* [34], antibodies to *Pg* were associated with higher serum level of ACPAs targeting citrullinated fibrinogen.

Harvey *et al.* [35] in their study reported that citrullinated proteins and ACPA have been noted in inflamed periodontal tissues. They had excluded RA to rule out the possibility that the presence of citrullinated proteins was owing to ongoing RA. They stated that as none of the participants in their study had RA, so the presence of anti-CCP antibodies in the GCF of patients may indicate preclinical phase of RA.

Regarding *Aa*, Konig *et al.* [14] studied the periodontal microenvironment in patients with PD to define mechanisms underlying mucosal inflammation and autoimmunity in RA. Among the microbial species associated with periodontal disease, they identified *Aa* as the only pathogen with the ability to reproduce the repertoire of citrullinated antigens found in the RA joint. They concluded that *Aa* may be a primary oral microbe that can trigger autoimmunity in RA.

Our findings support the relationship between PD and infectious trigger at the gingival site and RA. Further studies in this field are recommended aiming, specifically to define the exact origin of ACPA, primarily to initiate preventive measures for this destructive disease.

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Conflicts of interest

There are no conflicts of interest.

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