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ORIGINAL ARTICLE

Elevated serum rheumatoid factor, anti-citrullinated protein antibodies and active rheumatoid arthritis disease are not associated with chronic periodontitis

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Abstract

Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease of the joints with the involvement of other systems. Previous studies have demonstrated its association with chronic periodontitis (CP), a chronic inflammatory disease of tooth-supporting tissues. Positive rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA) in RA patients have been found to be associated with CP. The aim of this study is to determine the prevalence of CP in RA patients, and to investigate the association of ACPA, RF status and RA disease activity with CP and non-CP RA patients. *Materials and Methods*: A comparative cross-sectional study involving 98 RA patients was conducted at Hospital Universiti Sains Malaysia, Kubang Kerian, Malaysia. Clinical oral examination was carried out to determine the CP status of RA patients. RF, ACPA and erythrocyte sedimentation rate (ESR) were measured, and the 28-joint Disease Activity Score (DAS-28) was assessed. *Results*: Forty-five patients (45.9%) were found to have CP (95% CI: 0.36-0.56). No significant difference was observed in the prevalence of positive RF (p=0.989) or ACPA (p=0.431) in CP and non-CP RA patients. There was also no significant association between active RA disease (DAS-28 score ≥3.2) and RF positivity in CP (p=0.927) and non-CP (p=0.431) RA patients as well as ACPA positivity in CP (p=0.780) and non-CP (p=0.611) RA patients. *Conclusion*: In our cohort of RA patients, we did not find significant associations between elevated RF, ACPA, or active RA disease with the presence of CP. There were also no significant associations between elevated RF or ACPA with active RA disease.

Keywords: Rheumatoid arthritis, chronic periodontitis, rheumatoid factor, anti-citrullinated protein antibodies, 28-joint Disease Activity Score.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, autoimmune inflammatory disease characterised by joint tenderness, swelling and destruction leading to severe disability and premature death.[1-3] In RA, susceptible individuals develop loss of tolerance to citrullinated proteins, leading to the formation of anti-citrullinated protein antibody (ACPA) in the synovia, and ultimately to the development of RA.[4-6]

Periodontitis, defined as chronic inflammation of tooth-supporting tissues, occurs in more than 20% of the human population that causes tooth loss in its severe form.[7] Approximately 5 to 15% of the adult population is affected by severe periodontitis.[8] The main pathogenic organism *Porphyromonas gingivalis* (*P. gingivalis*) alongside with *Tannerella forsythia* and *Treponema denticola* colonise the dental plaque, leading to chronic periodontitis (CP).[9,10] These organisms are collectively known as the "red complex" due to their strong association with the disease. *P. gingivalis* produces porphyromonas gingivalis peptidyl-arginine deiminase (PPAD),

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a unique bacterial enzyme that converts arginine residues in proteins to citrulline via a process called protein citrullination. These citrullinated proteins have been shown to predispose susceptible patients to develop autoantibodies which result in autoimmunity in RA patients.[10]

The prevalence of CP in RA patients ranged from 51 to 97.5% according to the following studies: i) Ranade *et al*. reported that CP occurred in 97.5% of RA patients with 12.5% and 75% comprising the mild and moderate form of CP, respectively; ii) Mercado *et al*. reported the prevalence of CP in RA patients was 62.5% where 90% of them had moderate to severe disease; iii) Dissick *et al*. reported 51% of RA patients had moderate to severe CP.[11-13]

The 2010 American College of Rheumatology/ European League Against Rheumatism (ACR-EULAR) diagnostic criteria is currently used to diagnose the disease.[1] Rheumatoid factor and anti-citrullinated protein antibody (ACPA) such as anti-cyclic citrullinated peptide (CCP) are the most established laboratory marker for RA diagnosis.[14] Anti-citrullinated protein antibody is present in various phases of RA disease, from the preclinical stage to the established disease. Several studies identified that ACPA was present in the serum of 34-40% of RA patients, 12-14 years prior to the onset of RA and the detection was associated with patient's age.[15-18] ACPA test has evolved and shown to be a more sensitive and specific marker of RA compared to RF.[19-22]

Previous studies have shown that RA is conversely associated with periodontitis.[23-25] Induction of experimental arthritis in lab rats has resulted in the destruction of periodontal tissues and an increase in cytokines and matrix metalloproteinases in periodontal tissues.[26] In view of the interrelation between these two diseases, it has been postulated that there is an association between RA severity and periodontal status.

Khantisopon *et al*. in 2014 attempted to establish the association between RA and CP in 196 RA patients in Thailand.[27] Elderly male, with previous or current smoking habit and high plaque scores were found to be associated with severe periodontal diseases. However, no further associations were found for other parameters including the number of tender and swollen joints, ESR, the presence of rheumatoid factor (RF), hand x-ray, Disease Activity Index (DAS) and health status using the Thai Health Assessment Questionnaire (HAQ). The study by Dissick *et al*. also did not find the association between RA disease activity and periodontal status.[13]

The previous study demonstrated that CP was associated with RA as well as various systemic diseases such as diabetes mellitus and cardiovascular disease.[10] However, the associations of CP with RF, ACPA and RA disease activity have remained inconclusive. Thus, in this study, we set out to determine the prevalence of CP in RA patients, and to examine the association of RF, ACPA and RA disease activity with CP and non-CP RA patients.

MATERIALS AND METHODS

Patients and data collection

We screened a total of 138 RA patients who attended the Rheumatology Clinic at Hospital Universiti Sains Malaysia (USM) between June 2016 until December 2017. Patients were considered eligible for the study if they were 20 to 80 years old who fulfilled the 2010 ACR/ EULAR RA classification criteria.[1] Patients with the following features were excluded: edentulous, uncontrolled systemic diseases (*e.g*. systemic lupus erythematosus, diabetes mellitus, and blood disorders), diseases associated with inflammatory arthritis (*e.g*. psoriatic arthritis, osteoarthritis, and gout), smokers, pregnant, and on medication that affects periodontal tissues (*e.g*. phenytoin, cyclosporine, oral contraceptive pills, and nifedipine).

Clinico-demographic data of the patients were obtained during screening. RA disease activity was assessed using the 28-joint Disease Activity Score (DAS-28) score.[28] A DAS-28 score of greater than 3.2 implies active disease, while less than or equal to 3.2 indicates low disease activity. This study was approved by the ethics committee of Universiti Sains Malaysia (USM/ JEPeM/16030138).

Immunoassays

RF was assayed using RF Direct Latex Test according to the manufacturer's instructions (VEDALAB, Cerisé, France). RF value of more than 8 IU/mL was considered positive. ACPA level was measured using AESKULISA CCP Enzyme-Linked Immunosorbent Assay (ELISA) test (AESKU DIAGNOSTICS, GmbH & Co, Wendelsheim, Germany). A test value of more than 18 U/ml was considered positive.

Assessment of chronic periodontitis

Probing pocket depth (PPD) and clinical

attachment level (CAL) were the parameters used to determine the periodontal status. The PPD was measured from the gingival margin to the base of the pocket, and the CAL was measured from the cemento-enamel junction (CEJ) to the base of the pocket by using the periodontal probe (William's Probe). The measurements were done on six sites of each tooth (disto-buccal, mid-buccal, mesio-buccal, disto-lingual, mid-lingual, and mesio-lingua) excluding third molars. Those with PPD and CAL of more than 3 mm were considered as having CP.[29]

Statistical analysis

Data was analysed using IBM SPSS Statistics Version 22.0 (IBM, Armonk, NY, USA). Descriptive statistics of the variables were determined including frequency, percentage, and 95% confidence interval (CI). Chi-square tests were performed to determine the significance of the differences and associations between categorical variables. Two-tailed $p<0.05$ was considered as significant.

RESULTS

Clinico-demographic characteristics

A total of 138 patients were screened during the study duration and 98 patients fulfilled all inclusion and exclusion criteria, and hence were invited to participate in the study (Table 1). The majority of patients were female (85%; n=83/98). In terms of race, the majority of patients were Malay (87%; n=85/98), followed by Chinese (9%; n=9/98), Indian (2%; n=2/98), and Siamese (2%; n=2/98). Fourteen percent (n=15/98) of patients had hypertension, followed by hyperlipidemia (3%; n=3/98), asthma (1%; n=1/98), and hyperthyroidism (1%; n=1/98). Five patients had missing RF data, six patients had missing ACPA data and three had missing DAS-28 scores.

The association of RF, ACPA and disease activity with CP and non-CP RA patients

Forty percent (n=39/93) and 53% (n=52/92) of patients were positive for RF and ACPA, respectively. Forty-six percent (n=45/98) of the RA patients had CP (95% CI: 0.36–0.56) (Table 2). No significant association was found between RF positivity and CP (p=0.989), ACPA positivity and CP (p=0.431) as well as RA disease activity and CP (p=0.458) (Table 3).

The association of RF status and RA disease activity with CP and non-CP RA patients

Thirty-nine percent (n=7/18) of RF-positive RA patients with CP had active RA disease. No significant association was found between active RA disease and positive RF status in RA patients with CP (p=0.927). Among non-CP RA patients with positive RF, 25% (n=5/20) had active RA disease. No significant association was found between active RA disease and positive RF status in RA patients without CP (p=0.430) (Table 4).

TABLE 1: Sociodemographic characteristics and comorbidities of participants (n= 98)

Characteristics	Frequency (%)
Age group (years)	
20-29	1 (1.0)
30-39	20 (20.4)
40-49	15 (15.3)
50-59	28 (28.6)
60-69	25 (25.5)
70-79	8 (8.2)
80-89	1 (1)
Gender	
Male	15 (15.3)
Female	83 (84.7)
Race	
Malay	85 (87)
Chinese	9 (9.2)
Indian	2 (2.0)
Siamese	2 (2.0)
Comorbidities	
Hypertension	14 (14.3)
Asthma	1 (1)
Hyperlipidemia	1 (1)
Hyperthyroidism	1 (1)

The association of RF, ACPA and disease activity with CP and non-CP RA patients

TABLE 2: Prevalence of CP in RA patients (n=98)

Variables	RA patients; Frequency (%)	95% CI
CP	45 (45.9%)	0.36-0.56
Non-CP	53 (54.1%)	0.44-0.64

TABLE 3: Association of RF, ACPA and RA disease activity with CP and non-CP RA patients

Variables	CP; Frequency (%)	Non-CP; Frequency (%)	χ^2 statistics (df)	*p*-value
RF status				
Positive (n=39)	18 (46.2)	21 (53.8)	0.000 (1)	0.989
Negative (n=54)	25 (46.3)	29 (53.7)		
ACPA status				
Positive (n=52)	23 (44.2)	29 (55.8)	0.620(1)	0.431
Negative (n=40)	21 (52.5)	19 (47.5)		
Disease activity (DAS-28 score)				
Moderate to High (>3.2; n=33)	17 (38.6)	16 (31.4)	0.550 (1)	0.458
Low (≤3.2; n=62)	27 (61.4)	35 (68.6)		

The association of ACPA status and RA disease activity with CP and non-CP RA patients

Thirty-nine percent (n=9/23) of RA patients with CP and positive ACPA had active RA disease. No significant association was found between active RA disease and positive ACPA status in RA patients with CP (*p*=0.780). Among non-CP RA patients with positive ACPA, 33% (n=9/27) had active RA disease. No significant association was found between active RA disease and positive ACPA status in RA patients without CP (p=0.611) (Table 5).

DISCUSSION

Our study showed that there were no significant associations between elevated RF, ACPA or RA disease activity with the presence of CP. These findings differed with previous studies. Dissick *et al*. reported that there was a significantly higher prevalence of moderate to severe periodontitis in patients with positive RF or ACPA status compared to those negative for RA or ACPA.[13] An independent study by Hendler *et al*. also reported that elevated ACPA level was found in RA patients with aggressive periodontitis.[30] However, a study by Khantisopon *et al*. reported that there was no significant association between RA disease activity and CP status in RA patients.[27]

There were also no significant associations between elevated RF or ACPA with RA disease activity in CP and non-CP RA patients. To the

TABLE 4: RA disease activity and RF status in RA patients with CP (n=42) and without CP (n=48)

Variables	RF positive; Frequency (%)	RF negative; Frequency (%)	χ^2 statistics (df)	*p*-value
Disease activity with CP (DAS-28 score)				
Moderate – High (>3.2)	7 (43.8)	9 (56.3)	0.008 (1)	0.927
Low (≤3.2)	11(42.3)	15 (57.7)		
Disease activity without CP (DAS-28 score)				
Moderate – High (>3.2)	5 (33.3)	10 (66.7)	0.623 (1)	0.430
Low (≤3.2)	15 (45.5)	18 (54.5)		

TABLE 5: RA disease activity and ACPA status in RA patients with CP (n=43) and without CP (n=46)

Variables	ACPA positive; Frequency (%)	ACPA negative; Frequency (%)	χ^2 statistics (df)	*p*-value
Disease activity with CP (DAS-28 score)				
Moderate – High (>3.2)	9 (56.3)	7 (43.8)	0.078 (1)	0.78
Low (≤3.2)	14 (51.9)	13 (48.1)		
Disease activity without CP (DAS-28 score)				
Moderate – High (>3.2)	9 (64.3)	5 (35.7)	0.259 (1)	0.611
Low (≤3.2)	18 (56.3)	14 (43.8)		

best of our knowledge, our current study is the first to explore these associations.

Most of our patients were of Malay ethnic, reflecting the local population of the Kelantan state in Malaysia where Malays consist of 85% of the population. Approximately half of our RA patients had CP (46%) and this finding was comparable with the results from other studies as follows: Mercado *et al*. (n=35/56; 62.5%), Ranade *et al*. (n=39/40; 97.5%), Khantisopon *et al*. (n=194/196; 99%), Dissick *et al*. (n=35/69; 50.7%).[11,13,24,27] Dissick *et al*. reported higher proportion of male participants (82%), reflecting their study population of the United States Army Veterans.[13] In contrast to our study, 85% of our patients were female, similar to the study by Khantisopon *et al*. (87%).[27]

Limitations and conclusions

We acknowledge the limitations of our study as follows: (i) Considerable number of edentulous RA patients were present in our cohort (n=35/138; 25.4%), and majority of them were above 50 years old. However, we were unable to conclude whether CP was the cause of complete teeth loss in these patients, and the age of edentulous onset in RA patients was unclear; (ii) Relatively small sample size (n=98) partly due to incomplete clinico-demographical data and a number of edentulous RA patients that were not included in our main analyses.

In conclusion, our study suggests that status of RF, ACPA and disease activity are independent of the presence or absence of CP in RA patients. Nevertheless, future studies in independent cohort of RA patients and with larger number of patients are needed to establish the robustness of the associations explored in our study. Investigations on the prevalence of edentulous RA patients and the predisposing factors are also recommended.

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Authors' contributions

W.S.W.G., W.M.W.M and N.S developed the study design. M.J.C.R. engaged in collecting data, summarized the literature and drafted the manuscript. W.S.W.G., W.M.W.M, N.S., W.Z.W.A, H.T and K.K.W. revised and critically edited the manuscript. All authors have read and approved the final version of the manuscript.

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