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Original article

Oral status in patients with early rheumatoid arthritis: a prospective, case–control study

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Abstract

Objective. Patients with RA suffer from a higher risk of periodontal attachment loss and increased oral inflammation. We hypothesize that there are pathogenetic and immunological interactions between these diseases that go beyond impaired manual dexterity accompanying advanced RA. The primary objective of the present study was to determine whether a loss of alveolar bone can be detected in RA patients during the early course of the disease.

Methods. In this cross-sectional, epidemiological case–control study, 22 patients with early RA (ERA) were compared with 22 matched healthy controls. Oral and periodontal status, clinical activity, and socio-demographic parameters were determined. Oral microbiota were analysed using real-time quantitative PCR specific for leading oral pathogens.

Results. More advanced forms of periodontitis were found in ERA patients compared with controls. ERA patients had a greater number of missing teeth [ERA 5.7 (S.D. 5.0), controls 1.9 (S.D. 1.0), $P = 0.002$], deeper periodontal pockets [clinical attachment level: ERA 3.4 (S.D. 0.5 mm), controls 2.7 (S.D. 0.3 mm), $P < 0.000$], and greater bleeding on probing [ERA 18.6% (S.D. 9.0%), controls 10.5% (S.D. 5.1%), $P = 0.001$] despite comparable oral hygiene. *Tannerella forsythia* (6.77-fold, $P = 0.033$) subgingivally and *Streptococcus anginosus* (3.56-fold, $P = 0.028$) supragingivally were the characteristic pathogens in ERA.

Conclusion. Increased loss of periodontal attachment and alveolar bone can be detected in patients with ERA, therefore we propose that the consulting rheumatologists inform the patients that they have a higher risk of periodontal disease. It would be beneficial if these patients were referred directly for intensive dental care.

Key words: periodontal attachment loss, rheumatoid arthritis, case–control studies, oral hygiene, periodontal index, plaque index.

CLINICAL SCIENCE

Introduction

RA and periodontal disease (PD) are chronic inflammatory diseases that share striking similarities [1]. In both entities, proinflammatory cytokines such as IL-1β, IL-6 and TNF-α are up-regulated, promoting inflammation of soft tissue and destruction of bone. Patients with established RA have a greater prevalence of PD [2–5]. Furthermore, patients with PD have a greater risk of various systemic inflammatory diseases such as atherosclerosis, diabetes mellitus and RA [6, 7]. In recent studies, biologic DMARDs used in the treatment of RA, such as TNF and IL-6 inhibitors, not only improved signs and symptoms, but also inhibited the radiological progression of both RA and PD [8–10]. Whereas infection with the leading periodontal pathogens such as *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* is believed to cause and perpetuate PD [11], the aetiology of RA, because of its complexity, is still unclear. Genetic susceptibility and environmental factors such as smoking and distinct oral and intestinal microflora may act in concert to modulate the immune

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system. *P. gingivalis* infection of the oral cavity is common in both healthy and PD patients. Besides its high proteolytic activity and set of virulence factors that promote persistence, *P. gingivalis* is the only known periodontal pathogen that expresses bacterial peptidyldeaminase (PPAD). Recently PPAD has been shown to citrullinate other proteins, driving the generation of ACPAs as well as citrullinating itself [12, 13]. Thus PPAD, as a bacterial protein, adds to the ACPAs, possibly helping to break immunological tolerance in RA [14].

The primary objective of the present study was to determine whether a clinically significant loss of alveolar bone in terms of the clinical attachment level (CAL) was detectable in patients with early RA (ERA) compared with healthy controls. The secondary objective was to determine whether distinct oral pathogens were associated with ERA.

Materials and methods

This cross-sectional epidemiological case–control study involved a total of 44 subjects ($n = 44$). All RA subjects were recruited consecutively from the Division of Rheumatology, University Clinic of Heidelberg, and a rheumatology outpatient practice. Control subjects were first-time patients who were recruited consecutively from the Department of Conservative Dentistry, University of Heidelberg. Medical history (chart review and interview), diet, smoking, alcohol consumption and current medications were determined. Results of musculoskeletal exams and laboratory assessments were also documented. Patients with ERA formed the case group ($n = 22$). Healthy controls ($n = 22$) were statistically matched according to gender, age (30–44, 45–54, 55–64, 65–75 years) and smoking status (smoker, previous smoker, non-smoker).

The study was approved by the Ethics Committee of the Medical Faculty, University of Heidelberg (S-401/2009). Written informed consent was obtained from all subjects prior to enrolment. All subjects who met the study criteria were offered enrolment.

Inclusion criteria

RA patients met the 2010 ACR/European League Against Rheumatism (EULAR) criteria for RA [15]. Patients had to have active RA, defined as having a 28-joint DAS (DAS28) >3.2, and ERA, defined as the onset of symptoms <2 years prior. Patients had to be otherwise healthy. All patients were Caucasian and were between 30 and 75 years old.

Exclusion criteria

Exclusion criteria were previous or current therapy with biological DMARDs, poor oral hygiene or disabilities that interfere with adequate oral hygiene, periodontitis as a manifestation of systemic disease, periodontal therapy within the past 5 years and professional tooth cleaning, intake of antibiotics, or pregnant or nursing during the past 6 months.

Rheumatologic assessment

The DAS28 score system based on the ESR was used to assess rheumatoid disease activity in the RA subjects. Most ERA subjects (63.6%, $n = 14$) were given DMARDs and corticosteroids, one-third (31.8%, $n = 7$) received corticosteroids alone and one patient received DMARDs alone. Proton pump inhibitors were used in four patients (18.2%) at the time of enrolment. Control subjects did not receive any medication.

Oral assessment

Oral examinations were performed at the Department of Conservative Dentistry, University of Heidelberg. The number of decayed, missing and filled teeth (DMF-T) [16], gingival bleeding index (GBI) [17] and plaque index (PI) [18] were recorded. Periodontal status, including the CAL, probing pocket depth (PPD) and bleeding on probing (BOP), was assessed at six sites per tooth by an experienced examiner who was calibrated and trained prior to the investigation. Disease status was defined according to Armitage [19] as generalized mild, generalized mild localized moderate, generalized mild localized severe, generalized moderate, generalized moderate localized severe and generalized severe periodontitis.

Microbiological assessment

Samples of oral biofilm were assessed from supragingival and subgingival sites. Supragingival biofilm was swiped off the tooth surfaces with sterile curettes or paper points. Subgingival biofilm was extracted by inserting a sterile paper point into deep, active periodontal pockets (BOP positive) for 20 s. From each patient three supragingival and three subgingival samples were taken and pooled, respectively, by transferring them to 1.5 ml sterile tubes. Samples were immediately frozen (−25°C) until further analysis. Bacterial DNA was isolated and amplified following published protocols [20, 21]. Samples were analysed using a quantitative real-time PCR (RQ-PCR) assay screening for 43 health- and caries-associated bacteria [22]. Additionally, periodontal PCR primers for nine leading periodontal pathogens [23, 24] were added to the PCR assay. Bacterial DNA was isolated following published protocols [21, 25] and by using a modification of the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Since the amount of bacterial DNA from clinical sampling is rarely sufficient for molecular-based analysis, 50 ng of DNA was amplified according to a published protocol [20] and subsequently purified using the Marligen PCR Purification Kit (Marligen Biosciences, Ijamsville, MD, USA) according to the manufacturer's protocol.

The amount of isolated or amplified DNA used as input DNA for RQ-PCR was quantified spectrophotometrically. RQ-PCR was performed in a reaction volume of 12 μl containing 1× SYBR Green PCR Master Mix (ABsolute QPCR SYBR Green Mix, ABgene, Hamburg, Germany), 0.33 μm specific primer and 3 ng of isolated template DNA on an ABI PRISM 7900 Sequence Detection System (ABI 7900 HT, SDS 2.2 AbiPrism, Applied Biosystems, Foster City, CA, USA) in 384-well PCR plates (Thermo Scientific,

ABgene, Hamburg, Germany). The reaction conditions were 95°C for 15 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Data analysis was conducted with Sequence Detection Software version 2.2.2 (Applied Biosystems).

Statistics

All data were analysed using GraphPad InStat version 3.0 (GraphPad, San Diego, CA, USA) and GraphPad Prism version 5.0 as statistical software. An unpaired *t*-test with Welch correction and the Mann–Whitney *U*-test were applied for independent samples to test the significance of quantitative data. The association of categorical variables was assessed by the chi-square test. Statistical significance was declared if the *P*-value was < 0.05. Analysis of the relative abundance and hierarchical cluster analysis of oral bacteria were performed using DataAssist 3.0 (Applied Biosystems, Darmstadt, Germany). Differences of at least 2.5 in the abundance of pathogens in RQ-PCR were considered relevant.

Results

Advanced forms of periodontitis found in ERA

Of the 22 ERA subjects included in this study, 68% were female, with a mean age of 51.7 years. The mean disease duration was 5.9 months, the mean DAS28 was 4.55 and the mean of the HAQ was 0.79. Thirty-seven per cent of the patients were IgM-RF positive, with a mean titre of 60 IU/l. Fourteen per cent of ERA subjects were current smokers (Table 1). All ERA subjects had moderate to severe forms of PD. Most ERA patients ($n = 15$) had moderate-severe PD. Three ERA patients displayed severe PD. Therefore, according to Armitage [19], all ERA patients had a diagnosis of PD. In the healthy controls, the majority ($n = 15$) had mild-moderate or moderate PD (Fig. 1).

Loss of CAL in ERA

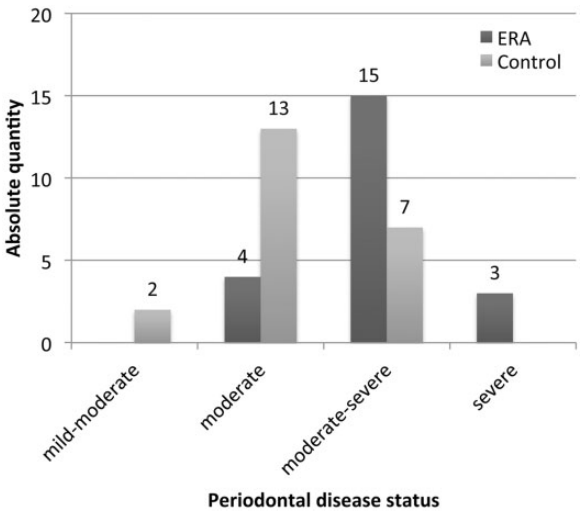
Although the DMF-T indexes of the ERA [17.6 (S.D. 6.4)] and control group patients [15.5 (S.D. 4.8)] were comparable, there was a significantly higher number of missing teeth in the ERA group [5.7 (S.D. 5.0)] compared with the healthy controls [1.9 (S.D. 1.0), $P = 0.002$]. The GBI was significantly higher in ERA [median 19.5% (95% CI 16.9, 28.7)] compared with controls [12.5% (95% CI 11.3, 21.0), $P = 0.022$]. The PI as a measure of oral hygiene was comparable between ERA [33.6% (S.D. 13.0%)] and controls [30.8% (S.D. 11.1%), $P = 0.445$]. BOP, indicating inflammation of the gingiva and periodontal attachment, was higher in the ERA group [18.6% (S.D. 9.0%)] than in the control group [10.5 (S.D. 5.1%), $P = 0.001$]. Also, PPD was significantly higher in the ERA group [2.9 (S.D. 0.4) mm] compared with the control group [2.4 (S.D. 0.3) mm, $P < 0.0001$]. The primary endpoint of the study—which was met—was to measure a clinically meaningful difference in the CAL between the ERA and control group. The difference in the CAL between the ERA group [3.4 (S.D. 0.5) mm] and the control group [2.7 (S.D.

Table 1 Demographic characteristics, data on ERA patients and controls

Parameter	ERA ($n = 22$)	Controls ($n = 22$)
Age, mean (S.D.), years[a]	51.7 (9.7)	51.9 (9.6)
Gender, female[a]	15	
Disease duration, mean (S.D.), months	5.9 (3.1)	
DAS28, mean (S.D.)	4.55 (1.1)	
HAQ, mean (S.D.)	0.78 (0.58)	
ESR, mean, mm/h	22.1	
CRP, mean, mg/l	10.8	
IgM-RF positive, %	37	
ACPA positive, %	41	
Smoker[a]		
Yes	3	
No	9	
In the past	10	
BMI		
< 25	7	9
25–30	10	10
> 30	5	3
Educational status		
Low	2	0
Medium	18	16
High	2	6
Consumption of alcohol		
Little	19	18
Moderate	2	4
Frequent	1	0

[a]Variables were matched between ERA patients and controls.

Fig. 1 Periodontal disease status in ERA patients and controls.



PD status classification according to Armitage [19].

Table 2 Oral and periodontal status, data on ERA patients and controls

Parameter	ERA	Controls	*P*-value
DMF-T[a]	17.6 (6.4)	15.5 (4.8)	0.216
Decayed[a]	0.9 (2.1)	0.8 (0.7)	0.932
Missing[a]	5.7 (5.0)	1.9 (1.0)	0.002
Filled[a]	11.5 (4.9)	12.7 (1.1)	0.405
GBI, %[b]	19.5 (16.9, 28.7)	12.5 (11.3, 21.0)	0.022
PI, %[a]	33.6 (13.0)	30.8 (11.1)	0.445
BOP, %[a]	18.6 (9.0)	10.5 (5.1)	0.001
PPD, mm[a]	2.9 (0.4)	2.4 (0.3)	<0.000
CAL, mm[a]	3.4 (0.5)	2.7 (0.3)	<0.000

[a]Parametric data, unpaired *t*-test with Welch correction, mean (S.D.). [b]Non-parametric data, Mann–Whitney *U*-test, median (95% CI).

Table 3 Microbiological data and quantitative real-time PCR measurements of abundance of subgingival microbiota in ERA patients and controls

Species	Relative quantity in ERA patients and controls	*P*-value
Fusobacteria species	0.33	0.036
Lactobacillus gasseri	0.19	0.010
Lactobacillus species	0.23	0.016
Leptotrichia species	0.34	0.041
Streptococcus gordonii	0.27	0.014
Tannerella forsythia	6.77	0.033

0.31) mm] was 0.7 mm ($P < 0.0001$). A group difference $\geqslant 0.5$ mm was considered clinically meaningful (Table 2).

Sub- and supragingival ERA microbiota compared with healthy controls

Subgingival microflora in ERA vs control group

T. forsythia (6.77-fold, $P = 0.033$) was the only enriched bacterium in ERA. *Fusobacteria* species 1 (0.33-fold, $P = 0.036$), *Lactobacillus gasseri* (0.19-fold, $P = 0.01$), *Lactobacillus* species 4 (0.23-fold, $P = 0.016$), *Leptotrichia* species (0.34-fold, $P = 0.041$) and *Streptococcus gordonii* (0.27-fold, $P = 0.014$) were underrepresented in ERA compared with the control group (Table 3).

Supragingival microflora in ERA vs control group

S. anginosus (3.56-fold, $P = 0.028$) was the only bacterium more abundant in ERA than in healthy subjects. *Rothia dentocariosa* (0.15-fold, $P = 0.006$), *Actinomyces* species 2 (0.19-fold, $P = 0.049$) and *Corynebacterium durum* (0.17-fold, $P = 0.040$) were underrepresented in ERA supragingivally (Table 4).

Table 4 Microbiological data and quantitative real-time PCR measurements of abundance of supragingival microbiota in ERA patients and controls

Species	Relative quantity in ERA patients and controls	*P*-value
Rothia dentocariosa	0.15	0.006
Actinomyces species	0.19	0.049
Corynebacterium durum	0.17	0.040
Streptococcus anginosus	3.56	0.028

Discussion

We showed that in a Caucasian ERA cohort with a mean disease duration of <6 months, a clinically significant loss of CAL is detectable compared with a matched healthy control cohort. All other important periodontal parameters, such as GBI, BOP, number of missing teeth and PPD, were also significantly elevated in ERA. With a mean of 5.7, the number of missing teeth in ERA patients was also considerably greater than in the average German population of comparable age, with a mean of 2.4 missing teeth (status 2005 [26]). Despite the high prevalence of PD in our healthy control cohort (90%), more advanced forms of PD were observed in the ERA cohort, leading to a diagnosis of PD in all ERA patients. A number of epidemiological studies showed higher odds ratios of PD prevalence in established RA patients [2, 4], however, there are few studies on ERA [27]. Our study strengthens the association of RA and PD in Caucasians. Results are in line with other recent case–control studies in American and Indian ERA/PD patients [5, 27, 28]. Despite similar baseline demographics, the prevalence of PD and CALs measured in patients both in our ERA cohort and in our healthy control cohort were substantially higher than those in the other case–control ERA/PD studies. This might be explained by different methods of measuring, different health care plans or regional differences. This difference, however, underlines the importance of further studies in different ethnic groups. Nevertheless, the prevalence of at least moderate forms of PD in our healthy cohort (90%) was consistent with the expected high prevalence of PD in the age-matched German population [26]. In our ERA cohort, the prevalence of PD was 100% and more advanced forms of PD were already present. PD was graded as severe in three ERA patients (none in the control) and moderate to severe in 16 ERA patients (seven in the control). As people with long-standing RA often suffer from impaired function of the hands, elbows and shoulders, it was suggested that this impairment leads to poor oral hygiene, which in turn explains the increased PD in RA patients [4]. To address this bias, we selected RA patients with short disease duration. Also, ERA patients had to be naive to biologic DMARDs—potent suppressors of inflammatory and erosive processes that would have suppressed the effects of the underlying

diseases. It was recently shown that anti-IL-6R and anti-TNF treatment of RA significantly improved the main periodontal parameters, such as CAL, compared with anti-rheumatic treatment without biologic DMARDs [29]. Because the PI, as a parameter of oral hygiene, was comparable between the groups, it seems unlikely that manual impairment explains the more advanced forms of PD in the ERA cohort.

It is hypothesized that oral pathogens trigger not only the pathogenesis and perpetuation of PD, but also RA. Recently it was shown that *P. gingivalis* peptidyl arginine deiminase acts not only as an enzyme to citrullinate other proteins to generate ACPAs, but also serves to autocitrullinate as an antigen itself [12, 13]. We therefore tried to determine whether the most common oral pathogens were associated with ERA. In subgingival plaque samples of ERA, there was a trend to enrich *P. gingivalis* (2-fold, $P = 0.08$); however, *P. gingivalis* infection was common in both cohorts. *T. forsythia* and *S. anginosus* were the only enriched bacteria in our ERA cohort. *T. forsythia* is a strictly anaerobic, gram-negative bacterium belonging to the red complex of bacteria [30]. It is associated with both severe and chronic periodontitis. The enrichment seen for these pathogens in ERA might be explained as an effect of either an advanced form of PD seen in ERA [27], the anti-rheumatic medication or a different oral milieu caused by RA. Given that in our study PD predisposed RA, another explanation could be that these pathogens are not only causing PD, but also acting as environmental factors causing or aggravating RA.

The major limitations of our study are that (i) patients with RA received anti-rheumatic medication that might have affected the oral microbiota, although current and previous therapy with biologic DMARDs was an exclusion criterion, and (ii) the limited patient number did not allow analysis of PD severity as a factor of variance.

In conclusion, patients with ERA should be informed by their consulting rheumatologist that there is a high risk of experiencing PD. Following the diagnosis of ERA, it is recommended that patients be referred to a dental specialist to provide intensive dental care. Whether this intensified dental care will also impact the course of RA is currently under investigation.

Rheumatology key messages

- Increased alveolar bone and periodontal attachment loss can be detected in patients with ERA.
- Intensified dental care might help to minimize mutual inflammatory inducement of periodontal disease and RA.

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