

EXTENDED REPORT

The association between periodontal disease and joint destruction in rheumatoid arthritis extends the link between the HLA-DR shared epitope and severity of bone destruction

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Objective: To evaluate a possible association between wrist and periodontal destruction in rheumatoid arthritis, and between periodontal destruction, dry mouth, and labial salivary gland biopsy and the contribution of genetic factors (the shared epitope (SE) and IL1B (+3954) or TNFA (−238 or −308) gene polymorphisms).

Methods: 147 patients with rheumatoid arthritis were enrolled. Periodontal damage was defined according to the Hugoson and Jordan criteria on panoramic dental x rays. Typing for the SE and cytokine polymorphisms was undertaken by enzyme linked oligosorbent assay. Odds ratios (OR), relative risk (RR), and χ^2 values were calculated to quantify associations.

Results: An association was observed between wrist and periodontal bone destruction ($\chi^2=11.82$; $p<0.001$): 63 patients had both wrist and periodontal destruction, 31 had wrist destruction alone, 20 had periodontal destruction alone, and 33 had no destruction at either site. An association was seen between a positive labial salivary gland biopsy and periodontal bone destruction (RR=2.73 (95% CI, 1.35 to 5.51), $p<0.01$, $n=41$) or wrist bone destruction (RR=4.52 (1.96 to 10.45), $p<0.001$, $n=41$). The SE was associated with wrist bone destruction (OR=2.5 (1.16 to 5.42), $p<0.05$) and periodontal bone destruction (OR=2.2 (1.04 to 4.84), $p<0.05$). No association was found between the selected cytokine polymorphisms and bone destruction.

Conclusions: A strong association was found between wrist and periodontal bone destruction. The destruction risk was further increased in patients with sicca syndrome. The SE appears to be a severity genetic marker for both wrist and periodontal bone destruction.

Rheumatoid arthritis is a chronic inflammatory joint disease with joint and bone destruction, affecting mainly the distal joints. In addition some patients have been found to be at high risk of periodontal disease,¹ thus suggesting that this could be a localised form of rheumatoid destruction. As periodontal disease can occur alone, our study was aimed at quantifying this feature in rheumatoid patients and examining a possible association between the two disorders.

Rheumatoid arthritis susceptibility and severity have been associated with genetic markers. The main genetic marker is carried by the highly polymorphic HLA-DRB1 locus. Some alleles coding for a conserved linear sequence of amino acids in the DR β 1 chain of the HLA-DR α / β heterodimer between amino acids 67 and 74 (QKRAA in *0401, QRRRAA in *0404, *0405, and *0101, and RRRRAA in *1001), are referred to as the "shared epitope" (SE).²

Cytokines such as interleukin 1 (IL1) and tumour necrosis factor α (TNF α) are key mediators of the inflammation which induces bone and joint destruction in rheumatoid arthritis. In some studies, some of the gene polymorphisms have been associated with joint destruction.^{3,4}

Periodontal disease is triggered by bacteria, predominantly Gram negative anaerobes, which produce inflammatory mediators leading to the destruction of connective gingival tissue and dental alveolar bone. However, as for rheumatoid arthritis, in the same overall population a similar association was described between the HLA-DRB1 subclasses encoded for the same SE and periodontal disease. In addition, TNF α and IL1 are key mediators of the inflammatory process and the

same cytokine gene polymorphisms have been associated with severe periodontal disease,^{5,6} although such results remain controversial. A case-control study had described an association between the SE and a rapidly progressive periodontal disease.⁷

In the present study we investigated an association between bone destruction in joint and periodontal sites in patients with rheumatoid arthritis. Second, we looked for an association between the SE and joint destruction to determine if the SE may also be associated with periodontal destruction. A possible association with bone destruction and a sicca syndrome was also evaluated. The results indicate that the SE is a severity marker for both wrist and periodontal bone destruction in rheumatoid arthritis.

METHODS

Patients

The protocol was approved by the local clinical ethics committee and all patients gave their written informed consent for the genetic analysis. From July 2002 to July 2003, 147 patients who met the American College of Rheumatology 1987 criteria for the diagnosis of rheumatoid arthritis were enrolled in the study. All patients were resident in the same geographical area and were followed at three different French university hospitals (Edouard Héribert Hospital, Lyon; Grenoble Hospital, Grenoble; and Saint-Etienne Hospital,

Abbreviations: DAS 28, 28 joint disease activity score; RF, rheumatoid factor; SE, shared epitope; SNP, single nucleotide polymorphism

Table 1 Association between rheumatoid arthritis associated variables and the presence of wrist or periodontal destruction

Variable	Periodontal destruction		p Value	Wrist destruction		p Value
	Yes (n=83)	No (n=64)		Yes (n=94)	No (n=53)	
Age (years)	51.9 (13.6)	55.9 (9.6)	NS	52.7 (12.8)	54.2 (10.9)	NS
Sex (% female)	84.6%	75.6%	NS	80.8%	75.6%	NS
Disease duration (years)	7.9 (7.2)	6.9 (6.7)	NS	7.8 (6.8)	7.3 (7.0)	NS
Tender joint count	9.1 (7.3)	7.4 (6.4)	NS	9.1 (7.1)	6.6 (6.1)	0.04
Swollen joint count	3.6 (4.0)	2.7 (3.1)	NS	3.9 (4.0)	1.8 (2.5)	0.0007
ESR (mm/h)	30.4 (21.2)	34.9 (25.4)	NS	36.6 (24.8)	26.8 (20.7)	0.01
Patient's global assessment	44.6 (21.2)	43.7 (21.1)	NS	46.2 (19.7)	39.6 (22.5)	0.07
DAS 28	4.8 (1.4)	4.5 (1.5)	NS	5.0 (1.2)	4.1 (1.4)	<0.001
Rheumatoid factor positive (%)	73.8%	79.5%	NS	78.5%	73.6%	NS

Values are mean (SD) unless stated otherwise.

DAS 28, 28 joint disease activity score; ESR, erythrocyte sedimentation rate.

Saint-Etienne). Clinical indices of disease activity included age, sex, disease duration, patient's global assessment of disease activity, and 28 joint tender and swollen joint counts. All patients were treated with methotrexate. Biological data included erythrocyte sedimentation rate (ESR) and rheumatoid factor (RF). The 28 joint disease activity score (DAS 28) was calculated. Joint damage evaluation by *x* rays and clinical examination was carried out at the same time. According to the Larsen wrist *x* ray score, patients were divided into two groups, as previously described⁷: destructive arthritis (that is, with a right Larsen wrist score ≥ 2), and non-destructive arthritis (a right Larsen wrist score < 2). To investigate intrareader agreement, radiographs of 100 patients were read twice by the same reader. The intrareader variability, as assessed by the correlation coefficient, was 0.96 (95% confidence interval (CI), 0.95 to 0.98). To investigate interreader agreement, radiographs of 100 patients were read twice by two readers. The correlation coefficient for the interreader variability was 0.98 (0.97 to 0.99).

Periodontal disease was assessed on the basis of a routine dental panoramic *x* rays film. Periodontal bone destruction was defined according to the Hugoson and Jordan criteria by an alveolar bone loss with a horizontal alveolysis over one third of the normal bone height.⁸ Alveolar bone destruction was measured at four sites, the four maxillary and mandibular first molars. All dental panoramic *x* rays were examined by the same examiner (PF), who was blinded for wrist destruction. To investigate intrareader agreement, 50 panoramic radiographs were read twice by the same reader. The correlation coefficient was 0.96 (95% CI, 0.93 to 0.98). One third of the cohort (41 patients) had a labial salivary gland biopsy because of suggestive symptoms of dry eyes and mouth. Labial salivary gland biopsies were assessed histologically, using criteria initially described by Chisholm and Mason.

Gene polymorphism oligotyping

Uncoagulated blood samples were taken from patients and stored frozen at -20°C until further DNA extraction. DNA was extracted using the QIAamp DNA blood mini kit (Qiagen GmbH, Hilden, Germany), as recommended by the manufacturer. All genotyping were undertaken using an enzyme linked oligosorbent assay (ELOSA) as previously described.⁹

Shared epitope

Exon 2 regions of both HLA-DR and HLA-B were PCR amplified, using a combination of DR specific and B specific primers. For DR specific primers, primer sequences were: forward primer, 5'CCG GAT CCT TCG TGT CCC CAC AGC ACG-3'; reverse primer, 5'TCG CCG CTG CAC TGT GAA G-3'. For B-specific primers, primer sequences were: forward primer, 5'GGG AGG AGC GAG GGG ACC G/CC AG -3';

reverse primer, 5'ATC TCG GAC CCG GAG ACT-3'. The amplification mixture was composed of: 50 mM Tris-HCl, pH 8.8; 15 mM ammonium sulphate; 1.5 mM MgCl₂; 50 μM EDTA; 0.01% (wt/vol) gelatin; 0.2 mM dNTPs; 2.5 U AmpliTaq (Perkin Elmer); 0.15 μM for HLA-DR primers, 0.3 for HLA-DR4 primer and 0.4 μM for HLA-B primers; in a final 100 μl volume reaction. We used 50 to 200 ng of the extracted DNA per amplification. Cycling conditions were as follows: two minutes of denaturation at 95°C ; then four cycles with 30 seconds at 95°C , 30 seconds at 68°C , and 30 seconds at 72°C ; then four cycles with 30 seconds at 95°C , 30 seconds at 57°C , and 30 seconds at 72°C ; then three cycles with 30 seconds at 95°C , 30 seconds at 64°C , and 30 seconds at 72°C ; then 30 cycles with 30 seconds at 95°C , 30 seconds at 60°C , and 30 seconds at 72°C ; then seven minutes at 72°C . Polymerase chain reaction (PCR) efficiency was checked by agarose gel electrophoresis. Amplicons were hybridised on specific capture probes coated in eight well strips assembled on a microtitre plate frame, followed by semiautomated washing, colorimetric detection, and reading.

IL-1B (+3954)

A single nucleotide polymorphism (SNP) was described at position +3954 in exon V. Common allele is C and rare allele is T. Primer sequences and PCR conditions were: forward primer, 5'TTC AGT TCA TAT GGA CCA GA-3'; reverse primer, 5'GTT GTC ATC AGA CTT TGA CC-3'. PCR cycles were: [95°C for two minutes] $\times 1$; [94°C for 30 seconds] $\times 40$; [55°C for 30 seconds] $\times 40$; [68°C for one minute] $\times 40$; and [68°C for 10 minutes] $\times 1$. Amplicons were hybridised on specific capture probes as described for the ELOSA method.

TNFA (-238)

This SNP was described in the promoter region of TNFA gene at position -238. The common allele is G and the rare allele is A. Primer sequences and PCR conditions were: forward primer, 5'TCA ACG GAC TCA GCT TTC TGA A-3'; reverse primer, 5'CGG AAA ACT TCC TTG GTG GAG-3'. PCR cycles were the same as for IL1B.

TNFA (-308)

This SNP was described in the promoter region of TNFA gene at position -308. The common allele is G and the rare allele is A. Primer sequences and PCR conditions were: Forward primer, 5'TCA ACG GAC TCA GCT TTC TGA A-3'; reverse primer, 5'CGG AAA ACT TCC TTG GTG GAG-3'. PCR cycles were the same as for IL1B.

Statistical analysis

We used χ^2 tests for comparisons involving dichotomous measures. Odds ratios (OR) and relative risk (RR) were calculated. Student's *t* tests were used for comparisons

Table 2 Relations between the presence of the shared epitope in patients with rheumatoid arthritis according to the presence of wrist or periodontal destruction

SE	Wrist destruction				Periodontal destruction				Overall destruction							
	No	Yes	Total	OR	χ^2	p Value	No	Yes	Total	OR	χ^2	p Value	Total	OR	χ^2	p Value
	18	16	34				20	14	34							
Yes	35	78	113	2.5	5.47	<0.05	44	69	113	2.2	4.20	<0.05	72	3.9	8.14	<0.001
Total	53	94	147				64	83	147				92			

OR, odds ratio; SE, shared epitope.

involving continuous variables. Stepwise methods were used to determine a multivariate model of independent predictors of wrist and periodontal destruction. All analyses was undertaken using the StatView statistical software.

RESULTS

Patient features

All 147 patients enrolled in the study had typical clinical and biological features of rheumatoid arthritis. Their (SD) mean age was 54.1 (11.7) years. Patients were predominantly women (78.9%) with a mean disease duration of 7.5 (7.0) years, and 77.6% were RF positive. Most patients had active disease with tender and swollen joint counts of 8.3 (6.9) and 3.2 (3.7), respectively. Patient global assessment was 44.2 (21.1). ESR was 32.9 (23.6) mm/h. The DAS 28 score was 4.7 (1.3), confirming active disease. All patients were treated with methotrexate, with a mean weekly dose of 14.2 (4.2) mg. Prednisone was used concomitantly by 38.1% (n = 56), with a mean daily dose at 8.6 (4.1) mg. Only 17 patients (13.6%) were receiving biphosphonates (risedronate (11), alendronate (6), and etidronate (3)). Most patients (67.3%) had severe rheumatoid disease with wrist joint destruction according to a right wrist Larsen index of ≥ 2 .

Association between wrist joint and periodontal destruction

Of the 147 patients, 83 (56.5%) had periodontal disease. A strong association was observed between periodontal disease and wrist destruction ($\chi^2 = 11.82$; $p < 0.001$). However, the destruction distribution at both sites was heterogeneous: 63 (42.9%) had destruction and 33 (22.4%) had no destruction at the two sites; 31 (21.1%) had only wrist destruction; and 20 (13.6%) had only periodontal destruction.

No relation was observed between periodontal disease severity and age, sex, disease duration, tender and swollen joint counts, ESR, patient's global assessment of disease activity, DAS 28, or RF (table 1). Conversely, tender and swollen joint counts, ESR, and DAS 28 score were associated with wrist destruction (table 1). Treatment with prednisone or biphosphonates was not associated with differences in periodontal destruction or wrist destruction.

Association between destruction and sicca syndrome

A trend was observed between dry mouth and periodontal disease (RR = 1.51 (95% CI, 0.94 to 2.43), $p = 0.09$) or wrist destruction (RR = 1.45 (0.94 to 2.23), $p = 0.09$). In the subset of patients who had a labial biopsy because of sicca syndrome, an association was observed between a positive labial salivary gland biopsy and periodontal disease (RR = 2.73 (1.35 to 5.51), $p < 0.01$, n = 41) or wrist destruction (RR = 4.52 (1.96 to 10.45), $p < 0.001$, n = 41).

Association between genetic markers and destruction

All 147 rheumatoid patients were tested for the SE. A significant association was observed between the SE and bone destruction at both the wrist and the periodontal sites. Patients with the SE had a 2.5 times greater risk of having wrist joint destruction than SE negative patients (OR = 2.5 (1.16 to 5.42), $p < 0.05$). Similarly, SE positive patients had a 2.2 times greater risk of having periodontal destruction compared with the SE negative patients (OR = 2.2 (1.04 to 4.84), $p < 0.05$). When comparing patients with destruction at both sites to patients without any destruction, patients with the SE had a 3.9 times increased risk of having such destruction (OR = 3.9 (1.53 to 9.96), $p < 0.001$) (table 2).

When considering cytokine gene polymorphisms affecting TNFA (-238 or -308) and IL1B (+3954), no association was

noted between these polymorphisms and the periodontal or wrist bone destruction.

A stepwise multiple logistic regression analysis was undertaken with identified independent predictors of wrist destruction. Active disease and the presence of the SE were independent predictors of wrist destruction. The same results were observed for periodontal disease with a stepwise multiple logistic regression analysis including in addition age and disease duration.

Association between sex, genetic markers and destruction: No association was observed between the sex and the SE status ($\chi^2 = 2.0$; NS). However, after stratification for sex, a strong association was observed in men between the SE status and periodontal destruction ($\chi^2 = 21.4$; $p < 0.001$). This association was not found between the SE status and wrist destruction.

DISCUSSION

The results of our study provide further evidence for an association between periodontal bone loss and joint destruction in rheumatoid arthritis. Our patients had the common rheumatoid arthritis characteristics (mean age, 54.1 (11.7) years; 78.9% female), implying that this group was representative of the general rheumatoid arthritis population. The prevalence of periodontal disease was high at 56.5%. In a previous study, the prevalence of rheumatoid arthritis in a periodontal disease population was 3.95% *v* 0.66% in a healthy group ($p < 0.05$).¹ The increased periodontal disease prevalence in the rheumatoid population and vice versa suggested a link between these two chronic inflammatory diseases. Destruction observed at the two sites may reflect a common underlying mechanism affecting bone destruction in rheumatoid arthritis.

Having confirmed this association between severe rheumatoid arthritis and periodontal disease, we next investigated some of the common severity markers involved in the mechanisms of these two chronic inflammatory disorders. Classical markers of rheumatoid arthritis activity (table 1) were associated with wrist destruction but not with periodontal disease, indicating that periodontal disease does not appear to be a localised form of rheumatoid arthritis. In this rheumatoid population, surprisingly, age and disease duration were not significantly associated with destruction. In previous studies, sicca syndrome, smoking, RF positivity, and familial or genetic background, such as the SE and IL1B or TNFA polymorphisms, have been associated with periodontal disease.^{1 5-7 10} Here, a similar trend was observed between dry mouth and periodontal disease or joint destruction. In addition, an association was found between a positive labial salivary gland biopsy and periodontal disease or joint destruction. The association between periodontal disease and sicca syndrome has been already documented.¹⁰ However, the possible association between rheumatoid arthritis severity and sicca syndrome remains more controversial.

Among the genetic factors, the SE is recognised as the major genetic factor for rheumatoid arthritis susceptibility and severity. The SE was also found as a prognostic factor for periodontal disease.⁷ Here, for the first time in a rheumatoid arthritis population, we observed that the SE was associated with destruction at both sites. We confirmed the previously described association between the SE and joint destruction and determined that the SE was also associated with periodontal destruction. The presence of the SE increased by more than twofold the risk of developing wrist or periodontal destruction. This is an indication of an association between the SE and bone destruction in the context of rheumatoid arthritis or another disease such as periodontal

disease, thus explaining the association between the two conditions observed here.

Both rheumatoid arthritis and periodontal disease are associated with an imbalance between pro-inflammatory and anti-inflammatory cytokines. IL1 β and TNF α are the main pro-inflammatory cytokines detected in rheumatoid joints and gingival tissues.¹¹ A correlation was observed between the IL1 β or TNF α levels in periapical exudates and periodontal disease.¹² Moreover, in experimental models of periodontal disease, periodontal disease progression was reduced with specific IL1 β and TNF α antagonists.¹³ Some genetic factors affecting these cytokines can modulate the imbalance between pro-inflammatory and anti-inflammatory cytokines. In relation to the +3954 IL1B polymorphism, the rare allele has previously been associated with increased IL1 β protein secretion.¹⁴ This rare allele was more frequent in non-smoking patients with periodontal disease.¹⁰ In contrast, other studies have failed to detect such an association in periodontal disease¹⁵ or in rheumatoid arthritis, whereas yet others have found a correlation with bone destruction at both sites.^{3 5} Similarly, the rare allele TNFA -308 has been associated with a high TNF α production. The findings of the present study did not show an association between TNFA gene polymorphisms and rheumatoid arthritis or periodontal disease severity, in contrast to some other studies.^{3 6} As previous studies on the association between TNFA and IL1B polymorphisms in rheumatoid arthritis are not concordant, these results suggest that their contribution to disease severity would be limited, and at the least much lower than that of the SE.

Conclusions

Although the two sites of bone destruction investigated here were commonly associated in our rheumatoid arthritis population, markers of rheumatoid disease activity were clearly associated with wrist destruction but not with periodontal disease. This suggests different initial mechanisms of induction, followed by a common pathway involving the contribution of cytokines and genetic markers. The SE appears in both diseases to be a marker of bone destruction severity and not directly associated with disease aetiology or induction mechanisms.

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