

Anti-Tumor Necrosis Factor-Alpha Therapy and Periodontal Parameters in Patients With Rheumatoid Arthritis

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Background: The aim of this study was to evaluate the influence of anti-tumor necrosis factor-alpha (TNF- α) therapy on the clinical and immunologic parameters of the periodontium.

Methods: Ten patients with rheumatoid arthritis (RA) who routinely received infusions of infliximab, 200 mg (RA+), 10 patients with RA without anti-TNF- α therapy (RA-), and 10 healthy controls (C) were included. Clinical parameters, including the plaque index (PI), gingival index (GI), probing depth (PD), clinical attachment loss (AL), and bleeding on probing (BOP), were assessed, and total gingival crevicular fluid (GCF) TNF- α level was determined using enzyme-linked immunosorbent assay. Analysis of variance with Scheffe modification and the Pearson correlation test were used for statistical analysis.

Results: The ages of the patients ranged from 22 to 76 years (mean, 50.73 ± 9.1 years). The mean PI was similar among the groups. However, mean inflammatory parameters in the three groups varied significantly; GI was greater in the RA- group compared to RA+ and C groups ($P = 0.0042$). The RA+ group exhibited less BOP than RA- and C groups ($21.1\% \pm 3.0\%$, $45.9\% \pm 6.2\%$, and $39.1\% \pm 7.2\%$, respectively; $P = 0.0146$). The mean PD in the RA+ group was shallower than in RA- and C groups (3.22 ± 0.13 mm, 3.85 ± 0.22 mm, and 3.77 ± 0.20 mm, respectively; $P = 0.055$). Clinical AL in the RA+ group was lower than in RA- and C groups (3.68 ± 0.11 mm, 4.52 ± 0.26 mm, and 4.35 ± 0.24 mm, respectively; $P = 0.0273$). TNF- α levels in the GCF of the RA+ group were the lowest compared to RA- and C groups (0.663 , 1.23 , and 0.949 ng/site, respectively; $P = 0.0401$). A significant positive correlation was found between TNF- α levels in the GCF and clinical AL ($r = 0.448$; $P = 0.0283$).

Conclusions: Patients with RA receiving anti-TNF- α medication had lower periodontal indices and GCF TNF- α levels. Thus, suppression of proinflammatory cytokines might prove beneficial in suppressing periodontal diseases. *J Periodontol* 2009;80:1414-1420.

KEY WORDS

Anti-inflammatory agents; cytokines; immunology; periodontium; pharmacology.

Periodontal diseases are characterized by the classic hallmarks of the inflammatory response, including erythema, and edema.¹ Late sequelae of periodontal diseases include the loss of alveolar bone, periodontal ligament attachment, and ultimately, teeth. Therefore, periodontal disease can be viewed as a chronic inflammatory process in which bacteria-induced localized gingival inflammation results in destruction of bone and the attachment apparatus of the teeth.² Rheumatoid arthritis (RA) is an inflammatory joint disease characterized by inflammatory infiltrate in the synovial tissue that leads to joint destruction and impaired function.³ The relationship between periodontitis and RA was examined by only a few studies^{4,5} in the last years. Recent studies^{6,7} showed that patients with RA have a higher prevalence of periodontitis.

Although the etiologies of these two diseases are distinctly separate, virtually all of the cytokines and inflammatory mediators involved in RA are paralleled in periodontal disease: high levels of proinflammatory cytokines, including interleukin (IL)-1 β and tumor necrosis factor alpha (TNF- α), and low levels of anti-inflammatory cytokines, such as IL-10 and transforming growth factor-beta. Abnormal cytokine regulation accompanied by low levels of tissue inhibitors of metalloproteinases and high levels of matrix metalloproteinases (MMPs), coupled with prostaglandin E₂ (PGE₂),

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are associated with the active stages of periodontitis.

The destruction of extracellular matrix in both conditions is determined by the balance between MMPs and their inhibitors. TNF- α is one of the key cytokines in rheumatoid inflammation. In both diseases, TNF- α contributes to the upregulation of osteoclastogenesis and the downregulation of osteoblastogenesis.⁸

The term “tumor necrosis factor” refers to two associated proteins, TNF- α and lymphotoxin- α , also known as TNF- β . TNF- α is produced mainly by macrophages in response to antigens, such as lipopolysaccharides.⁹ TNF- α is present in serum and inflammatory tissues; its levels correlate with disease activity and the degree of tissue damage.¹⁰ TNF- α can also induce the expression of other mediators that amplify or sustain the inflammatory response (e.g., prostaglandins), stimulate the production of lytic enzymes (e.g., collagenase), and enhance bacterial killing and phagocytic activity.¹¹

Gingival crevicular fluid (GCF) is a serum ultrafiltrate of blood originating from the vasculature subjacent to the sulcus. GCF flow rates and the increased concentration of molecules that mediate innate and adaptive immune responses correlate with the severity of periodontal inflammation. As such, numerous GCF constituents have been characterized to identify biomarkers that may be used to monitor the initiation and progression of gingival inflammation and the immune response.¹²

Inhibition of TNF- α activity leads to clinical improvement in RA and periodontitis.¹³⁻¹⁵ The purpose of the present study was to evaluate the influence of anti-TNF- α therapy on the periodontal health of patients with RA and to study the association of GCF TNF- α and periodontal inflammatory parameters. The null hypothesis was that anti-TNF- α therapy has a beneficial effect on periodontal parameters.

MATERIALS AND METHODS

Patient Selection

Twenty consecutive patients with RA attending the B. Shine Rheumatology outpatient clinic were enrolled in the study. All patients fulfilled the American College of Rheumatology criteria for RA.¹⁶ The study took place at the Department of Periodontology, Rambam Health Care Campus, between July and December 2008. The nature of the study was conveyed, and informed written consent was obtained from all subjects prior to commencement. The study protocol was approved by the institutional Helsinki committee.

The subjects were divided into three groups. The test group (RA+) included 10 patients with RA (seven females; mean age, 51.9 ± 6.74 years) who received an infusion of infliximab, 200 mg, every 8 weeks. Ten

patients with RA (five females; mean age, 48.8 ± 13.64 years), treated without biologic agents, made up the first control group (RA-). The second control group (C) included 10 healthy subjects (five females; mean age, 51.5 ± 6.8 years) without RA or any other systemic inflammatory disease. Age, gender, and smoking status were recorded. Data on the immunologic and erosive joint changes in the patients with RA were collected from medical records.

At the time of the periodontal examination, there were no differences between RA+ and RA- groups with respect to the RA disease activity score (DAS28).¹⁷ DAS28 is a measure of the activity of RA. The parameters included in the calculations are the number of joints tender to touch, the number of swollen joints, the erythrocyte sedimentation rate, and the patient's assessment of disease activity measured by a visual analog scale.¹⁸

Patients <18 years of age and pregnant and lactating woman were not included in the study. Subjects were excluded if they received medications, such as antibiotics, within the previous 6 months or had periodontal treatment in the past 12 months.

Periodontal Examination

The periodontal examination was performed by the same calibrated examiner (YM) at the beginning of the dental visit. Assessment of clinical parameters included probing depth (PD), clinical attachment loss (AL), plaque index (PI),¹⁹ gingival index (GI),²⁰ and bleeding on probing (BOP). To avoid contamination of the filter paper strips with blood, GI, BOP, PD, and clinical AL were measured after the GCF collection. The deepest pockets were detected in an early screening session. All periodontal parameters were measured in all teeth present, excluding third molars. PD and clinical AL were recorded in millimeters and were measured at six sites per tooth using a manual periodontal probe. PD and clinical AL are presented as mean patient values. BOP was recorded as present or absent within 30 seconds of probing at six sites per tooth, and the mean percentage of bleeding sites was calculated for each patient.

TNF- α Sampling and Assay

A single investigator (YM) obtained all samples. GCF samples from each subject were collected from the two deepest periodontal pockets. At these sites, supragingival plaque was carefully removed using Gracey curets,[§] after which the sample sites were isolated with cotton rolls. Each sterile paper strip^{||} was inserted into the pocket for 30 seconds. Samples were wrapped in aluminum foil and stored at -20°C .

§ Hu-Friedy, Chicago, IL.

|| Periopaper, Proflow, Amityville, NY.

All specimens were masked prior to the laboratory assay.

A single laboratory technician, masked to the patient and the health status, performed all assays. Total GCF TNF- α level was determined using a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) kit,[¶] as we described previously.²¹ Briefly, filter papers were unwrapped and inserted into a sterile test tube containing 1.0 ml distilled water. The tubes stood at room temperature for 30 minutes and were agitated every 5 minutes to facilitate extraction of the sample from the filter paper. A monoclonal antibody specific for TNF was precoated on a microplate. Standards and samples were pipetted into the wells, where the immobilized antibody binds the cytokine. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for TNF- α was added to the wells. The absorbance values were determined using an ELISA reader at 450 nm. A standard curve was constructed by using standards provided in the kit, and the cytokine concentration was calculated from the standard curve. The color intensity results were obtained using a microplate reader.[#]

Statistical Analysis

Means \pm SD of the clinical and immunologic parameters were calculated. Analysis of variance (ANOVA) with Scheffe modification was used to test the differences between the clinical periodontal parameters and TNF- α level in the GCF among the groups. The Pearson correlation coefficient test was used to analyze the correlation between TNF- α level in the GCF and the various periodontal parameters. Results were considered statistically significant at $P < 0.05$.

RESULTS

The mean age, gender, smoking status, DAS28, and disease duration of the patients are shown in Table 1. Patient age ranged from 22 to 76 years (mean, 50.73 \pm 9.1 years); the means were not significantly different among RA+, RA-, and C groups.

Likewise, there were no differences among the groups with respect to gender, smoking status, and DAS28. However, patients treated with infliximab had significantly longer disease duration (mean, 16.4 \pm 12.68 years versus 4.6 \pm 2.12 years; $P = 0.018$).

Table 1.
Characteristics of Subjects

Characteristics	RA+ (n = 10)	RA- (n = 10)	C (n = 10)	Difference (P value)*
Age (years; mean \pm SD)	53.6 \pm 9.23	47.1 \pm 16.1	51.5 \pm 9.97	NS
Females/males (n)	7/3	5/5	5/5	NS
Smokers (n)	3	3	5	NS
DAS28 (mean \pm SD)	4.80 \pm 0.91	5.05 \pm 1.08	–	NS
Duration of RA (years; mean \pm SD)	16.4 \pm 12.68	4.6 \pm 2.12	–	0.018
Positive for RF and/or anti-CCP antibodies (n)	7	6	–	NS
Erosive joint disease (n)	7	5	–	NS
DMARDs (n; mean \pm SD)	1.8 \pm 0.6	2.1 \pm 0.7	–	NS

NS = not statistically significant; – = not applicable.

* Analysis of variance with Scheffe modification.

At the time of the periodontal examination, patients in both RA groups were taking additional medications as part of their standard therapeutic regimen. These included non-steroidal anti-inflammatory drugs, glucocorticoids, and disease-modifying antirheumatic drugs (DMARDs; methotrexate and/or sulfasalazine and/or hydroxychloroquine). There was no difference in the medication protocol between RA+ and RA- groups, except for the anti-TNF- α therapy, which was only used by the RA+ group. The mean duration of infliximab therapy was 26 \pm 8 months, and it was the only biologic drug (including patient history) in use by patients in the RA+ group.

According to the American Academy of Periodontology classification of periodontal disease,²² 25 patients (83%) had moderate-advanced chronic periodontitis, four (13%) had slight chronic periodontitis, and one (3%) had gingivitis. Using this classification, the severity of periodontal disease was categorized based on the amount of clinical AL as follows: slight = 1 to 2 mm, moderate = 3 to 4 mm, and severe \geq 5 mm. The number of patients in each periodontal disease category did not vary significantly among the study groups (data not shown).

The mean PI was very similar among the groups (1.95 \pm 0.134, 1.82 \pm 0.11, and 1.90 \pm 0.137, respectively). To the contrary, mean inflammatory parameters in the three groups varied significantly; GI was significantly greater in the RA- group compared to RA+ and C groups (2.01 \pm 0.12, 1.45 \pm 0.09, and 1.59 \pm 0.11, respectively; $P = 0.0042$; (Table 2).

¶ Human TNF- α /TNFSF1A Immunoassay, R&D Systems, Minneapolis, MN.

SUNRISE, Magellan, Tecan Group, Männedorf, Switzerland.

Table 2.
Periodontal Status of Study Subjects (mean ± SEM)

Periodontal Parameters	RA+ (n = 10)	RA- (n = 10)	C (n = 10)	Difference (P value)*
PI	1.95 ± 0.134	1.82 ± 0.11	1.90 ± 0.137	NS
GI	1.45 ± 0.09 ^a	2.01 ± 0.12 ^{a,b}	1.59 ± 0.11 ^b	0.0042
BOP (% sites)	21.1 ± 3.0 ^{a,c}	45.9 ± 6.2 ^a	39.1 ± 7.2 ^c	0.0146
PD (mm)	3.22 ± 0.13	3.85 ± 0.22	3.77 ± 0.20	0.0554
Clinical AL (mm)	3.68 ± 0.11 ^{a,c}	4.52 ± 0.26 ^a	4.35 ± 0.24 ^c	0.0273

ANOVA was used to analyze differences among groups, and the unpaired *t* test was used for bilateral comparisons.

NS = not statistically significant; a = difference between RA+ and RA- (*P* < 0.05); b = difference between RA- and C (*P* < 0.05); c = difference between RA+ and C (*P* < 0.05).

* Analysis of variance with Scheffe's modification.

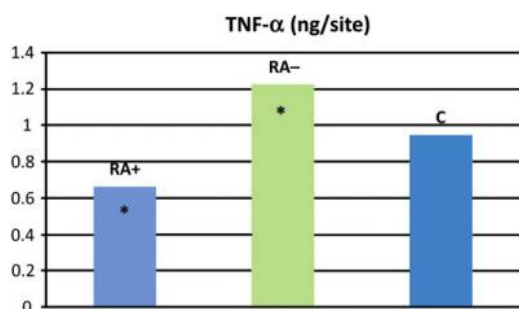


Figure 1.
TNF-α levels (ng/site) in the GCF of the three groups. **P* = 0.04.

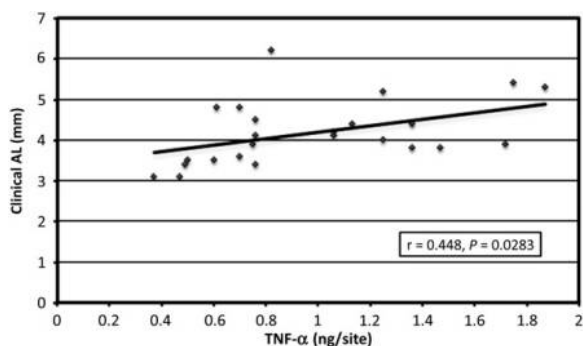


Figure 2.
Relationship between TNF-α levels in the GCF and clinical AL.

Likewise, patients receiving anti-TNF-α therapy (RA+) exhibited less BOP than RA- and C groups (21.1% ± 3.0%, 45.9% ± 6.2%, and 39.1% ± 7.2%, respectively; *P* = 0.0146).

The mean periodontal PD in the RA+ group was shallower than in patients with RA who were not receiving the medication and the healthy controls

(3.22 ± 0.13 mm, 3.85 ± 0.22 mm, and 3.77 ± 0.20 mm, respectively). The difference between RA+ and RA- groups was borderline significant at *P* = 0.0554.

Also, the clinical AL in the RA+ group was lower than that in RA- and C groups (3.68 ± 0.11 mm, 4.52 ± 0.26 mm, and 4.35 ± 0.24 mm, respectively; *P* = 0.0273).

TNF-α in the GCF was evaluated by ELISA to determine the effect of the anti-TNF-α therapy on this proinflammatory cytokine in the gingival fluid (Fig. 1). Five patients were excluded from this

analysis because of contamination of the paper strips with blood (three RA+, one RA-, and one C). TNF-α in the GCF of patients in the RA+ group was lower than in RA- and C groups (0.663 ng/site versus 1.23 and 0.949 ng/site, respectively); however, the difference was only significant between RA+ and RA- groups (*P* = 0.0401).

A significant positive correlation was observed when TNF-α levels in the GCF were plotted against the corresponding clinical AL (*r* = 0.448; *P* = 0.0283; Fig. 2). No correlations were observed between the TNF-α levels and any of the other clinical parameters.

DISCUSSION

The results of the present study suggest that patients with RA receiving anti-TNF-α therapy exhibited lower GCF levels of TNF-α and milder periodontal disease compared to matched patients with RA who did not receive this medication. Patients in both RA groups (RA+ and RA-) did not show differences in their immune profile (rheumatoid factor [RF] and/or anti-cyclic citrullinated peptide [CCP] antibodies), disease activity (according to DAS28), or DMARDs. However, patients in the RA+ group had much longer disease duration. This difference could be explained by the fact that in Israel, anti-TNF-α agents are available to patients free of charge only after the failure of at least three antirheumatic (DMARD) drugs. As is typical with RA, hand deformities were present in both groups. Patients with RA receiving anti-TNF-α therapy exhibited lower GCF levels of TNF-α and milder periodontal disease compared to matched patients with RA who did not receive this medication. These findings suggest that anti-TNF-α may influence the destruction processes (as reflected by the greater PD and clinical AL). Infliximab also seems to influence the vascular gingival status (as represented by the higher BOP and GI).

The total amount of TNF- α in GCF of patients in the RA+ group was significantly lower than that in RA- and C groups. The consensus is that analysis of GCF represents the most practical approach for the biochemical analysis of the host response in periodontal disease.^{23,24} GCF is an inflammatory exudate derived from the periodontal tissues. Some studies^{25,26} on GCF protein concentration suggested that inflamed gingiva had a protein concentration similar to that of serum. Most proteins were significantly lower in GCF but with a strong covariation between the two fluids, suggesting that GCF represents an inflammatory exudate of the serum.²⁷ We analyzed the total amount of TNF- α collected from the two deepest periodontal pockets by insertion of paper points for 30 seconds in the same site. Studies^{28,29} indicated that the expression of GCF data as the total amount per standardized sampling time is more sensitive than reporting them as concentration and should be used when estimating periodontal disease activity.

A positive and significant correlation was established between TNF- α levels (ng/site) in the GCF and clinical AL ($r = 0.448$; $P = 0.0283$). Clinical AL is defined as the distance from the cemento-enamel junction to the most apical penetration of the probe. Our results are in agreement with recent studies. Engebretson et al.³⁰ studied 46 patients with type 2 diabetes and chronic periodontitis to determine the relationship between plasma TNF- α levels and clinical measures of periodontitis. They showed a significant positive correlation between TNF- α and AL ($r = 0.40$; $P = 0.009$). Kurtiş et al.³¹ investigated the presence of TNF- α in the GCF and the clinical parameters in patients with chronic or aggressive periodontitis. They also detected a positive statistical correlation between clinical AL and TNF- α levels ($r = 0.487$; $P < 0.001$). Furthermore, these latter studies³¹ also found a correlation with other clinical parameters, such as PD, PI, and GI.

Patients with RA who received anti-TNF- α medications had lower periodontal indices (GI, PD, and clinical AL) compared to patients with RA who received a similar medication protocol, except for the anti-TNF- α . Multiple randomized clinical trials³²⁻³⁵ showed the therapeutic efficacy of anti-TNF- α agents combined with methotrexate in patients with RA. Assuma et al.¹⁵ assessed the effectiveness of IL-1 and TNF- α antagonists in a *Macaca fascicularis* primate model of experimental periodontitis. Injection of soluble receptors to IL-1 and TNF- α inhibited the recruitment of inflammatory cells in close proximity to bone by ~80%; the formation of osteoclasts was reduced by 67%, and the amount of bone loss was reduced by 60%. In the same primate model, Delima et al.¹⁴ showed, by using histomorphometric analysis, that IL-1 and TNF- α antagonists significantly reduced the

loss of connective tissue attachment by ~51% and the loss of alveolar bone height by almost 91%. To the best of our knowledge, only one study in humans examined the effect of anti-TNF- α treatment on coexisting periodontitis: Pers et al.³⁶ evaluated patients with RA before and after receiving nine infusions of infliximab. They reported that blocking of TNF- α activity resulted in the inhibition of bone resorption. However, contrary to our findings, they showed that infliximab tended to aggravate gingival inflammation, as indicated by the assessment of bleeding indices before and after treatment. Their explanation for this dichotomy between alveolar bone loss, which was halted, and gingivitis, which was aggravated, was based on a concept that destruction and inflammation are two separate components. Our results are in agreement with Nilsson and Kopp,³⁷ who conducted a study on 19 patients with RA. Their work revealed that gingivitis and periodontitis were associated with high levels of circulating TNF- α . Patients with high levels of TNF- α from repeated plasma samples had a higher frequency of BOP as well as increased clinical AL and PD compared to those with low levels. These findings suggested that circulating TNF- α is related to periodontal inflammation with regard to tissue destruction and vascular reaction in patients with RA.

In our study, patients with RA exhibited overall worse periodontal and gingival condition compared to patients without systemic inflammatory disease. However, only the differences in GI were statistically significant. One should bear in mind that the patients in all three groups had high plaque scores, as reflected by the PI, and most of them did not visit dental clinics frequently, except for emergency dental treatment (data not shown). Furthermore, subjects in the C group were selected from patients who came to the periodontal department for screening, suggesting that their periodontal condition was worse than average. This may explain the lack of differences in the periodontal clinical indices between the patients with RA and the healthy subjects. Some studies^{4-7,38,39} found that periodontal disease was more common and more severe in patients with RA compared to patients without RA. In 51 patients with RA with long-standing active arthritis, Kässer et al.³⁹ reported a higher rate of gingival bleeding (increased by 50%), greater PD (increased by 26%), greater AL (increased by 173%), and greater number of missing teeth (increased by 29%) compared to controls. In our study, the benefit of infliximab on gingival status was prominent, despite long-standing joint inflammatory disease. Despite these reports, the literature is still lacking studies correlating the severity of RA with the severity of periodontitis.

The similar pattern and natural history of RA and periodontitis provide useful insight into these

diseases. In both diseases, antigenic challenge to the monocytic/lymphocytic axis results in the secretion of proinflammatory cytokines, mainly PGE₂, IL-1 and -6, and TNF- α . In both diseases (periodontitis and RA), bone destruction results from uncoupling of the normally coupled process of bone resorption and bone formation mediated by PGE₂, IL-1 and -6, and TNF- α . It seems that anti-TNF- α agents may halt periodontal inflammation and bone resorption by suppressing proinflammatory cytokines and restoring the cytokine balance in GCF.

CONCLUSIONS

Our data showed that patients with RA exhibited overall worse periodontal and gingival conditions. Treatment of patients with RA with infliximab significantly reduced the levels of TNF- α in GCF and led to an improvement in periodontal status. We suggest that by suppressing proinflammatory cytokines and restoring the cytokine balance in GCF, anti-TNF- α agents may halt periodontal inflammation and bone resorption. The effect of anti-TNF- α therapy on periodontitis seems promising. However, in light of the relatively small sample size, this needs to be substantiated in future studies.

ACKNOWLEDGMENTS

The authors are grateful to Mrs. Margarita Filatov, Laboratory of Endocrinology, Rambam Health Care Campus, for her laboratory analysis. They also thank the nurses of the B. Shine Department of Rheumatology, Rambam Health Care Campus, for their great assistance. The authors report no conflicts of interest related to this study.

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Submitted January 8, 2009; accepted for publication April 12, 2009.