Effect of TNF-α Blockade in Gingival Crevicular Fluid on Periodontal Condition of Patients with Rheumatoid Arthritis

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ABSTRACT

Background: Periodontitis and rheumatoid arthritis (RA) share a number of clinical and pathologic features, one of which is the presence of the tumor necrosis factor alpha (TNF-α)-induced bone resorption that is involved in the pathogenesis of both. **Objective:** To investigate the effect of TNF-α blockade on periodontal conditions in patients with active RA. **Method:** The periodontal statuses of 36 patients (26 females, 10 males) diagnosed with active RA were evaluated both before and after anti-TNF-α therapy. Gingival index, bleeding on probing (BOP), probing pocket depth (PPD), oral hygiene index (OHI), and levels of TNF-α in gingival crevicular fluid (GCF) were measured at the baseline and 6 weeks after the treatment. Wilcoxon signed ranked test was used for statistical analyses. **Results:** Based on OHI (p=0.860), the level of plaque control did not change during the study period, but there was a significant reduction in gingival inflammation based on the mean BOP (p=0.049) and GI (p=0.036) before and after 6 weeks of anti-TNF-α therapy. The mean PPD index did not significantly differ at the baseline and 6 weeks after treatment (p=0.126). **Conclusion:** Anti-TNF-α therapy might have a desirable effect on periodontal conditions and might reduce TNF-α level in GCF of patients with RA.

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Keywords: Anti-TNF Alpha, Periodontal Diseases, Rheumatoid Arthritis

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INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune systemic inflammatory disease, characterized by synovial hyperplasia and intra-articular fibrin deposition that normally leads to joint destruction (1). Periodontitisis categorized as an infectious disease, is characterized by gingival inflammation and tooth supporting tissue destruction. Although periodontitis is initiated by bacteria, it is believed that disease progression relies heavily on the production of inflammatory mediators in response to pathogens or their products (2,3).

The relationship between RA and periodontal diseases has been well documented (4). It has been reported that RA is more prevalent in patients with moderate to severe periodontitis and vice versa (5-7). This bidirectional relationship between the 2 diseases could be explained by the similar immuno-pathology and host responses. The ample degradation of the connective tissue and bone is a common pathologic pathway in chronic periodontitis and RA. Dysregulation of inflammatory responses and overproduction of pro-inflammatory cytokines, in particular, TNF-α have been suggested as the most common features of the two diseases (8). Mainly secreted by mesenchimal cell lineage and immune cells, TNF-α can induce bone resorption through different mechanisms. TNF-α also has a negative impact on bone formation and interferes with bone coupling phenomenon as it reduces the osteoblast numbers, inhibits the osteoblast differentiation, or decreases the bone matrix production (9,10). In the shade of such effects, anti TNF-α therapy has been studied for managing some diseases including rheumatoid arthritis and periodontitis. It has been demonstrated that TNF-α antagonists could reduce the loss of both connective tissue and bone in the experimental periodontitis in primate (11,12) and lead to the clinical improvement of periodontal status of RA patients (13-15). One of such medications, Etanercept, is a recombinant fusion protein, linked to human type II TNF receptor Fc portion. Recently, the Iranian version of Etanercept, Altebrel® (AryoGen Biopharma Company, Iran) has been introduced to manage signs and symptoms of active RA. This study was conducted to assess the effect of Altebrel® on periodontal status of RA patients who had periodontal diseases.

The gingival crevicular fluid (GCF) could represent an accurate profile of tissue and serum concentration of infla mmatory mediators. Since these mediators have key roles in the disease pathogenesis and could be effective in periodontal disease monitoring (16,17), the TNF-α level in GCF was also evaluated to investigate the correlation between periodontal condition and crevicular TNF-α level after Etanercept consumption.

MATERIALS AND METHODS

Subjects. In this study, the periodontal health of 128 Iranian patients with active Rheumatoid Arthritis, treated with Altebrel® was examined in a randomized clinical trial (code no= IRCT201206266302N3). After the initial oral examination, thirty six patients including 26 females and 10 males (aged 25-63 years) were enrolled. The patients all suffered the periodontal diseases and met the inclusion criteria of being 18 years of age and having at least six Ramford's teeth (18).

Periodontal diseases are defined to appear with generalized gingival inflammation and redness concomitant with bleeding on probing, with or without pocket depth ≥ 5 mm. There were also exclusion criteria including the history of antibiotic use and reception of any periodontal treatment 3 months before the time of experiment. The study was carried out in the rheumatology clinic of Shariati hospital, Tehran, Iran (November to December, 2014). All participants signed an informed consent.

Diagnosis of RA was confirmed in all participants according to the 2010 RA classification criteria of the American college of rheumatology and EULAR (19). In brief, active Rheumatoid Arthritis is defined as the presence of 12 or more tender joints, 10 or more swollen joints, and at least one of the followings: erythrocyte sedimentation rate of at least 28 mm/h, C-reactive protein level greater than 20 mg/L, or morning stiffness for at least 45 minutes, and diagnosed by a rheumatologist. Patients have been taking disease-modifying anti-rheumatic drugs, including methotroxide, hydroxychloroquine and prednisone. None of the patients had ever taken anti-TNF-α drugs before.

Clinical Assessment. The periodontal status was assessed through the following measurements: bleeding on probing (BOP), Gingival index (GI) (20), oral hygiene index (OHI) (21) and probing pocket depth. GI, BOP and OHI were recorded at four sites of each Ramford's teeth. The percentage of sites bleeding after probing was also calculated and reported as BOP. Probing pocket depth measurements were performed via Williams probe at six sites around Ramford's teeth and the mean recorded to the nearest millimeter.

After the periodontal analyses at baseline, all patients received Etanercept at a dosage of 25 mg subcutaneously twice weekly and 6 weeks later; the periodontal status was reassessed. No periodontal treatment including scaling and oral hygiene instruction were introduced before or during the study period. In addition, there was no change in the oral hygiene regimens of all participants during the study period.

GCF Samples. GCF samples were collected using paper strips (Periopaper- oraflow-Inc., USA) from the deepest pocket at the baseline and 6 weeks after Etanercept therapy. The selected area was air dried and isolated with cotton pellets. Paper strips were maintained in the sulcus for 30 seconds. The samples were immediately transferred into vials containing phosphate-buffer saline. In the laboratory, after being shaken for 5 minutes in the room temperature, the strips were removed and the extract was centrifuged (22). The samples were stored at -20°C until the time of assay. The TNF-α levels were determined using enzyme-linked immunosorbent assay at the Department of Immunology, Tehran University of Medical Science. The ELISA procedure was carried out using Human TNF-alpha Platinium Kit (eBioscience Inc., San Diego, CA, USA)

Statistical Analysis. Mean ± SD of clinical parameters were calculated. Wilcoxon signed ranked test was used to test the differences between the periodontal parameters and TNF-α level in the GCF at baseline and reassessment. Data were analyzed using SPSS 16 (SPSS Inc., Chicago, IL, USA). P values less than 0.05 were considered to be statistically significant.

RESULTS

Thirty six RA patients (mean ± SD) 40.8 ± 12.3 years have been evaluated in the study. No adverse effect of the Etanercept was observed in participants' periodontal tissues (e.g. dentoalveolar abscess (23), lichenoid drug reaction or gingival ulcers) during the study period. As shown in Table 1, Etanercept therapy led to significant improvements in periodontal indices of inflammation, GI, BOP ($p<0.05$). Other periodontal parameters including OHI and PD did not undergo any significant changes between the baseline and reassessment ($p>0.5$). The GCF- level of TNF-α decreased after 6 weeks of anti-TNF-α therapy.

Table 1. Periodontal parameters (mean ± SD) and GCF-level of TNF-α in rheumatoid arthritis patients.

	Baseline	Reassessment	P Value
TNF-α (pg/ml)	2.09 ± 0.59	1.73 ± 0.2	0.04 *
OHI	0.52 ± 0.38	0.5 ± 0.38	0.8
GI	2.09 ± 0.53	1.73 ± 0.46	0.036*
BOP	0.35 ± 0.62	0.05 ± 0.1	0.049*
PD (mm)	2.04 ± 0.61	1.8 ± 0.39	0.12

*significantly different as assessed by Wilcoxon signed rank test (P<0.05)

DISCUSSION

Periodontal condition as determined by GI and BOP was improved after administration of TNF-α blocking agent ($p<0.05$). Due to the comparable level of plaque control between baseline and reassessment, it seemed that the reduction of gingival inflammation was independent of oral hygiene status of the patients. The mean periodontal pocket depth was not changed during the study. This could be explained by initial shallow mean pocket depths and short duration of Etanercept usage. The results were consistent with the findings of other studies (14,15,24,25). In a prospective study, Kobayashi *et al.* evaluated the impact of a 3-month administration of Adalimumab (humanized anti-TNF-α monoclonal antibody) on periodontal condition of RA patients. Although the gingival inflammation reduced, the plaque level was even during the study. They suggested that TNF inhibition had a beneficial effect on periodontal condition and these improvements might be related to the difference in serum protein profile after Adalimumab therapy (14). Mayer *et al.* observed that periodontal indices and the concentration of GCF TNF-α were lower in RA patients, who received infliximab (a chimeric mouse/human anti-TNF-α monoclonal antibody), than the others with other autoimmune diseases who did not receive TNF-α blocker (13). The positive effect of Etanercept on reduction of inflammation and tissue injury was shown in an

experimental periodontitis rat model. Administration of Etanercept, resulted in significant decrease in the infiltration of neutrophils and expression of TNF-α in periodontal lesions (26). In a further study, the effect of Infliximab on the periodontal status of 40 participants with RA was evaluated. Although gingival inflammation aggravated after administration of anti-TNF-α medication, periodontal breakdown was limited as determined by the reduction of the clinical attachment loss (27).

In contrast, Ortiz *et al.* showed that anti-TNF-α treatment could not significantly improve periodontal condition without periodontal therapy in RA patients with severe periodontitis. They also observed that non-surgical periodontal therapy might reduce the severity of RA signs and symptoms as well as the TNF-α level in serum (24). Another 6 months prospective study suggested that the administration of 3 different types of anti-TNF-α drugs could not improve the periodontal condition of the participants, but it should be noted that just 45% of the subjects had mild periodontal diseases (23). Taken together, the beneficial impact of TNF-α blockers appears to be more evident in mild to moderate forms of periodontal diseases. It could also be concluded that anti-TNF-α therapy without periodontal treatment did not significantly improve the periodontal condition of RA patients with severe periodontitis.

In this study, we also found that the level of TNF-α in GCF decreased after 6 weeks administration of Etanercept. In two other studies, lower crevicular and salivary levels of TNF-α were reported in RA patients receiving anti TNF-α medications compared with non-treated RA patients (15,25). Gingival crevicular fluid is an exudate from periodontal tissues and it has been suggested that protein concentrations in GCF of inflamed gingiva resembled to those of the serum (28). As the host response is a critical determinant in the pathogenesis of periodontitis and rheumatoid dermatitis, the assessment of the inflammatory mediator levels in gingival crevicular fluid might, to some extent, indicate the relevant levels of these mediators in the serum (17,29).

The results showed that TNF-α blockade could improve gingival inflammation in RA patients. This could be possibly explained by the reduction of TNF-α level in GCF. Long term studies are needed to compare the serum and crevicular level of TNF-α in RA patients treated by anti-TNF-α medications. We suggest that GCF has the potential to be used as an alternative diagnostic test to evaluate the efficacy of the TNF-α blockade therapy in RA patients.

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