

Effects of vector ultrasonic system debridement and conventional instrumentation on the levels of TNF- α in gingival crevicular fluid of patients with chronic periodontitis

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Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online)

Adv Clin Exp Med. 2017;26(9):1419–1424

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Funding sources

Scientific Research Projects Agency,
Coordination Unit of Dicle University,
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Conflict of interest

None declared

Acknowledgments

The present study was supported
by the Scientific Research Projects Agency,
Coordination Unit of Dicle University,
Diyarbakır, Turkey. We thank the Dicle
University for its contributions.

Received on March 18, 2016

Reviewed on July 28, 2016

Accepted on September 28, 2016

Abstract

Background. Tumor necrosis factor alpha (TNF- α) is an inflammatory mediator whose levels are increased in the gingival crevicular fluid and blood serum in the case of chronic periodontitis.

Objectives. The aim of this study was to determine the effect of vector ultrasonic system (VUS) on the levels of TNF- α in gingival crevicular fluid (GCF) and the clinical parameters in patients with chronic periodontitis.

Material and methods. The study protocol was conducted using split-mouth design in 30 patients with chronic periodontitis. VUS and scaling and root planing (S/RP) were applied separately to 2 quadrants, including the upper and the lower jaws. At baseline and after 6 months, clinical parameters including plaque index (PI), gingival index (GI), probing depth (PD), clinical attachment level (CAL) were recorded, and concentrations of TNF- α in GCF were determined by enzyme-linked immunosorbent assay (ELISA). Intergroup comparisons were evaluated by the independent Students' t-test, and the Pearson correlation was used to determine the relationship between parameters. The level of significance was set at 5%.

Results. Both treatment modalities provided statistically significant improvements in clinical periodontal parameters and TNF- α levels after 6 months ($p < 0.05$). Also, there were no significant correlations between the TNF- α levels in GCF and the clinical parameters in both treatment group at baseline and at the end of 6 months period ($p > 0.05$).

Conclusions. The use of the vector ultrasonic system in the non-surgical treatment of chronic periodontitis presents beneficial improvements for the clinical attachment level and the probing pocket depth as well as TNF- α levels in GCF.

Key words: non-surgical periodontal debridement, tumor necrosis factor alpha, chronic periodontitis

DOI

10.17219/acem/65410

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Introduction

Chronic periodontitis (CP) is a multifactorial inflammatory disease affecting the supporting tissues of teeth and is associated with loss of gingival attachment, destruction of the alveolar bone and periodontal ligament, leading to eventual tooth loss. Immune-inflammatory response has an important role in the course of chronic periodontitis.¹ Immune-inflammatory products appear in gingival crevicular fluid (GCF) and saliva, and these markers carry diagnostic information related to periodontal diseases. GCF contains oral bacteria, enzymes, leukocytes, the structure cells of periodontium, and complex-structured substances that expressed from serum. The presence of pro-inflammatory cytokines in GCF, especially interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF- α), may be an indicator of the activity of the periodontal disease.^{2,3}

TNF- α , which is an inflammatory cytokine belonging to the TNF family, has been reported to play important roles in bone resorption and the inhibition of bone formation. In animal models, it has been reported that the level of TNF- α was increased in cases of periodontitis.⁴

Hand instrumentation is known as conventional periodontal therapy, and is considered the gold standard for the non-surgical treatment of periodontal diseases. Based on conventional periodontal treatment which includes scaling and root planing (S/RP), numerous studies have reported favorable developments in both the clinical and microbial parameters.^{5,6} In spite of the successful clinical outcomes, hand instrumentation has several disadvantages, including being time-consuming and exhausting for patients as well as clinicians.⁷

Vector ultrasonic system (VUS) (Dürr Dental, Bietigheim-Bissingen, Germany) is used non-surgically for the procedures of subgingival debridement. VUS generates vibrations at a frequency of 25 kHz and has metal and fibre tips which are used on the buccal, lingual, and interdental surfaces, and in the furcation area. This instrument comprises a ring-shaped resonant body vibrated by an ultrasonic drive. The energy of vertical vibration converted by the resonating ring of the device is transmitted from the working tip to the root surface and the periodontal tissues by means of the hydroxyapatite-contained suspension and water. Thus, the root surfaces of teeth are hydrodynamically cleaned rather than coming into direct contact with the working tips.^{8–10} A study has indicated that the effects of the new vector device are the decrease of infection and a significant acceleration of the tissue healing process in peri-implantitis cases.¹¹ Moreover, in several studies, there was a significant decrease in the probing depth and bleeding upon probing and an increase of clinical attachment gain in patients with severe periodontitis treated with both VUS and hand instruments.^{12–14}

Numerous studies have demonstrated decreased levels of TNF- α following the non-surgical periodontal treatment

performed with the hand instruments.^{15,16} In the literature, there are no reports associated with the effects of vector ultrasonic system on the concentration of inflammatory mediators in patients with periodontal diseases. In light of this data, the aim of this study was to determine the effect of VUS on the levels of TNF- α in gingival crevicular fluid of patients with chronic periodontitis.

Material and methods

Patient selection

In the study, 30 patients with CP (12 females and 18 males, aged 27–66 years) were selected from among individuals who applied to the Clinic of Periodontology, Faculty of Dentistry, Dicle University (Diyarbakır, Turkey). All participants signed an informed consent form before beginning the study. The study was approved by the Ethical Committee of Dicle University and conducted in accordance with the Helsinki Declaration of 1975, as revised in 1983.¹⁷ Inclusion criteria for patient selection were: a) no systemic diseases; b) no use of antibiotics and/or anti-inflammatory drugs within 6 months prior to the treatment; c) no smoking; d) no treatment of periodontitis within 12 months prior to the treatment; e) no pregnancy and no lactation in females; and f) the presence of bone loss as detected radiographically. Patients who had at least 14 teeth and at least 2 teeth with ≥ 5 mm probing depth in each quadrant were included in the study.

Study groups

Our study was performed according to split-mouth design. Study groups were as follows:

- I – scaling and root planing (S/RP Group) by hand instruments: randomly to the selected 2 quadrants in upper and lower jaws;
- II – vector ultrasonic system (VUS Group): to the remaining 2 quadrants in upper and lower jaws (used hydroxyapatite-particled suspension for irrigation).

Study design and clinical parameters

Following oral hygiene instruction, the routine clinical periodontal indexes (plaque index (PI), gingival index (GI), probing depth (PD), and clinical attachment level (CAL) measurements) were obtained from all patients at baseline. Before treatment, GCF samples were collected from the approximal site of a single-rooted tooth at least 5 mm or more probing depth. Teeth that had any fixed prosthesis, endodontic-periodontal lesion, filling materials, or caries were excluded for the GCF sampling. The measurement of clinical parameters and GCF samplings were repeated after 6 months by 1 blinded examiner.

Measurement of clinical parameters

Silness and Löe Plaque Index (PI) and Löe and Silness Gingival Index (GI) were taken from all existing teeth out of the 3rd molars in each patient.^{18,19} Probing depth measurement, which is the distance from gingival margin to the bottom of the gingival sulcus, was obtained from the mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual surfaces of each tooth. Also, the clinical attachment level was measured from the 6 surfaces in the same manner as the probing depth, but its value was the distance between the cemento-enamel junction and the bottom of the gingival sulcus. Clinical measurements such as PD and CAL were obtained using a periodontal probe (PCPUNC 15® Hu-Friedy, Chicago, USA).

Non-surgical periodontal treatment

On the 1st day of treatment, S/RP was performed using the Gracey curettes (Hu Friedy, Chicago, USA) under local anesthesia in the 2 quadrants as mentioned above until the visible and detectable deposits on the root surfaces of teeth no longer remained. Twenty-four hours after the 1st appointment, the remaining 2 quadrants were treated using VUS (Dürr Dental, Bietigheim-Bissingen, Germany). Seven LED lights and metal Paro Probe tips were used for the VUS group.

The premature contacts were removed and false restorations, e.g., fillings and fixed partial prosthesis, were corrected. Because the medication may positively affect the healing of periodontal tissues, any antibiotic and antimicrobial drugs were not prescribed. All the treatment procedures were performed by the same surgeons.

Collection of GCF samples

Two absorbent paper strips (Periopaper, Amityville, USA) were used to collect the GCF from 2 quadrants of each patient in this study. Before the collection of GCF, the sites were isolated with cotton rolls and gently dried using an air syringe. To collect GCF samples, periopaper strips were placed into the gingival sulcus for 30 s according to the shallow intra-crevicular technique.²⁰ Then, the periopaper strips were delivered into the eppendorf tubes filled with 200 µL of phosphate buffer saline (pH = 7.4). They were preserved at -80°C until the evaluation of TNF-α levels.

Determination of TNF-α levels

Sandwich enzyme immunoassay of ELISA (Human TNF-alpha Platinum ELISA-Bender Medsystems, GmbH, Vienna, Austria) was used to evaluate the concentration of TNF-α in the gingival crevicular fluid. The test was performed according to kit instructions. A total of 50 µL

from each standard fluid or patient sample was added to each well in duplicate, and 50 µL of the sample diluent was added to each well. Then 50 µL of the biotinylated antibody reagent was added to each well and incubated for 1 h at room temperature, 20–25°C. Plates were then washed 4 times with a washing solution. Then, 100 µL of TMB substrate solution was added and incubated for 10 min at room temperature, in the dark. In order to stop the reaction, 100 µL of stop solution was added to each well. Absorbances were measured on an automated ELISA plate reader (Dynex, DSX, Chantilly, USA) set at 450 nm wavelength. The standard curve was generated by plotting the average absorbance obtained for each standard concentration on the vertical (Y) axis vs the corresponding TNF-α concentration on the horizontal (X) axis. The amount of TNF-α in each sample was determined with this curve as pg/µL.

Statistical analysis

Normal distribution and homogeneity of the data were verified with the Kolmogorov-Smirnov test and the Levene's test, and Repeated Measures ANOVA test was performed for pairwise comparisons. For intergroup comparisons, the Student's t-test for dependent and independent samples was used to analyze the data. Pearson's correlation was used to determine the relationship between parameters. Statistical analyses were performed using the SPSS v. 15.0 for Windows (SPSS, Inc., Chicago, USA). The level of significance was set at 5%.

Results

At baseline, this study began with 35 participants. But 3 patients did not return for the follow-up visits and 2 patients took medication that was excluded by the study, resulting in total of 5 patients being excluded from the study. Therefore, the study was pursued with 30 patients throughout the 6-month follow-up period. Gender, age range, and mean values belonging to the subjects are shown in Table 1.

The clinical parameters of the full-mouth and the sampling sites include PI, GI, PD, and CAL, significantly decreased in the 2 groups. Moreover, the levels of TNF-α

Table 1. Demographic characteristics of the patient population

Gender	n	Age range		mean ±SD
		min	max	
Females	12	27	48	34.66 ±5.38
Males	18	32	66	42.61 ±8.99
Total	30	27	66	39.43 ±8.61

n – number of subjects; min – minimum; max – maximum; SD – standard deviation.

obtained from the sampling sites also decreased depending on the improvement of clinical parameters (Tables 2, 3).

Based on improvements in PD and gain in CAL for the full-mouth and sampling sites, there were significant differences in the 6 months compared to the baseline

Table 2. Comparison of full-mouth clinical parameters and TNF- α levels belonging to S/RP and VUS group at baseline and after 6 months between and within the groups

Parameters/ treatment groups	Baseline (mean \pm SD)	6 months (mean \pm SD)	p-value
PI			
VUS	1.95 \pm 0.57	0.41 \pm 0.23	<0.001
S/RP	2.01 \pm 0.52	0.44 \pm 0.22	<0.001
p-value	0.699	0.584	
GI			
VUS	1.72 \pm 0.37	0.28 \pm 0.20	<0.001
S/RP	1.74 \pm 0.35	0.27 \pm 0.16	<0.001
p-value	0.820	0.827	
PD			
VUS	3.62 \pm 0.71	2.30 \pm 0.32	<0.001
S/RP	3.74 \pm 0.55	2.27 \pm 0.23	<0.001
p-value	0.464	0.693	
CAL			
VUS	3.96 \pm 0.80	3.07 \pm 0.62	<0.001
S/RP	4.07 \pm 0.65	3.06 \pm 0.65	<0.001
p-value	0.560	0.922	
TNF-α			
VUS	3.65 \pm 0.42	3.04 \pm 0.20	<0.001
S/RP	3.75 \pm 0.56	3.01 \pm 0.16	<0.001
p-value	0.437	0.353	

PI – plaque index; GI – gingival index; PD – probing depth; CAL – clinical attachment level; TNF- α – tumor necrosis factor alpha; VUS – vector ultrasonic system; S/RP – scaling and root planning; SD – standard deviation.

Table 3. Comparison of clinical parameters relating to sampling sites at baseline and after 6 months between and within the groups

Parameters/ treatment groups	Baseline (mean \pm SD)	6 months (mean \pm SD)	p-value
PI			
VUS	2.17 \pm 0.53	0.30 \pm 0.46	<0.001
S/RP	2.20 \pm 0.40	0.37 \pm 0.49	<0.001
p-value	0.861	0.585	
GI			
VUS	1.77 \pm 0.50	0.40 \pm 0.62	<0.001
S/RP	1.97 \pm 0.41	0.40 \pm 0.62	<0.001
p-value	0.943	1.00	
PD			
VUS	6.30 \pm 1.60	2.93 \pm 0.11	<0.001
S/RP	6.43 \pm 1.31	2.87 \pm 0.68	<0.001
p-value	0.502	0.716	
CAL			
VUS	6.37 \pm 1.58	3.47 \pm 0.54	<0.001
S/RP	6.63 \pm 1.62	3.57 \pm 1.71	<0.001
p-value	0.442	0.927	

PI – plaque index; GI – gingival index; PD – probing depth; CAL – clinical attachment level; VUS – vector ultrasonic system; S/RP – scaling and root planning; SD – standard deviation.

measurements for the 2 groups. When the groups were compared to each other, these parameters had no statistically significant differences. The average amounts of PD reduction and CAL gain at 6-month time point is presented in Table 4.

The correlation between the levels of TNF- α with the clinical parameters of both the full-mouth and sampling sites was analyzed. There was no statistically significant correlation between TNF- α levels and PI, GI, PD, and CAL (Tables 5, 6).

Discussion

The causes of chronic periodontitis may be microbial plaque biofilm, food debris, and/or dental calculus accumulating on the surface of teeth. The mechanical removal of these deposits, which is performed by hand and power-driven instruments, is essential for the treatment of chronic periodontitis.²¹ Several studies showed that hand instrumentation could promote microbial and clinical periodontal parameters. The efficiency of VUS for the removal of microbial biofilms has been demonstrated to be as good as conventional periodontal treatment in a study performed by some investigators.²² The elimination of predisposing factors, including calculus, dental stains, false restorations, and similar factors for the retention of dental plaque in both the VUS and S/RP groups showed to facilitate the effective brushing of the patients. The findings of our study related to dental plaque scores were in accordance with various other studies in which different methods were applied for the non-surgical periodontal treatment.^{13,22,23}

During inflammation, observable changes occur in the gingival tissues. To determine the extent of these gingival changes subjectively, different gingival indexes are used, such as the Löe and Silness Gingival index.¹⁹ With respect to our gingival index scores, it was determined that the S/RP and VUS groups had statistically significant improvements in their gingival tissues at the end of 6 months, but there was a similarity between the 2 groups in gingival and dental plaque scores. These results comply with those of several other studies.^{22,24}

It was observed that there was a statistically significant reduction in the mean values of PD from the baseline to the 6th month in the S/RP and VUS groups. In the comparison of the groups, the mean values of PD in the S/RP group declined from 3.74 \pm 0.55 mm to 2.27 \pm 0.23 mm. Similarly, the VUS group demonstrated a reduction of mean PD values from 3.62 \pm 0.71 mm to 2.30 \pm 0.32 mm. In the split-mouth design study performed by Christgau et al. who examined the efficiency of VUS and S/RP in a group of 20 patients with chronic periodontitis, similar differences were observed between the 2 groups at the end of 6-month period.²² In another study,

the results showed that mean PD changed in the VUS group from 4.5 ± 0.5 mm to 3.7 ± 1.2 mm and in the SRP group from 4.5 ± 0.3 mm to 3.4 ± 1.1 mm.¹⁴ In the study conducted by Guentsch et al., a decrease of probing depth was observed from 5.20 ± 0.70 mm to 2.40 ± 0.57 mm in the VUS group, and from 5.12 ± 0.60 mm to 2.33 ± 0.32 mm in the S/RP group.²⁵ These decreases were statistically significant at the end of 6-month period in comparison to the baseline. In the present study, the results associated with PD reduction were similar to those of the above-mentioned studies. Especially, the PD reductions related to the sampling sites were more pronounced.

The difference between the average values of CAL at baseline and 6 months is interpreted as a gain in attachment level. The full-mouth mean attachment gain was statistically significant at the 6th month as compared to baseline both in the VUS and in the S/RP, 0.88 ± 0.66 mm and 1.01 ± 0.63 mm, respectively ($p < 0.05$). There were no statistically significant differences between

the 2 groups ($p > 0.05$). Based on the sampling sites, mean CAL values were reduced from 6.37 ± 1.58 mm at baseline to 3.47 ± 0.54 mm at 6 months in the VUS group, whereas in the S/RP group they were reduced from 6.63 ± 1.62 mm to 3.57 ± 1.71 mm. The measurement of CAL gains obtained at the end of 6-month period were 2.90 ± 1.24 mm in the VUS group and 3.06 ± 1.31 mm in the S/RP group. These changes were statistically significant at the 6th month for the 2 groups. Numerous studies demonstrated significant improvements in terms of clinical attachment levels which are similar to those of our study. In a study, especially at deep sites (>5 mm probing depth), the S/RP group showed a CAL gain of 0.7 ± 0.4 mm, while the VUS group showed a CAL gain of 0.6 ± 0.4 mm at the end of 6-month period postoperatively. The findings of CAL gain in this study showed that the non-surgical periodontal treatment carried out with the hand instruments and the VUS may result in significant improvements.^{10,14,26} Some studies showed better results in terms of full-mouth CAL gain

when compared with our study.^{12,27}

These disparities in the gain of attachment level are based on differences in the initial probing pocket depth. As the probing pocket depth increases without gingival recession, the clinical attachment gain increases after proper periodontal therapy.¹⁰

Although some periodontal parameters including plaque index, gingival index, bleeding on probing, probing pocket depth, clinical attachment level, alveolar bone loss, etc. might provide useful information about the severity of periodontal disease, these parameters are not the indicators of the activity of the disease. Therefore, different methodologies have been used, such as biochemical and immunologic diagnostic tests, which analyze the levels of numerous inflammatory mediators in GCF samples.²⁸ In particular, the increase of TNF- α levels in GCF is a sign of periodontal inflammation.²⁹ Gamonal et al. detected an increase in the levels of TNF- α in the GCF of patients with chronic periodontitis as compared to healthy subjects.³⁰

In the present study, a decrease of GCF-TNF- α levels was observed at the end of 6-month period, when compared to baseline ($p < 0.05$). However, at the same time, there were no statistically significant

Table 4. Mean values of PD reduction and CAL gain from baseline to 6th month

Parameters	Full-mouth			Sampling sites		
	S/RP (mean \pm SD)	VUS (mean \pm SD)	p-value	S/RP (mean \pm SD)	VUS (mean \pm SD)	p-value
PD	1.46 ± 0.49	1.31 ± 0.52	0.261	3.56 ± 1.35	3.36 ± 1.21	0.572
CALg	1.01 ± 0.63	0.88 ± 0.66	0.448	3.06 ± 1.31	2.90 ± 1.24	0.671

PD – probing depth; CALg – clinical attachment level gain; VUS – vector ultrasonic system; S/RP – scaling and root planning; SD – standard deviation.

Table 5. Correlations between full-mouth clinical parameters and TNF- α levels at different time points

Parameters	Baseline				6 months			
	S/RP		VUS		S/RP		VUS	
	r	p	r	p	r	p	r	p
PI-TNF- α	-0.151	0.427	-0.027	0.888	-0.304	0.102	0.232	0.218
GI-TNF- α	-0.125	0.511	0.079	0.678	-0.153	0.421	0.174	0.359
PD-TNF- α	-0.141	0.475	0.145	0.445	-0.110	0.563	0.337	0.069
CAL-TNF- α	-0.222	0.237	0.068	0.721	0.191	0.313	0.145	0.446

PI – plaque index; GI – gingival index; PD – probing depth; CAL – clinical attachment level; TNF- α – tumor necrosis factor alpha; VUS – vector ultrasonic system; S/RP – scaling and root planning; significance level $p < 0.05$; r – Pearson's correlation coefficient.

Table 6. Correlations between clinical parameters of sampling sites and TNF- α levels at baseline and after 6 months

Parameters	Baseline				6 months			
	S/RP		VUS		S/RP		VUS	
	r	p	r	p	r	p	r	p
PI-TNF- α	0.125	0.510	0.043	0.822	-0.204	0.280	0.265	0.157
GI-TNF- α	-0.016	0.934	-0.113	0.553	-0.025	0.897	0.059	0.757
PD-TNF- α	0.083	0.664	-0.008	0.966	-0.067	0.725	0.238	0.205
CAL-TNF- α	0.085	0.654	0.071	0.709	0.171	0.368	0.253	0.177

PI – plaque index; GI – gingival index; PD – probing depth; CAL – clinical attachment level; TNF- α – tumor necrosis factor alpha; VUS – vector ultrasonic system; S/RP – scaling and root planning; significance level $p < 0.05$; r – Pearson's correlation coefficient.

differences between the groups ($p > 0.05$). Unfortunately, because there were no similar reports using the method of our study in the literature, we could not compare the results related to TNF- α levels in the VUS group. There was no correlation between GCF-TNF- α levels and the clinical periodontal parameters, neither at baseline nor after 6 months for the 2 groups. In the study, which performed a non-surgical treatment of chronic periodontitis by S/RP, Erdemir et al. did not observe any correlation at the 6-month follow-up.³¹ These findings support the view that local expressions of inflammatory mediators vary from site to site and from subject to subject.³²

In conclusion, the use of the vector ultrasonic system for non-surgical periodontal treatment presents beneficial improvements in the clinical attachment level and the probing pocket depth as well as TNF- α levels. Although no significant differences were found in the 2 groups, the decrease of TNF- α levels in the S/RP group was a slightly better than in the VUS group. To reach a definitive judgment on the relationship between the levels of TNF- α and treatment type, we believe that further studies are needed.

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