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The effect of low-level laser therapy as an adjunct to non-surgical periodontal treatment on gingival crevicular fluid levels of transforming growth factor-beta 1, tissue plasminogen activator and plasminogen activator inhibitor 1 in smoking and non-smoking chronic periodontitis patients: A split-mouth, randomized control study

F. Pamuk[1,2] | M. Lütfioğlu[3] | A. Aydoğdu[4] | C. Z. Koyuncuoglu[2] | E. Cifcibasi[5] | O. S. Badur[6]

[1]Department of Periodontology, Faculty of Dentistry, Yeditepe Univesity, Istanbul, Turkey
[2]Department of Periodontology, Faculty of Dentistry, Istanbul Aydin University, Istanbul, Turkey
[3]Department of Periodontology, Faculty of Dentistry, Ondokuzmayis University, Samsun, Turkey
[4]Department of Periodontology, Faculty of Dentistry, Istanbul Research and Application Center, Baskent University, Istanbul, Turkey
[5]Department of Periodontology, Faculty of Dentistry, Istanbul University, Istanbul, Turkey
[6]Division of Virology and Immunology, Department of Microbiology, Faculty of Medicine, Istanbul University, Istanbul, Turkey

Correspondence
Muge Lütfioğlu, Department of Periodontology, Faculty of Dentistry, Ondokuzmayis University Samsun, Turkey.
Email: mugelutfioglu@hotmail.com

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Background and Objective: This study aimed to investigate the effects of low-level laser therapy (LLLT) as an adjunct to scaling and root planing (SRP) on smoking and non-smoking patients with chronic periodontitis.

Material and Methods: The study was conducted using a split-mouth design with 30 patients with chronic periodontitis (15 smokers, 15 non-smokers) and 30 healthy individuals matched for age, sex and smoking status as controls. Groups were constituted as follows: Cp+SRP+Sham: non-smokers with chronic periodontitis treated with SRP; Cp+SRP+LLLT: non-smokers with chronic periodontitis treated with SRP+LLLT; SCp+SRP+Sham: smokers with chronic periodontitis treated with SRP; SCp+SRP+LLLT: smokers with chronic periodontitis treated with SRP+LLLT; C: control group comprised of periodontally healthy non-smokers; SC: control group comprised of periodontally healthy smokers. LLLT was first applied on the same day as SRP and again on days 2 and 7 after SRP treatment. Clinical parameters were recorded before non-surgical periodontal treatment (baseline) and on day 30. Gingival crevicular fluid samples were collected before periodontal treatment (baseline) and during follow-up visits on days 7, 14 and 30. Gingival crevicular fluid transforming growth factor (TGF)-β1, tissue plasminogen activator (tPA) and plasminogen activator inhibitor 1 (PAI-1) levels were measured using enzyme-linked immunosorbent assay.

Results: All clinical parameters showed significant reductions between baseline and day 30 following SRP treatment in both the LLLT and sham groups ($P<.001$). No significant differences were observed between the LLLT and sham groups of either the smokers or non-smokers ($P>.05$). Gingival crevicular fluid PAI-1 levels decreased significantly in the SCp+SRP+sham and SCp+SRP+LLLT groups ($P<.05$), and gingival crevicular fluid tPA levels decreased significantly in the Cp+SRP+sham, Cp+SRP+LLLT and SCp+SRP+LLLT groups ($P<.05$). Gingival crevicular fluid TGF-β1 levels decreased significantly in all treatment groups ($P<.05$). Although no significant differences were found between the gingival crevicular fluid PAI-1, tPA and TGF-β1 levels of the LLLT versus sham groups (P>.05) at any of the time points measured, both LLLT groups showed significant reductions in tPA/PAI-1 ratios over time.

Conclusion: Within the limits of this study, LLLT may be understood to play a role in the modulation of periodontal tissue tPA and PAI-1 gingival crevicular fluid levels, particularly in smoking patients with chronic periodontitis, and may thus be recommended as an adjunct to non-surgical periodontal treatment.

KEYWORDS

low-level laser therapy, periodontitis, plasminogen activator inhibitor 1, smoking, tissue plasminogen activator, transforming growth factor

1 | INTRODUCTION

Periodontitis is an infectious oral disease characterized by clinical attachment loss, alveolar bone resorption, periodontal pocketing and gingival inflammation. Treatment aims include the elimination of infection, arrest of disease progression and regeneration of the periodontium. These aims can be achieved through non-surgical periodontal treatment that involves (i) altering or eliminating the microbial etiology and other contributing factors by performing scaling and root planing (SRP) to debride pockets/roots effectively, and (ii) establishing an appropriate self-performed supragingival plaque-control regimen.[1] Because furcations, grooves and deep pockets can be difficult to access with conventional non-surgical periodontal treatment,[2,3] low-level laser therapy (LLLT) has been proposed as an adjunct or alternative. Also known as soft laser therapy, biostimulation or photobiomodulation, LLLT entails exposure to low-level laser light with the main aims of achieving tissue ablation and hemostasis, eliminating periodontal pathogens, enhancing tissue growth and regeneration, resolving inflammation, reducing pain and promoting wound healing.[3-5]

LLLT has been shown to possess biostimulatory action on various cell and tissue types through its ability to affect the mitochondrial respiratory chain, thereby increasing adenosine triphosphate production, which in turn facilitates fibroblast proliferation, angiogenesis, growth factor release and collagen synthesis.[6] However, few clinical trials have investigated the benefits of LLLT used as an adjunct to non-surgical periodontal treatment, and conflicting results have been reported.[7-10]

The proteolytic events occurring in the extracellular matrix of periodontal tissue are controlled by complex biological processes regulated by interactions between cells and growth factors that trigger a series of intracellular events culminating in new tissue formation.[8] Growth factors such as transforming growth factor-beta 1 (TGF-β1) and enzymes such as tissue plasminogen activator (tPA) and plasminogen activator inhibitor 1 (PAI-1) are responsible for regulating the local inflammatory reactions as well as the synthesis of specific extracellular matrix molecules by fibroblasts, angiogenesis and re-epithelization that occur during tissue repair and remodelling.[8,11] TGF-β1 possesses both proinflammatory and anti-inflammatory characteristics and plays a role in cell proliferation and differentiation as well as regulation of inflammatory responses, making it an important cytokine in terms of wound healing, tissue remodeling and tissue regeneration.[12] Not only does TGF-β1 inhibit destruction of extracellular matrix proteins, it is also a potent stimulator of extracellular matrix protein synthesis and PAI-1 secretion.[12,13]

The plasminogen-activating (PA) system remains in balance through the activities of PAs such as urokinase and tPA and PAIs such as PAI-1 and PAI-2.[14,15] These components of the PA system have been shown to contribute to periodontal connective-tissue degradation and cell migration as well as the entire process of periodontal wound healing.[14,15] PAs and PAIs have previously been detected and studied in gingival crevicular fluid.[16] Based on the increases in tPA levels found in the gingival tissue and gingival crevicular fluid samples taken from patients with periodontal disease, Kinnby[16] suggested that the PA system could contribute to the tissue destruction seen in periodontal disease.

At the same time, smoking has been shown to be an important risk factor for periodontal disease and is known to affect the disease's pathogenesis.[17-19] By impairing neutrophil function, antibody production, fibroblast activity and inflammatory mediator production, smoking affects the oral environment and ecology, including the inflammatory response, immune response and homeostasis of gingival tissue and vasculature and the healing potential of periodontal tissue.[17,20] Thus, while periodontal pathogens are more or less the same in smoking and non-smoking patients,[11,21] smokers tend to respond less favorably to periodontal treatment.[11,22]

To the best of our knowledge, the relationship among smoking, the PA system and LLLT in patients with chronic periodontitis receiving non-surgical periodontal treatment has not been evaluated. Therefore, the present study investigated the effects of LLLT used as an adjunct to non-surgical periodontal treatment by evaluating clinical parameters and TGF-β1, tPA and PAI-1 gingival crevicular fluid levels in smoking and non-smoking patients with chronic periodontitis.

2 | MATERIAL AND METHODS

2.1 | Subject selection and study design

The study was designed as a split-mouth randomized controlled clinical study. The study protocol was approved by the Local Ethics

Committee of Istanbul University (no. 2013/1225), and written informed consent was obtained from all study participants in accordance with the Helsinki Declaration (1975; revised, 2002). Participants were recruited from among those individuals applying consecutively to the Aydin University Dental Faculty's Department of Periodontology between April and September 2014.

Inclusion criteria were as follows: (i) ≥35 years of age and having ≥16 teeth and generalized chronic periodontitis; (ii) no periodontal therapy in the 6 months before data collection; and (iii) no systemic problems or chemotherapy within the 6 weeks before data collection. Exclusion criteria were as follows: (i) medical history of cancer, rheumatoid arthritis, diabetes mellitus or cardiovascular disease; (ii) compromised immune system; (iii) pregnancy, menopause or lactation; (iv) ongoing drug therapy that might affect the clinical characteristics of periodontitis; (v) use of systemic antimicrobials during the 6 weeks before data collection; and (vi) dental treatment during the 6 months before data collection.

Periodontal status was assessed by clinical examination, with classifications determined according to criteria proposed by the 1999 International World Workshop for a Classification of Periodontal Disease and Conditions.[23] Participants were classified as periodontally healthy if they had a mean gingival index <1, mean percentage of bleeding on probing ≤25%, and no sites of attachment loss. Participants were classified as having generalized chronic periodontitis if they had ≥30% of sites with both probing depth ≥5 mm and clinical attachment levels ≥5 mm as well as ≥30% radiographically observable alveolar bone loss. Participants were classified as smokers if they smoked ≥15 cigarettes per day for ≥5 years and as non-smokers if they had no previous history of smoking.

Of a total 356 individuals screened, 60 were included in the split-mouth designed study and the sites were split due to application of different treatment modalities as follows:

Cp/SRP+sham (n=15, split-mouth): non-smokers with chronic periodontitis receiving SRP only

Cp/SRP+LLLT (n=15, split-mouth): non-smokers with chronic periodontitis receiving SRP and LLLT

SCp/SRP+sham (n=15, split-mouth): smokers with chronic periodontitis receiving SRP only

SCp/SRP+LLLT (n=15, split-mouth): smokers with chronic periodontitis receiving SRP and LLLT

C (n=15): periodontally healthy non-smoking age-/gender-matched control group

SC (n=15): periodontally healthy smoking age-/gender-matched control group

2.3 | Non-surgical periodontal treatment

All participants with chronic periodontitis received non-surgical periodontal treatment from the same periodontist blinded to the study groups. Non-surgical periodontal treatment consisted of full-mouth SRP performed in a single appointment under local anesthesia using scalers, curettes and ultrasonic devices. Supragingival polishing was also performed; however, subgingival irrigation, mouth rinsing with chlorhexidine and tongue brushing with chlorhexidine were not performed. Oral hygiene instruction was provided and consisted of a demonstration of a modified Bass brushing technique and the use of dental floss and interdental brushes. Subjects were instructed not to use any antimicrobial mouth rinsing solutions for the duration of the study.

2.4 | Low-level laser treatment and sham application

Following SRP, either the left or right side of each patient with chronic periodontitis was randomly selected (by coin toss) to receive LLLT or sham treatment, both of which were provided by a single periodontist who did not perform the SRP treatment. LLLT was performed three times (on the same day as SRP and on the second and seventh days after treatment) using a 940 nm indium gallium arsenide phosphorous diode laser (Epic Biolase, Irvine, CA, USA) applied perpendicularly to the periodontal pocket for 20 seconds at a constant distance of 15 mm and with a continuous wavelength (3.41 J/cm^2 delivery with a 1.76 cm^2 spot and average output of 0.3 W). Sham application was provided by simulating laser application without pushing the start button on the laser device.

2.5 | Clinical measurements and gingival crevicular fluid sampling

Clinical measurements and gingival crevicular fluid sampling were performed by a single clinician who did not perform the LLLT/sham treatment. Clinical measurements were performed before SRP and on day 30 of follow-up. The following clinical parameters were recorded: Silness and Löe plaque index[24]; Löe and Silness gingival index[25]; probing depth; clinical attachment level; bleeding on probing. A Williams periodontal probe (Nordent Manufacturing Inc., Elk Grove Village, IL, USA) calibrated in mm was used to measure six sites on each tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, disto-lingual locations). For each subject, the five teeth with the highest probing depth and clinical attachment level values were identified, and the additional clinical measurements and gingival crevicular fluid sampling were performed on these teeth and the contralateral teeth (total: 10 teeth). (Radiographically observed alveolar bone loss confirmed the clinical identification of the probing depth of the sampling sites.) Gingival crevicular fluid samples were collected before SRP treatment and on days 7, 14 and 30 of follow-up. Sampling sites were gently air-dried, isolated with cotton rolls, saliva contamination was prevented and supragingival plaque, if present, was removed using a sterile curette. Gingival crevicular fluid was sampled by placing a strip of filter paper (Periopaper; ProFlow, Inc., Amityville, NY, USA) into the crevice until mild resistance was felt and then leaving it in position for 30 seconds. The strips contaminated with blood were discarded. The gingival crevicular fluid volume of each strip was determined by electronic impedance (Periotron 8000; ProFlow, Inc.), and samples were then placed in sterile polypropylene tubes and stored at −70°C until analysis.

2.6 | Gingival crevicular fluid enzyme-linked immunosorbent assay analysis for tissue plasminogen activator, plasminogen activator inhibitor 1 and transforming growth factor

Gingival crevicular fluid elution was performed according to Curtis et al.[26] with a slight modification. A total of 150 μL 2% bovine serum albumin (0.01 M, pH 7.2) in phosphate-buffered saline was added to each tube, and the samples were incubated at 4°C for 60 minutes. Following incubation, a sterile drill was used to bore a hole in the bottom of each tube, which was then placed inside a 1.5 mL tube, and the nested tubes were centrifuged at 10 000 *g* for 10 minutes at 4°C.

Amounts of tPA (tPA enzyme-linked immunosorbent assay [ELISA] kit; American Diagnostica catalog no. 860; Biocompare, South San Francisco, CA, USA), PAI-1 (IMUBIND PAI-1 ELISA Kit catalog no. 822; Sekisui Diagnostics, Lexington, KT, USA) and TGF-β1(TGF-β1 ELISA Kit Diaclone, catalog no. 650.010.096; Diaclone SAS, Besancon Cedex, France) in each gingival crevicular fluid sample were evaluated using standard ELISA according to the manufacturers' instructions. Reactions were terminated by the addition of an acid solution, and color change was measured spectrophotometrically at a wavelength of 450 nm. TGF-β1, tPA and PAI-1 were identified using the standard curves. Total amounts (pg/30 s) of tPA, PAI-1 and TGF-β1 collected from each sample in a 30-second period were calculated and recorded for analysis.

2.7 | Statistical analysis and sample-size calculation

Statistical analysis was performed using the statistical software program SPSS (SPSS v.21.0; IBM Corp., Armonk, NY, USA). A Shapiro-Wilk test showed non-normal distribution; thus, non-parametric analysis was used to compare results among groups, which are presented as medians and 25-75 percentiles (quarters). Differences within groups (by time) were evaluated by the Friedman test and Wilcoxon signed rank test with Bonferroni correction; differences between groups were evaluated with a Kruskal-Wallis test and Mann-Whitney *U* test with Bonferroni correction; and differences between genders were evaluated with a chi-squared test. $P<.05$ was considered statistically significant. The sample size required to ensure adequate power for this study was calculated based on changes in clinical attachment levels.[27] A minimum of 15 patients per group was identified to ensure an accuracy of $\alpha=0.05$ at a confidence level of 82%.

3 | RESULTS

All participants completed the 1 month treatment and follow-up period without missing any appointments. Healing was uneventful in all cases. No adverse effects (eg, burning sensation, pain) related to laser irradiation were reported.

3.1 | Clinical assessments

Pre-treatment and post-treatment clinical measurements are given in Table 1.

All clinical parameters showed statistically significant reductions between baseline and day 30 in the LLLT as well as the sham groups. Reductions in clinical parameters did not vary significantly between the LLLT and sham groups or between the smoking and non-smoking groups.

3.2 | Biochemical analysis

The results of biochemical analysis are given in Table 2.

3.2.1 | Gingival crevicular fluid tissue plasminogen activator

Gingival crevicular fluid tPA levels showed significant reductions at all times in each of the chronic periodontitis groups except the SCp+SRP+sham group. Changes in gingival crevicular fluid tPA levels did not vary significantly between LLLT and sham groups (Table 2). When compared to the control groups, gingival crevicular fluid tPA levels were significantly higher in the chronic periodontitis groups at baseline; however, these differences between the treatment and control groups were no longer observed after day 7 in the smoking and non-smoking LLLT groups and after day 14 in the smoking and non-smoking sham groups (Figure 1).

3.2.2 | Gingival crevicular fluid plasminogen activator inhibitor 1

Gingival crevicular fluid PAI levels showed steady reductions over time in both the LLLT and sham groups of smokers as well as non-smokers, but the differences between time points were statistically significant ($P<.05$) only for smokers (SCp/SRP+sham and SCp/SRP+LLLT) (Table 2). Changes in gingival crevicular fluid PAI-1 levels did not vary significantly between LLLT and sham groups. When compared to the control groups, gingival crevicular fluid PAI-1 levels remained significantly higher in the chronic periodontitis groups at all times (Figure 2).

3.3.3 | Gingival crevicular fluid transforming growth factor-beta

Gingival crevicular fluid TGF-β1 levels showed significant reductions at all times in each of the chronic periodontitis groups, with no significant differences in the changes observed between the LLLT and sham groups (Table 2). When compared to the control groups, the chronic periodontitis groups had significantly higher gingival crevicular fluid TGF-β1 levels at all times while these differences between the treatment and control groups were no longer observed after day 14 (Figure 3).

TABLE 1 Clinical assessments of sampling sites of the study groups before and after treatment

Groups	Before treatment					After treatment					P**
	GI	PI	BOP	PPD	CAL	GI	PI	BOP	PPD	CAL	
Cp/SRP+Sham	2.00 (2.00-2.00)	1.33 (0.84-2.00)	100 (100-100)	6.67 (5.17-7.00)	7.0 (5.83-7.33)	1.68 (1)	0.67 (0.00-0.69)	66.67 (50.00-83.34)	4.33 (3.00-4.84)	5.00 (4.00-5.84)	**<.001****
Cp/SRP+LLLT	2.00 (2.00-2.00)	2.00 (1.0-2.00)	100 (100-100)	6.00 (5.00-6.84)	6.68 (5.67-7.01)	1.00 (0.68-1.51)	0.33 (0.00-1.33)	33.33 (25.33-66.67)	3.68 (3.33-4.33)	5.00 (4.00-5.33)	**<.001****
P*	>.05	>.05	>.05	>.05	>.05	>.05	>.05	>.05	>.05	>.05	
SCp/SRP+Sham	2.00 (2.00-2.00)	2.00 (1.67-2.00)	100 (100-100)	6.17 (5.67-7.33)	7.34 (6.33-9.00)	1.3 (1.33-1.67)	0.67 (0.33-1.33)	58.97 (33.33-66.67)	4.97 (3.67-6.00)	6.33 (4.67-8.00)	**<.001****
SCp/SRP+LLLT	2.00 (2.00-2.00)	2.00 (1.67-2.00)	100 (100-100)	6.67 (5.33-7.33)	7.17 (5.66-8.33)	1.00 (0.67-1.67)	0.67 (0.00-1.67)	50.00 (0.00-66.67)	5.50 (3.33-6.00)	6.52 (4.33-7.00)	**<.001****
P*	>.05	>.05	>.05	>.05	>.05	>.05	>.05	>.05	>.05	>.05	

BOP, bleeding on probing; CAL, clinical attachment level; GI, gingival index; PI, plaque index; PPD, probing depth.Values are given as median (first and third quartiles).
Bold indicates statistically significant paired-group comparisons before-after treatment with Wilcoxon Signed Rank Test (parameters in the columns).
*Inter-group comparisons due to study groups with Kruskal-Wallis test (parameters in the lines).
**Paired-group comparisons before-after treatment with Wilcoxon signed rank test (parameters in the columns).

3.3.4 | Tissue plasminogen activator/plasminogen activator inhibitor 1 ratios

In both LLLT groups, tPA/PAI-1 ratios showed a significant decrease (*P*<.05) from baseline, indicating a reduction in fibrinolytic activity. However, this decrease was detected on day 7 in smokers and, on both days 7 and 14 in non-smokers (Table 3).

4 | DISCUSSION

The use of diode lasers or any kind of low-level laser irradiation either as a monotherapy or as an adjunct to SRP is still somewhat controversial.[28] Ineffective results from LLLT have been reported mainly when performed on healthy individuals,[29,30] whereas several studies have suggested that improved healing occurs in compromised wounds, such as diabetic wounds or wounds in which healing is delayed.[31-34] Given the current lack of clarity on this subject, this study looked at the effects of LLLT used as an adjunct to SRP in smoking and non-smoking patients with chronic periodontitis by examining clinical parameters as well as inflammatory markers. In all cases, postoperative healing was uneventful, and no complications (abscesses, infections, dentin hypersensitivity) were observed. While LLLT did not result in any additional clinical benefits, decreases observed in tPA and PAI-1 gingival crevicular fluid levels suggest that LLLT may play a role in the healing of periodontal tissue, particularly in smokers with chronic periodontitis.

In the present study, clinical parameters of patients receiving non-surgical periodontal treatment showed statistically significant improvements over a 1-month period following initial treatment. These changes were not affected by the use of LLLT as an adjunct therapy and did not vary between smoking and non-smoking subjects. These findings are in line with Lai et al.,[9] Calderin et al.[10] and De Micheli et al.,[35] who also reported that diode lasers did not provide any additional clinical benefits over conventional treatment. In contrast, several previous studies[7,8,27] have reported significantly better outcomes when laser treatment is used as an adjunct to non-surgical treatment. The differences in findings could be due to the short examination period in our study, which might not have been long enough to detect re-epithelization, regeneration of epithelial attachment and healing of connective tissue.[36] Differences in study findings could also be related to differences in clinical characteristics of the study populations; for example, in our study, the patients with periodontitis had relatively moderate mean pocket depths (<7 mm) and attachment losses, whereas previous studies[37] have reported the effects of conventional non-surgical periodontal treatment with or without adjunct laser treatment to be more apparent in subjects with deep pockets.

The PA system has been implicated in wound healing and tissue remodeling as well as in the initiation and progression of periodontal disease.[38] Since, in addition to fibrin, plasmin also cleaves to other components of the extracellular matrix, such as fibronectin, laminin and proteoglycans, strict control of plasminogenic activity is needed to maintain tissue integrity.[14] Previous studies have suggested that

TABLE 2 Biochemical assessment of the gingival crevicular fluid of the sampling sites

Variables	Groups					
	Cp/SRP+Sham N=15	Cp/SRP+LLLT N=15	SCp/SRP+Sham N=15	SCp/SRP+LLLT N=15	C N=15	SC N=15
tPA						
Baseline	537.17 (476.93-662.74)	557.92 (439.02-609.16)	711.73 (535.95-968.08)	760.79 (661.79-957.74)	268.71 (195.06-396.18)	414.33 (393.24-555.85)
Day 7	469.88 (381.43-524.09)	387.51 (244.65-472.19)	678.42 (488.08-905.06)	473.74 (367.31-654.29)		
Day 14	364.98 (327.41-465.96)	309.35 (208.63-347.07)	530.78 (421.98-787.06)	490.28 (308.72-658.47)		
Day 30	392.17 (217.84-479.29)	327.54 (277.66-439.82)	554.48 (367.39-921.02)	479.53 (403.42-632.29)		
*P**	**.007***	**.000***	>.05	**.000***		
PAI-1						
Baseline	703.02 (662.74-783.95)	693.63 (608.91-852.82)	1010.50 (778.27-1296.6)	982.89 (731.83-1376.6)	406.68 (244.66-427.67)	514.77 (294.15-578.38)
Day 7	659.72 (528.84-826.10)	627.83 (471.28-797.61)	890.14 (647.15-1082.4)	774.56 (666.01-1184.9)		
Day 14	529.09 (469.68-650.36)	558.93 (528.42-642.55)	693.09 (572.79-869.32)	725.26 (496.02-898.87)		
Day 30	534.92 (393.23-783.93)	550.83 (447.35-595.89)	600.79 (453.91-745.91)	541.06 (409.77-738.28)		
*P**	>.05	>.05	**.000***	**.000***		
TGF-β1						
Baseline	606.87 (404.01-664.96)	613.53 (517.51-810.96)	599.87 (363.77-704.13)	588.26 (355.21-724.29)	218.81 (137.07-300.55)	294.69 (217.79-352.58)
Day 7	497.89 (347.49-619.45)	561.83 (411.74-750.48)	442.11 (302.82-529.48)	523.33 (346.11-661.90)		
Day 14	270.28 (199.47-369.20)	356.92 (206.87-544.83)	266.42 (246.25-296.57)	266.39 (203.47-398.58)		
Day 30	198.26 (158.95-244.06)	225.89 (200.03-278.36)	225.89 (200.03-278.36)	211.96 (158.98-282.96)		
*P**	**.000***	**.000***	**.000***	**.000***		

PAI-1, plasminogen activator inhibitor 1; TGF-β1, transforming growth factor beta1; tPA, tissue plasminogen activator.
Values are given as median (first and third quartiles).
*Bold indicates Friedman test, statistically significant paired comparison due to sampling time points.

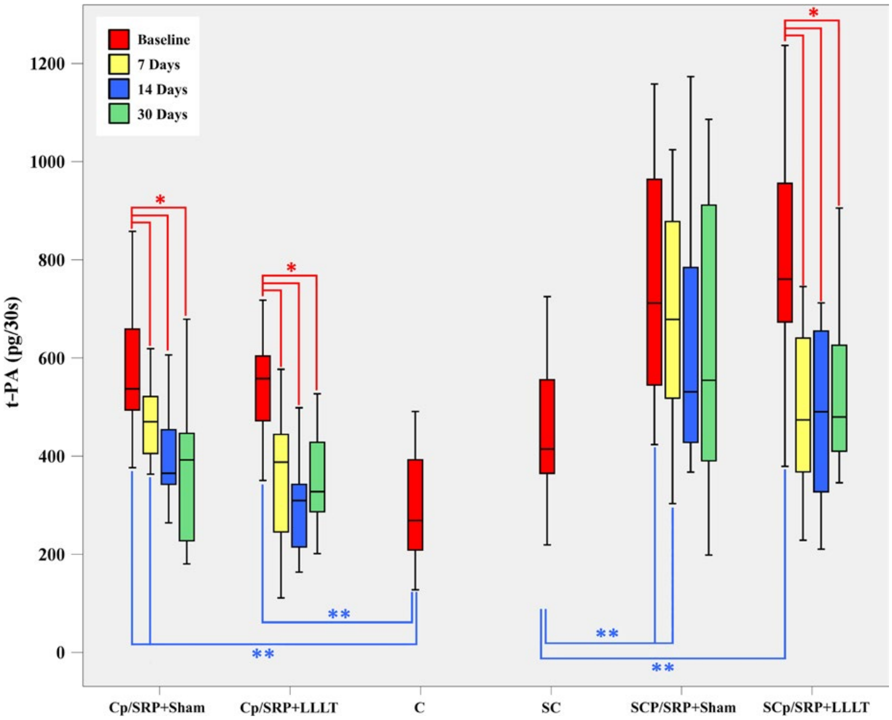

FIGURE 1 Comparison of gingival crevicular fluid tPA levels of the control and study groups at baseline, on days 7, 14 and 30 after treatment with LLLT and SRP. Box plots show the median, first and third quartiles, minimum and maximum values (whiskers). *Wilcoxon signed rank test with Bonferroni adjustment; **Mann-Whitney *U* test; LLLT, low-level laser therapy; SRP, scaling and root planing; tPA, tissue plasminogen activator

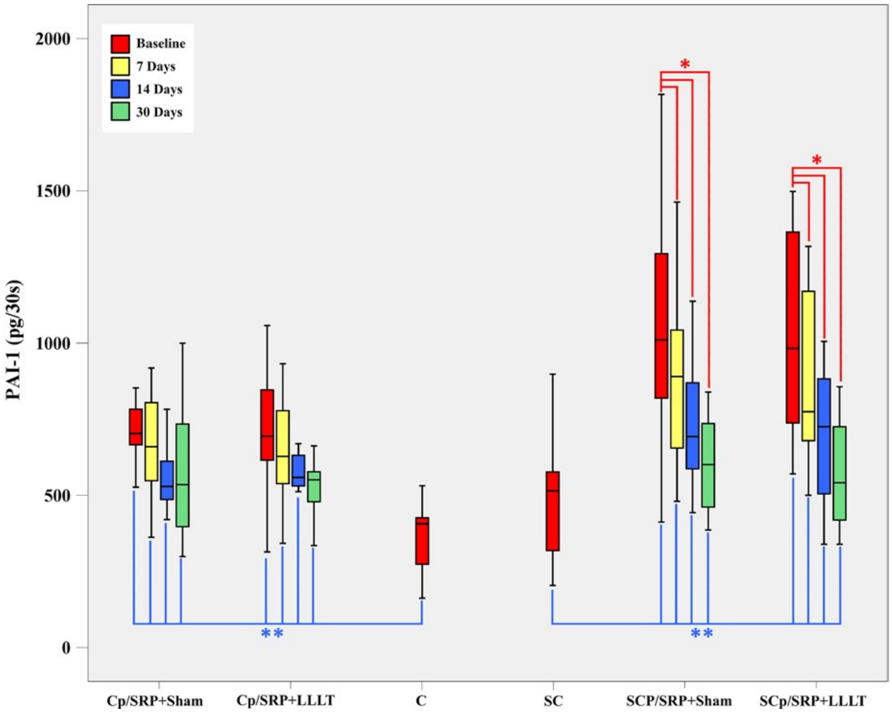

FIGURE 2 Comparison of gingival crevicular fluid PAI-1 levels of the control and study groups at baseline, on days 7, 14 and 30 after treatment with LLLT and SRP. Box plots show the median, first and third quartiles, minimum and maximum values (whiskers). *Wilcoxon signed rank test with Bonferroni adjustment; **Mann-Whitney *U* test; LLLT, low-level laser therapy; PAI-1, plasminogen activator inhibitor 1; SRP, scaling and root planing

PAs and inhibitors are produced locally in gingival tissue and gingival crevicular fluid.[11,16,39] Not only have gingival crevicular fluid tPA levels been shown to be higher in patients with gingivitis and periodontitis when compared to healthy controls,[15,39,40] higher PAI-1 levels have been found in gingival biopsies of patients with periodontal disease when compared to healthy individuals.[41] The present study also found higher baseline gingival crevicular fluid tPA and PAI-1 levels in patients with chronic periodontitis when compared to healthy controls.

The present study found gingival crevicular fluid tPA levels decreased significantly in both smokers and non-smokers who received LLLT as an adjunct to SRP and in non-smokers treated with SRP alone; however, no changes were found in the gingival crevicular fluid tPA levels of the smokers who were treated with SRP alone. Moreover, while gingival crevicular fluid PAI-1 levels decreased in all the chronic periodontitis groups, these decreases were significant only for smokers. There are few previous studies evaluating changes in tPA in relation to

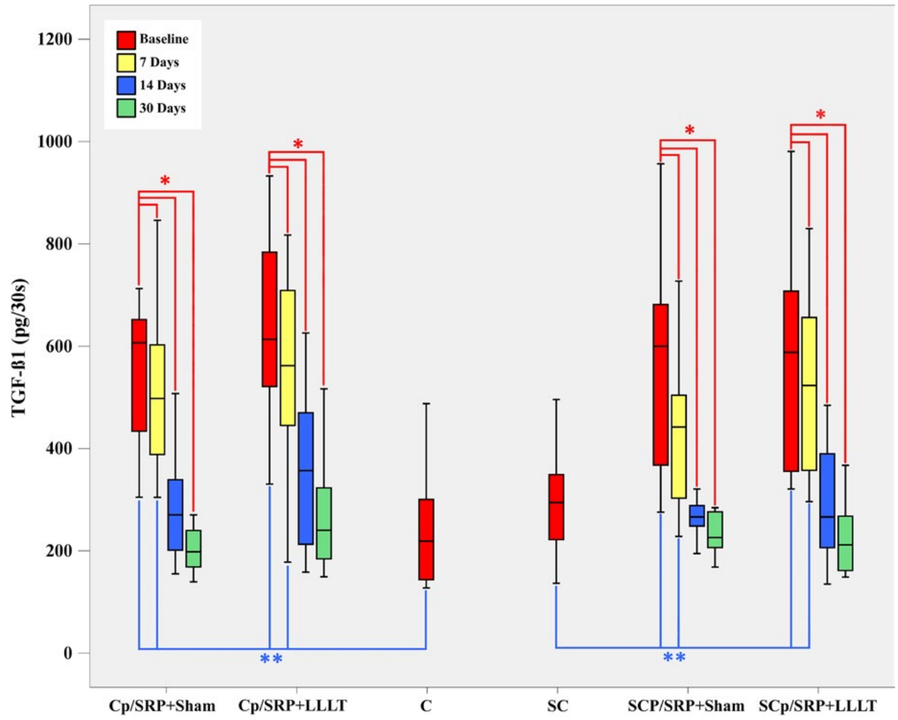

FIGURE 3 Comparison of gingival crevicular fluid TGF-β1 levels of the control and study groups at baseline, on days 7, 14 and 30 after treatment with LLLT and SRP. Box plots show the median, first and third quartiles, minimum and maximum values (whiskers). *Wilcoxon signed rank test with Bonferroni adjustment; **Mann-Whitney *U* test; LLLT, low-level laser therapy; SRP, scaling and root planing; TGF-β1, transforming growth factor beta1

TABLE 3 The tPA/PAI-1 ratio of the study groups due to sampling time pointsb and their statisitical comparison

	Groups			
	Cp/SRP+Sham N=15	Cp/SRP+LLLT N=15	SCp/SRP+Sham N=15	SCp/SRP+LLLT N=15
tPA/PAI-1 -baseline	0.73 (0.55-1.08)	0.72 (0.67-0.82)	0.78 (0.60-0.94)	0.69 (0.51-1.12)
-Day 7	0.74 (0.61-0.92)	0.60 (0.39-0.71)	0.81 (0.60-1.01)	0.54 (0.38-0.77)
-Day 14	0.71 (0.56-0.87)	0.51 (0.39-0.75)	0.89 (0.61-1.12)	0.69 (0.48-0.96)
-Day 30	0.81 (0.33-0.91)	0.66 (0.50-0.90)	1.10 (0.53-1.77)	0.92 (0.60-1.41)
(*P**)	>.05	**.015***	>.05	**.001***
	(*P***)			
Baseline -Day 7	**.009****		**.001****	
-Day 14	**.004****		>.05	
-Day 30	>.05		>.05	
Days 7-14	>.05		>.05	
Days 14-30	>.05		>.05	

PAI-1, plasminogen activator inhibitor 1; tPA, tissue plasminogen activator.
Values are given as median (first and third quartiles).
*Friedman test,statistically significant paired comparison due to sampling time points.
**Wicoxon signed rank test with bonferonni adjustment, statistically significant paired comparison due to sampling time points.

periodontal treatment. Kinnby et al.[42] reported gingival crevicular fluid tPA levels in patients with gingivitis decreased significantly after non-surgical periodontal therapy. Tuter et al.[40] also reported reductions in gingival crevicular fluid tPA levels of patients with periodontitis after periodontal treatment, but the decreases were not significant. A recent study by Kardesler et al.[32] that evaluated the effects of periodontal treatment on several inflammation markers in patients with type 2 diabetes and systemically healthy subjects with periodontitis reported statistically significant reductions in gingival crevicular fluid tPA levels at 1 and 3 months after the initiation of periodontal treatment in both groups. The present results are in line with those findings of the previous studies.[32,40,42]

The present study is the first to evaluate gingival crevicular fluid PAI-1 levels before and after periodontal treatment. However, Behle et al.[43] examined serum PAI-1 levels in patients with periodontitis receiving comprehensive periodontal treatment and found significant reductions within a 6-week period, which is consistent with the findings of the present study. Similarly, Taylor et al.[44] reported reductions in serum PAI-1 levels within 3 months of the initiation of periodontal treatment. Both Bizzarro et al.[45] and Akman et al.[46] documented the importance of serum PAI-1 as a marker in periodontal disease, with PAI-1 levels increasing with increases in inflammation and disease progression. The reduction of gingival crevicular fluid PAI-1 in our study reflects the reductions in tissue inflammation achieved through periodontal treatment.

TGF-β1 exerts both anti-inflammatory and proinflammatory effects on host cells during the onset and progression of periodontal disease.[12] It is a critical mediator in the resolution of inflammation and an indication of ongoing wound-healing as well as chronic inflammation during host response.[12] Khalaf et al.[47] showed TGF-β1 levels to be higher in gingival tissue and gingival crevicular fluid at sites of inflammation when compared to healthy tissue, and Skaleric et al.[48] demonstrated a positive correlation between gingival crevicular fluid TGF-β1 concentrations and periodontal disease. In line with these earlier findings, the present study found baseline gingival crevicular fluid TGF-β1 levels in patients with chronic periodontitis to be higher than those in healthy controls were. Skaleric et al.[48] hypothesized that low levels of TGF-β1 activity stimulate inflammation during the initial stages of the disease process, but that the effects of TGF-β1 activity are reversed as the inflammation progresses.[48] It is possible that the role of TGF-β1 alternates between proinflammatory and anti-inflammatory axes to regulate the immune inflammatory response and limit tissue degradation as periodontal disease progresses to a more destructive state, such as periodontitis.[49] In other words, TGF-β1-dependent mechanisms may be involved in both the initiation and regulation of inflammation and connective tissue destruction that is observed with periodontal disease. Gürkan et al.[12] investigated gingival crevicular fluid TGF-β1 levels in subjects with various types of periodontal disease and found that gingival crevicular fluid TGF-β1 levels increased in line with increases in disease severity and concomitant periodontal destruction. In a recent in vitro study by Yoshimoto et al.,[50] TGF-β1 was found to mediate apoptosis in human gingival epithelial cells, thereby facilitating destruction of the gingival epithelium and, thus, the progression of periodontitis. The present study, in line with Vikram et al.,[51] found that initiation of periodontal treatment led to reductions in gingival crevicular fluid TGF-β1 levels. While the exact mechanism behind the decrease in gingival crevicular fluid TGF-β1 levels observed with periodontal treatment is unclear, this decrease may reflect the resolution of inflammation, and it might help to induce the remodeling events observed in all groups in the present study over the course of treatment.

To the best of our knowledge, no published study has evaluated the use of LLLT as an adjunct to non-surgical periodontal treatment in relation to changes in the plasminogen system in the periodontal tissue of smokers versus non-smokers. In fact, there is scant research in general on how LLLT affects the plasminogen system in terms of periodontal healing and inflammation. Those studies that have been conducted have demonstrated the ability of low-level laser irradiation to downregulate tPA expression. Ozawa et al.[52] found low-energy diode-laser irradiation applied to human periodontal ligament cells in a culture medium significantly inhibited the production of stress-induced tPA, and Takema et al.[53] reported that low-level laser irradiation reduced tPA mRNA levels, leading to significant decreases in lipopolysaccharide-induced PA activity in human gingival fibroblasts. Changes in periodontal tissue PAI-1 have yet to be evaluated in relation to laser irradiation. In the present study, gingival crevicular fluid tPA levels decreased significantly below baseline in both treatment groups receiving adjunctıve LLLT therapy as well as in the non-smoking group receiving SRP only, and gingival crevicular fluid PAI-1 levels decreased in all treatment groups, but the differences were significant only for smokers. Although changes in gingival crevicular fluid tPA and PAI-1 levels did not vary significantly between the groups receiving adjunctive LLLT therapy and the sham groups, the overall study data suggest that LLLT therapy adds to the effectiveness of SRP in terms of altering inflammatory mediators in smokers with chronic periodontitis, with PAI-1 playing an important role in the initial response of periodontal tissue to treatment, particularly in smokers. Considering that smoking plays a role in hypoxia and that hypoxia has been reported to have a significant role in PAI-1 expression, with hypoxia mediating the stimulation of PAI-1,[13,54] it is possible to suggest that the combination of periodontal disease and smoking is important in creating a hypoxic environment and reducing blood flow in gingival tissue.[55] Given the findings of the present study as well as earlier studies, it can be hypothesized that periodontal tissue response to treatment, particularly in smokers, may be related to oxygen resaturation and the restoration of gingival blood flow, and that this explains why smokers showed greater initial improvements following periodontal treatment, regardless of whether LLLT is used as an adjunct.

The PA system is regulated not only by the tissue levels of individual components, but also by net PA capacity, as reflected in the balance between PAs and PAIs.[11,56] Previous studies have used PA/PAI ratios as an indirect measure of fibrinolytic activity,[39,42,56] with high gingival crevicular fluid PA/PAI ratios identified in those sites that show ongoing deterioration in patients with periodontitis during the maintenance phase.[56] It is possible that SRP and LLLT might affect PA/PAI ratios by stimulating vascular flow rates and the development of new vasculation. The present study showed significant decreases in tPA/PAI ratios in the LLLT groups occurred earlier in smokers (on day 7) than in non-smokers (on day 14), indicating that LLLT may significantly modulate plasmogenic activity during the proteolytic events leading to periodontal tissue breakdown. This could occur through inhibition of the tPA, as the results of Ozawa et al.[52] and Takema et al.[53] suggest; however, given the numerous other types of cells in the gingival area capable of directly or indirectly regulating tPA[57,58] and PAI-1[13] production, the observed changes in fibrolytic activity could also occur through other interactions. Moreover, in diseases such as periodontitis that involve close interactions between inflammatory proteins, endothelial activation and prothrombotic alterations that are commonly linked to chronic prothrombotic[43-45] and low-grade inflammation that “prime” the host, LLLT application could affect several cell types and initiate a chain reaction of reciprocal interactions in the area of tissue receiving treatment.

Clinical studies examining the effect of LLLT on growth factors are also extremely rare. A number of in vitro and animal studies have been conducted on this subject,[5,59] and while such studies cannot duplicate the complex cellular and molecular interactions that occur in vivo, they can help identify the mechanism of action of LLLT. There is only one clinical study in the literature reporting on how LLLT used as an adjunct to SRP affects TGF-β1 regulation. In that study, Aykol et al.[8] reported that gingival crevicular fluid TGF-β1 levels decreased when LLLT was applied after the elimination of inflammation by non-surgical periodontal treatment methods; however, the decreases were not statistically significant when compared to controls that did not receive LLLT. The present results are in line with those findings of Aykol et al.[8]

The present study has some methodological limitations. First, the small sample size of the study may affect the reproducibility of the results, which must therefore be interpreted with caution. Second, the difficulties in establishing study groups with similar tissue conditions at baseline and not able to verify the accuracy of the declaration of smokers and non-smokers by saliva cotinine concentration analysis represents a limitation, given the importance of the description of disease severity in this study. Third, due to the lack of adequate studies, there is no agreement as to optimal treatment parameters for laser therapy; in this regard, standardized criteria for periodontal laser therapy need to be developed that address energy levels, application time, modes of irradiation, power settings and laser types.

In conclusion, based on the results of the present study, the application of LLLT as an adjunct to non-surgical periodontal treatment may help to resolve inflammation and support healing of periodontal tissue particularly in patients with chronic periodontitis who smoke by modulating gingival crevicular fluid tPA and PAI-1 levels. Further controlled clinical studies conducted on a larger scale can provide a better understanding of the associations between the PA system, periodontal tissue healing and LLLT.

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