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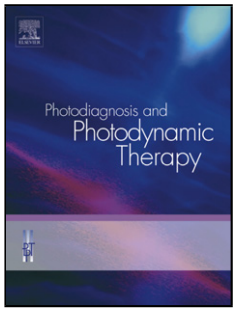

Title: Effect of laser-assisted scaling and root planing on the expression of pro-inflammatory cytokines in the gingival crevicular fluid of patients with chronic periodontitis: A systematic review

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Title: Effect of laser-assisted scaling and root planing on the expression of pro-inflammatory cytokines in the gingival crevicular fluid of patients with chronic periodontitis: A systematic review.

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Running Title: Laser-assisted scaling and root planing and cytokines

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Highlights

- A total of 22 randomized control trials were included in the present systematic review.
- Nine studies used low level laser therapy and 6 studies used high intensity laser therapy as adjunct to scaling and root planing (SRP).
- Seven studies assessed the efficacy of aPDT as adjunct to SRP on down-regulating the expression of pro-inflammatory cytokines.
- The outcomes of the studies included based upon the reduction in the levels of pro-inflammatory cytokines were inconsistent
- The role of laser-assisted SRP on the expression of pro-inflammatory cytokines in the GCF of patients with chronic periodontitis remains unclear.

Abstract

Background: The aim of the present systematic review was to assess the efficacy of laser-assisted (low level laser therapy [LLLT], high intensity laser therapy [HILT], or antimicrobial photodynamic therapy [aPDT]) scaling and root planing (SRP) compared with SRP alone on the expression of inflammatory cytokines in the gingival crevicular (GCF) of patients with chronic periodontitis (CP).

Methods: In order to address the focused question: "What is the efficacy of SRP with and without laser and/or aPDT on the expression of pro-inflammatory cytokines in the GCF of patients with CP?" an electronic search without time or language restrictions was conducted up to and including February 2017 in indexed databases using various key words.

Results: Twenty-two randomized control trials were included in the present systematic review. Nine studies and six studies assessed the efficacy of LLLT and HILT, as adjunct to SRP, respectively. Seven studies assessed the efficacy of aPDT as adjunct to SRP on down-regulating the expression of pro-inflammatory cytokines in the GCF among patients with CP. The outcomes of the studies included based upon the reduction in the levels of pro-inflammatory cytokines were inconsistent.

Conclusion: The role of laser-assisted SRP on the expression of pro-inflammatory cytokines in the GCF of patients with CP remains unclear. Further long term and well-designed randomized clinical trials are needed in this regard.

KEY WORDS: Cytokines; photochemotherapy; periodontal diseases; chronic periodontitis; lasers.

1. **Introduction**

Traditionally, scaling and root planing (SRP) is performed for the treatment of periodontal diseases such as chronic periodontitis (CP) [1, 2]. SRP involves the mechanical debridement of plaque and calculus deposits (which harbor pathogenic microbes) from the teeth and root surfaces using hand instruments such as curettes [3, 4]. Although SRP is an effective means to remove dental plaque and calculus deposits, the technique may be unable to eliminate pathogenic microbes [2]. However, therapies such as low level laser therapy (LLLT) [5-13], high intensity laser therapy (HILT) [14-19] and antimicrobial photodynamic therapy (aPDT) [20-26] have been proposed as adjuncts to SRP for the treatment of CP. Diode lasers operate in the red and near-infrared region (wavelengths between 600 and 1000 nm) to provide a low-energy output which maintains the tissue temperature below 36.5 °C or normal body temperature [27, 28]. LLLT increases adenosine triphosphate (ATP) production in the mitochondria membrane through a non-thermal biomodulative effect on the respiratory chain system [29]. Moreover, LLLT has been shown effective increasing local microcirculation by stimulating angiogenesis (primordial for cell migration) and reducing the inflammatory phase [30]. On other hand, HILT delivers a high energy output into the tissues, resulting in tissular dynamic vibration, increased mitochondrial oxidation and ATP levels which lead to the removal of exudates through increased metabolism and blood circulation [31]. HILT may contribute to root bio-modification, reduced number of pathogens in periodontal pockets and the ablation of calculus and granulation tissue [18, 32-34]. In aPDT the interaction between a photosensitizer and light results in the production of reactive oxygen species which are

lethal to pathogenic microbes and their products [35-37]. Moreover, aPDT reduces the biological activities of toxic lippolysaccharides produced by these microbes [38].

The classical signs of CP include increased plaque index (PI), gingival bleeding, probing depth (PD) ≥4mm, clinical attachment loss (CAL) and marginal bone loss (MBL) [39]. However, raised levels of pro-inflammatory cytokines have also been identified in the gingival crevicular fluid (GCF) and saliva of patients with CP [40, 41]. Results by Shimada et al. [42] showed increased levels of interleukin (IL)-1β in the GCF of patients with CP. Similar results were reported in a recent systematic review and meta-analysis in which raised GCF levels of IL-1β, IL-6, and interferon (IFN)-γ were reported in patients with CP compared to individuals without CP [41].

Studies [10, 14, 22] have already shown that SRP using LLLT, HILT or aPDT is more effective in reducing the GCF pro-inflammatory cytokines load in patients with CP as compared with SRP alone. However, to our knowledge from indexed literature the effect of various laser-assisted therapies (LLLT, HILT, or aPDT) as adjuncts to SRP in reducing the levels of GCF pro-inflammatory cytokines among patients with CP have not been systematically reviewed. With this background, the aim of the present systematic review was to assess the efficacy of laser-assisted (LLLT, HILT, or aPDT) SRP compared with SRP alone on the expression of inflammatory cytokines in the GCF of patients with CP.

2. Material and methods

2.1. Focused question

The present systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [43]. A specific

question was developed according to the Participants, Interventions, Control, and Outcomes (PICO) format. P: patients with CP; I: treatment of CP using SRP with adjunct laser therapy or aPDT; C: patients that underwent SRP without adjunct laser therapy or aPDT; and O: expression of pro-inflammatory cytokines in GCF. The focused question was "What is the efficacy of SRP with and without laser and/or aPDT on the expression of pro-inflammatory cytokines in the GCF of patients with CP?"

2.2. Eligibility criteria

The inclusion criteria were as follows: (a) randomized controlled clinical trials; (b) conducted in adult patients (>18 years) diagnosed with CP; (c) presence of control group (patients receiving SRP without adjunctive laser therapy or aPDT); (d) interventions evaluating efficacy of laser therapy or aPDT as adjunct to SRP; and (e) studies reporting one or more GCF cytokines levels as outcome. The reasons for exclusion included: (a) qualitative and/or quantitative reviews; (b) laboratory (*in vitro)* and experimental (animal models) studies; (c) case reports and/or case-series; (d) commentaries, letters to the editor and/or interviews; (e) studies in which the intervention group received laser therapy or aPDT alone (without SRP); (f) studies with other adjunct therapies (such as local delivery of antibiotics) in addition to laser therapy or aPDT; (g) studies in which biomarkers were collected from fluids other than GCF (such as serum, saliva or gingival tissue); and (h) studies were patients were diagnosed with aggressive periodontitis.

2.3. Literature search protocol

In order to identify studies relevant to the focused question, two authors (SVK and FJ) conducted a structured and logical electronic search without time or language restrictions up to and including February 2017 in PubMed (National Library of Medicine), Google-Scholar, Scopus, EMBASE, MEDLINE (OVID) and Web of Knowledge databases. The following Medical Subject Headings (MeSH) were used: (1) periodontal debridement, (2) periodontal diseases, (3) periodontitis, (4) lasers, (5) cytokines and (6) photochemotherapy. Other related non-MeSH terms were used in the search strategy to detect articles discussing periodontal parameters and periodontal treatment. These included: (7) non surgical periodontal therapy, (8) mechanical curettage, (9) bleeding on probing, (10) clinical attachment loss and (11) probing depth. These keywords were used in the following combinations: (a) 1 or 7 or 8, and 2 or 3, and 4 or 6; (b) 1 or 7 or 8, and 2 or 3, and 4 or 6 and 5; (c) 2 or 3, and 9, 10 or 11, and 4 or 6; (d) 2 or 3, and 9 or 10 or 11, and 4 or 6, and 5.

To minimize the potential for reviewer bias, titles and abstracts of studies identified using the above-described protocol were independently screened by 3 reviewers (SVK, FJ and VRM) and checked for agreement. Full-texts of studies judged by title and abstract to be relevant were read and independently evaluated for the stated eligibility criteria. Reference lists of original studies were hand searched to identify any articles that could have been missed during the initial search. Hand searching of the following journals was performed: Clinical Oral Investigations, Journal of Clinical Periodontology, Journal of Periodontology, Photodiagnosis and Photodynamic Therapy and Lasers in Medical Science. Any disagreements in the study selection were resolved via discussion and consensus. Cohen's kappa value [44] was used to determine the inter-reviewer reliability between the 3 reviewers. The kappa coefficient for inter-reviewer agreement was 0.81.

2.4. Quality assessment

In order to increase the strength of the present systematic review the studies that were included underwent a quality assessment following the recommendations of the Consolidated Standards of Reporting Trials (CONSORT) statement [45]. The CONSORT tool uses a systematic approach based on 7 specific criteria which are: (A) sample size calculation (minimum number of participants required to detect a significant difference among compared groups); (B) randomization and allocation concealment methods; (C) clear definition of inclusion and/or exclusion criteria; (D) complete follow-up; (E) experimental and control groups comparable at study baseline; (F) presence of masking; and (G) appropriate statistical analysis. After determining the scores, an overall estimation of risk of bias (low, moderate or high) was estimated for each selected study. When all the criteria were met, a low risk of bias was estimated; those studies which partly met one or more criteria were estimated as moderate risk of bias; and the risk of bias was estimated as high when one or more criteria were not met [46].

3. Results

3.1. Study selection

Through the initial search 1237 potential articles were identified. After screening the titles and abstracts of these studies, 1168 articles which did not fulfill the eligibility criteria or did not answered the focused question were excluded. In the next step, 47 more articles were excluded as they either did not answer the focused question, were experimental studies or review articles. A total of 22 studies [5-26] were included in the present systematic review and processed for data extraction (Figure 1). It is pertinent to mention that the significant heterogeneity among the included studies [5-26] did not allow pooling of the results and statistical analysis.

3.2. General characteristics of included studies

All studies [5-26] were conducted under healthcare or university settings between 1999 and 2017, in the following countries: Brazil, China, Egypt, Hong Kong, Iran, Spain, Sweden, Taiwan, Turkey, and United States of America. All studies [5-26] were randomized controlled trials, out of which 13 studies [5, 7, 10, 12, 14, 15, 17, 19, 21-25] presented a split-mouth design and 9 studies [6, 8, 9, 11, 13, 16, 18, 20, 26] were conducted with a parallel design.

3.2.1. Low level laser therapy

Nine studies [5-13] assessed the efficacy of LLLT as adjunct to SRP compared with SRP alone in the down-regulation of pro-inflammatory cytokines in GCF among patients with CP. In total 252 patients were included, out of which 130 participants were female and 122 were male. The number of participants in these primary studies [5-13] ranged between 16 and 60 individuals, with ages ranging between 22 years and 81 years, and a mean age ranging between 40.23±10.18 years to 61.8 years. Eight studies [5-12] included systemically healthy individuals and addressed confounding variables including pregnancy and lactation, antibiotics or anti-inflammatory medication, and/or recent periodontal treatment. Kocak et al. [13] evaluated the efficacy of LLLT as adjunct to SRP in patients with diabetes mellitus type 2 (T2DM). Self-reported smokers were excluded in 6 studies [7-10, 12, 13]; whereas, 3 studies [5, 6, 11] reported the effect of SRP with adjunct LLLT among cigarette smokers (Table 1).

Eight studies [6-13] included a control group which received SRP alone. In the study by Qadri et al. [5] the control group (SRP alone) was exposed to a placebo laser (low-powered red light-emitting diode). In all studies [5-13] the follow-up ranged between 6 weeks and 24 weeks after periodontal therapy. Eight studies [5-9, 11-13] assessed pro-inflammatory biomarkers levels in the GCF of patients with CP treated with SRP with and without adjunctive LLLT using enzyme-linked

immunosorbent assay (ELISA); whereas, Üstün et al. [10] determined the concentration of GCF IL-1β using flow cytometry (Table 2).

All studies [5-13] employed diode lasers with wavelengths ranging between 635 nm and 980 nm, and power average between 0.01 W and 1.5 W. Qadri et al. [5] used a diode laser with 2 separate wavelengths (635 nm and 830 nm). Two studies [9, 13] and 2 studies [6, 12] used aluminium gallium indium phosphide (AlGaInP) and gallium aluminum arsenide (GaAlAs) diode lasers, respectively. Four studies [7, 9, 10, 13] used an optic fiber with 3 mm diameter. Gundogar et al. [12] used a 10 mm diameter optic fiber. In 4 studies [5, 6, 8, 11] optic fiber dimensions were not reported. In eight studies [5-10, 12, 13] duration of LLLT irradiation per tooth ranged between 10 seconds and 90 seconds. Nguyen et al. [11] did not report the irradiation time. In 5 studies [6, 9-11, 13] adjunctive LLLT was applied at baseline after SRP (a single session irradiation). Calderin et al. [8] presented 2 different laser treatment modalities; test group one received SRP and one day after a LLLT single session; whereas, test group 2 received 5 adjunctive LLLT sessions during 2 weeks (days 1, 2, 4, 7 and 11) after SRP [8]. Qadri et al. [5] applied LLLT once a week for 6 weeks after initial SRP. Makhlouf et al. [7] performed 3 LLLT sessions in the first and second weeks, followed by 2 sessions during the third week and weekly sessions during weeks 4 and 5 (a total of 10 sessions). Gundogar et al. [12] applied 4 LLLT sessions (immediately after SRP, and first day, third day, and seventh day post-SRP) (Table 3).

3.2.2. High intensity laser therapy

Six studies [14-19] evaluated HILT efficacy as adjunct to SRP on reducing the GCF expression of pro-inflammatory biomarkers in patients with CP. A total of 139 participants were included, out of which 73 patients were female and 58 were male. In the study by Liu et al. [14] the participants' gender remained unclear. The number of participants in these studies [14-19] ranged

between 8 and 30 individuals, with ages ranging between 26 years and 70 years, and a mean age ranging between 43 years and 51 years. In one study [14] participants' age was not reported. All studies [14-19] included systemically healthy individuals and addressed confounding variables including pregnancy and lactation, antibiotics or anti-inflammatory medication, and/or recent periodontal treatment. In 3 studies [15, 16, 18] , patients self-reported as smokers were excluded. Qadri et al. [17] included 5 cigarette smokers and 1 patient which used smokeless tobacco. In two studies [14, 19], the inclusion/exclusion criteria of smokers remained unclear (Table 1).

All studies [14-19] included a control group (patients with CP) which received only SRP. In the study by Lopes et al. [15] a negative control group (patients without CP) was included. In 5 studies [15-19] HILT was used as adjunctive after SRP. Liu et al. [14] compared the efficacy of 4 different treatment modalities (HILT alone, SRP alone, HILT at baseline and SRP 6 weeks later, and SRP at baseline and HILT 6 weeks later). In all studies [14-19] the follow-up ranged between 4 weeks and 36 weeks after periodontal therapy. ELISA was used in all the studies [14-19] to measure biomarkers levels in the GCF of patients treated with SRP with and without adjunctive HILT (Table 2).

Four studies [14, 17-19] used neodymium-doped yttrium aluminum garnet (Nd:YAG) lasers with a wavelength of 1064 nm; and 2 studies [15, 16] used erbium (ER):YAG lasers with a 2940 nm wavelength. All studies [14-19] reported the optic fiber diameter which ranged between 0.2 and 0.6 mm. Four studies [15, 17-19] reported duration of HILT irradiation per tooth which ranged between 30 seconds and 240 seconds. In 2 studies [14, 16] irradiation time was not reported. In all studies [14-19] a single HILT irradiation session was conducted at baseline after SRP. Liu et al. [14] applied a single HILT irradiation in one of the test groups after 6 months of SRP (Table 3).

3.2.3. Antimicrobial photodynamic therapy

Seven studies [20-26] assessed the efficacy of aPDT as adjunct to SRP on reducing the expression of pro-inflammatory biomarkers in the GCF among patients with CP. One hundred seventy-nine patients were included (88 females and 79 males). In the study by Teymouri et al. [25] participant's gender remained unclear. The number of participants among the studies [20-26] ranged between 12 and 58 individuals, with ages ranging between 18 years and 69 years. In all studies [20-26] systemically healthy individuals were included and confounding variables including antibiotics or anti-inflammatory medication, and/or recent periodontal treatment were assessed.

All studies [20-26] included a control group which received only SRP. In 5 studies [20, 22-24, 26], test group received aPDT as adjunctive treatment after SRP. Teymouri et al.[25] used a three-arm parallel design with a control group (SRP alone), test group 1 (LLLT before SRP) and test group 2 (aPDT before SRP). Lui et al. [21] treated patients with combined LLLT and aPDT after SRP. In all studies [20-26] the follow-up ranged between 4 weeks and 52 weeks. All studies [20-26] assessed pro-inflammatory levels in GCF using ELISA (Table 2).

Two studies [22, 24] used 10mg/ml phenotiazine chloride as photosensitizer. Three studies [20, 21, 26] applied methylene blue with concentrations ranging between 0.1 mg/ml and 10 mg/ml; whereas, Pourabbas et al. [23] and Teymouri et al. [25] filled periodontal pockets with toluidine blue. Photosensitization period prior laser application ranged between 10 seconds and 300 seconds [20-24, 26]. One study [25] did not report the photosensitization time pre-irradiation. In all studies [20-26] diode lasers with wavelengths ranging between 638 nm and 940 nm were used. Five studies [22-25, 47] applied adjunct aPDT to SRP at baseline (only one application). Ge et al. [20] used 2 aPDT sessions, baseline and 42 days after initial therapy. Whereas, da Cruz Andrade et al.

[26] applied aPDT in residual pockets at baseline (after 6 weeks of SRP), and 3, 6 and 9 months (Table 3).

3.3. Biomarkers main outcomes

3.3.1. Low level laser therapy

Level of GCF IL-1β was assessed in 8 studies [5, 7-13], out of which 2 studies [8, 10] reported lower GCF IL-1β levels in patients treated with SRP and adjunct LLLT compared with SRP alone. Six studies [5, 7, 9, 11-13] reported no significant difference in the GCF IL-1β concentrations between control and test groups. The expression of IL-6 and IL-8 was assessed in 3 studies [9, 12, 13]. Saglam et al. [9] and Gundogar et al. [12] reported no difference in IL-6 and IL-8 levels in patients receiving SRP with or without adjunctive LLLT; whereas, Kocak et al. [13] identified significantly lower GCF IL-6 levels, but not significant difference in IL-8 levels among patients treated with adjunct LLLT to SRP compared with SRP alone. Calderin et al. [8] assessed levels of tumor necrosis factor (TNF)- α, reporting lower GCF concentration among patients treated with adjunct LLLT compared with control after 8 weeks follow-up. Gundogar et al. [12] reported comparable levels of TNF-α, IL-2, IL-4, IL-5, IL-7, IL-9, IL-10, IL-12, IL, 13, IL-15, IL-17, IL-1 receptor antagonist (ra), and IFN-γ among patients treated with SRP and adjunct LLLT compared with SRP alone.

Two studies [6, 9] assessed expression of GCF matrix metalloproteinase (MMP)-1, reporting similar down-regulation in control and test groups after treatment. Two studies [5,

9] reported MMP-8 levels in GCF among patients treated with and without adjunctive LLLT, out which one study reported comparable levels between groups after treatment. Qadri et al. [5] reported increased levels of MMP-8 on the control sites compared with LLLT sites. One study [6] reported similar reduction in the GCF concentration of tissue inhibitor of metalloproteinase (TIMP)-1 between control and test groups at follow-up. Saglam et al. [9] reported significant lower TIMP-1 levels in patients treated with SRP and adjunct LLLT. Aykol et al. [6] reported similar reduction in the GCF concentration of transforming growth factor (TGF)-β1 and basic fibroblast growth factor (b-FGF) between control (SRP) and test (SRP + LLLT) groups at 24 weeks follow-up. One study [12] reported similar levels of b-FGF, eotaxin, granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor, IFN-γ-induced protein 10, monocyte chemoattractant protein-1, macrophage inflammatory protein (MIP)-1α, MIP-1β, platelet-derived growth factor, regulated on activation normal T cell expressed and secreted (RANTES) and vascular endothelial growth factor (VEGF), between control and test group after 6 months post-treatment. Calderin et al.[8] reported a significant reduction in receptor activator of nuclear factor kappa-β ligand/osteoprotegerin (RANKL/OPG) ratio at 8 weeks follow-up in the SRP+LLLT group compared with control. One study [13] reported GCF intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) levels. Results showed lower VCAM levels and similar ICAM concentrations in the adjunct LLLT to SRP group compared with controls 12 weeks post-treatment [13].

3.3.2. High intensity laser therapy

All studies [14-19] assessed GCF IL-1β levels. Five studies [14, 16-19] reported lower IL-1β levels in patients treated with SRP and adjunct HILT compared with SRP

alone. Results by Lopes et al. [15] showed no significant difference in IL-1β levels between control and test groups after 4 weeks post-treatment. Qadri et al. [17] reported comparable GCF IL-4, IL-6 and IL-8 concentrations between control and HILT groups after 12 weeks follow-up. Two studies [16, 18] reported lower GCF TNF-α expression among patients treated with adjunct HILT compared with SRP alone after 8 weeks follow-up. Two studies [17, 19] assessed levels of GCF MMP-8, identifying lower concentrations among groups treated with adjunct HILT to SRP compared with controls at follow-up.

3.3.3. Antimicrobial photodynamic therapy

Six studies [20, 21, 23-26] assessed the concentration of IL-1β, out of which 3 studies [20, 24, 26] reported lower IL-1β levels in the GCF of patients treated with adjunct aPDT to SRP compared with controls. In 3 studies [21, 23, 25], there was no significant difference in the IL-1β concentrations among patients with or without adjunct aPDT to SRP. Levels of TNF- α were explored in 2 studies [23, 26], out of which, 1 study [23] reported lower levels of TNF- α in the GCF of patients treated with aPDT compared with controls, and 1 study [26], reported no significant difference in TNF- α levels between test and control groups. The expression of MMP-8 in GCF among patients treated with and without adjunctive aPDT was reported in 3 studies [20, 23, 24], out of which 2 studies [20, 24] reported significantly lower MMP-8 levels in test group compared to control. Pourabbas et al. [23] identified comparable MMP-8 and MMP-9 concentration in GCF of patients treated with SRP versus SRP and aPDT. Teymouri et al.[25] identified a significant reduction of IL-17 levels in the GCF of patients treated with SRP and adjunct aPDT or LLLT compared with SRP alone. Souza et al. [22] reported lowerTGF-β1 levels after aPDT

adjunct to SRP compared to SRP alone. One study [26] reported lower IL-1α, IL-8, VEGF, IL-1ra, IFN-γ and IL-10 GCF concentrations among patients treated with SRP and adjunct aPDT; whereas, FGF and IL-4 levels showed no statistical difference between control and test groups.

3.4. Periodontal main outcomes

3.4.1. Low level laser therapy

All studies [5-13] reported improvement on periodontal parameters in both groups (SRP alone and SRP+LLLT) at follow-up compared with baseline. Four studies [7, 8, 11, 13] reported comparable outcomes in terms of periodontal parameters (PD, CAL, bleeding on probing [BOP], PI, and/or gingival index [GI]) among individuals in control and test groups at follow-up. Five studies [5, 6, 9, 10, 12] and four studies [6, 9, 10, 12] reported significant reduction on PD and CAL respectively, in LLLT groups compared with controls at follow-up. Three studies [5, 9, 12] reported significantly lower GI and PI among patients treated with adjunct LLLT to SRP compared with SRP alone.

3.4.2. High intensity laser therapy

All studies [14-19] showed improvement of periodontal parameters in control and treatment groups (SRP and SRP+HILT) at follow-up compared with baseline. In 3 studies [14, 16, 18] similar outcomes in terms of periodontal parameters (PD, BOP, PI, and/or GI) were reported among individuals in control and test groups at follow-up. Three studies [15, 17, 19] reported lower GI in patients treated with adjunct HILT to SRP compared with SRP alone. In 2 studies [17, 19], and 1 study [19] significant PD and CAL reduction were

reported respectively in LLLT groups compared with controls. Lopes et al. [15] and Qadri et al. [17] reported reduced PI in SRP+HILT groups compared with controls.

3.4.3. Antimicrobial photodynamic therapy

All studies [20-26] reported improvement in periodontal parameters in both groups (SRP alone versus SRP and aPDT) at follow-up compared with baseline. Six studies [20, 22-26] reported comparable results in terms of periodontal parameters (PD, CAL, BOP) among individuals in control and test groups at follow-up. In the study by Lui et al. [21] sites receiving SRP and aPDT presented lower BOP and PD after 4 weeks follow-up compared to sites receiving SRP alone.

3.5. Quality assessment

All the included studies [5-26] in the present systematic review were randomized controlled trials. Quality score of the included studies [5-26] according to CONSORT guidelines ranged between 6 and 12. Quality assessment identified that in general, recruitment of the patients (inclusion-exclusion criteria), complete follow-up, comparability of control and test groups at baseline for periodontal and inflammatory parameters, and appropriate statistical analysis were adequately performed in these studies [5-26].

In 15 studies randomization was performed by the use of random number tables or lists [7, 8, 15, 16, 18, 22, 24], random identification cards [13], random allocation software [26] or coin toss [6, 10-12, 17, 23]. Seven studies [5, 9, 14, 19-21, 25] did not report the method used for randomization. Eleven studies [9-11, 13, 15, 17, 18, 22-24, 26] described the power and sample size calculation. In 11 studies [5-8, 12, 14, 16, 19-21, 25] the sample size calculation remained unclear. Low risk of bias was regarded as low in 6 studies [10,

11, 13, 15, 18, 26] since these studies received a CONSORT score of 12. Four studies [9, 17, 22, 24] were graded as moderate risk of bias because partly met one criterion; whereas the remaining twelve studies [5-8, 12, 14, 16, 19-21, 23, 25] were catalogued as high risk of bias because one or more criteria were not met. Quality assessment of the studies [5-26] included in the systematic review is summarized in Table 4.

4. Discussion

Based upon the beneficial effects of laser therapy on periodontal tissues it was expected that SRP with adjunct laser assisted therapies (LLLT, HILT or aPDT) would significantly reduce the levels of pro-inflammatory cytokines in GCF compared with SRP alone. However, it was interesting to know that the outcomes of the studies [5-26] based upon the reduction in the levels of pro-inflammatory cytokines were inconsistent. For example, in the study by Üstün et al.[10] levels of GCF IL-1β were significantly lower when LLLT was used as adjunct to SRP compared with SRP alone; whereas, Makhlouf et al. [7] reported no significant difference in the levels of GCF IL-1β after SRP with adjunct LLLT compared with SRP alone. Likewise, Dominguez et al. [16] reported that IL-1β concentrations in GCF were significantly lower among patients treated with SRP and adjunct HILT compared with controls; however, Lopes et al. [15] showed comparable GCF IL-1β levels using the same treatment protocol. The same trend was seen in patients that received SRP with or without aPDT. Several factors may have influenced these results. The number, frequency and duration of LLLT varied significantly among the included studies [5-13]. For instance, Gundogar et al. [12] applied 4 LLLT sessions (baseline, days 1, 3 and 7), for 15 seconds per tooth; whereas, Nguyen et al. [11] used a single LLLT session and the

irradiation duration remained unclear. These primary studies [11, 12] reported that low level laser assisted SRP failed to reduce GCF cytokines at follow-up compared to SRP alone. This could possibly be associated with the duration of LLLT, its frequency and case selection criteria. Calderin et al. [8] reported lower GCF IL-1β, TNF-α and RANKL-OPG ratio among patients with CP after 5 LLLT sessions (days 1, 2, 4, 7 and 11), for 60 seconds per tooth. Pinheiro and Gerbi [48] suggested that LLLT is more effective at early treatment stages when high cellular proliferation occurs. Therefore, it is hypothesized that increased number of LLLT applications with a longer duration would have significantly reduced the expression cytokines in the GCF of patients included in the studies by Gundogar et al.[12] and Nguyen et al.[11]. Secondly, the variation among laser parameters (such as power, power density, energy fluence and optic fiber diameter) makes challenging to draw specific guidelines for laser assisted SRP, in order to obtain the most predictable outcomes in terms of clinical and immune-inflammatory parameters. For example, Aykol et al. [6] used 0.25 W power average and did not report the optic fiber diameter; Gundogar et al.[12] employed a 10 mm diameter optic fiber with a 0.4 W power; and Makhlouf et al.[7] used a 3 mm diameter fiber with 0.1 W. These factors may have influenced the results reported. To our knowledge there is not agreement among researchers and clinicians regarding the ideal laser parameters that would yield optimal outcomes. Further studies are warranted to test this hypothesis.

It is noteworthy that all studies [5-26] presented a short-time follow-up period (up to 52 weeks). It has been proposed that short-term modifications in the pocket microbiota can predict sustained periodontal stability [49]. However, longitudinal evaluation of the control and test groups is necessary to evaluate the long-term efficacy of laser assisted SRP compared to SRP alone.

It is pertinent to mention that there was no standardization towards the definition of CP amongst the studies included in the present systematic review. For example, in the study by Üstün et al.[10] CP was defined as the presence of a 4-7 mm pocket in 2 anterior teeth; whereas, in the study by Gundogar et al.[12] CP was defined as the presence of pockets over 5 mm in at least 2 bilateral premolars. According to the American Academy of Periodontology (AAP) CP is defined as the presence of probing depths of at least 4 mm in at least 30% sites [39]. In the present systematic review only one study [6] defined CP according to the AAP guidelines. Moreover, the severity of CP among the studies [5-26] included in the present systematic review remained unclear. Therefore, it is hypothesized that due the discrepancy of lasers parameters used and unclear definition of CP a variation in the reduction of GCF pro-inflammatory cytokines load was observed in the studies investigated.

5. Conclusion

The role of laser-assisted SRP on the expression of pro-inflammatory cytokines in the GCF of patients with CP remains unclear. Further long term and well-designed randomized clinical trials are needed in this regard.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article

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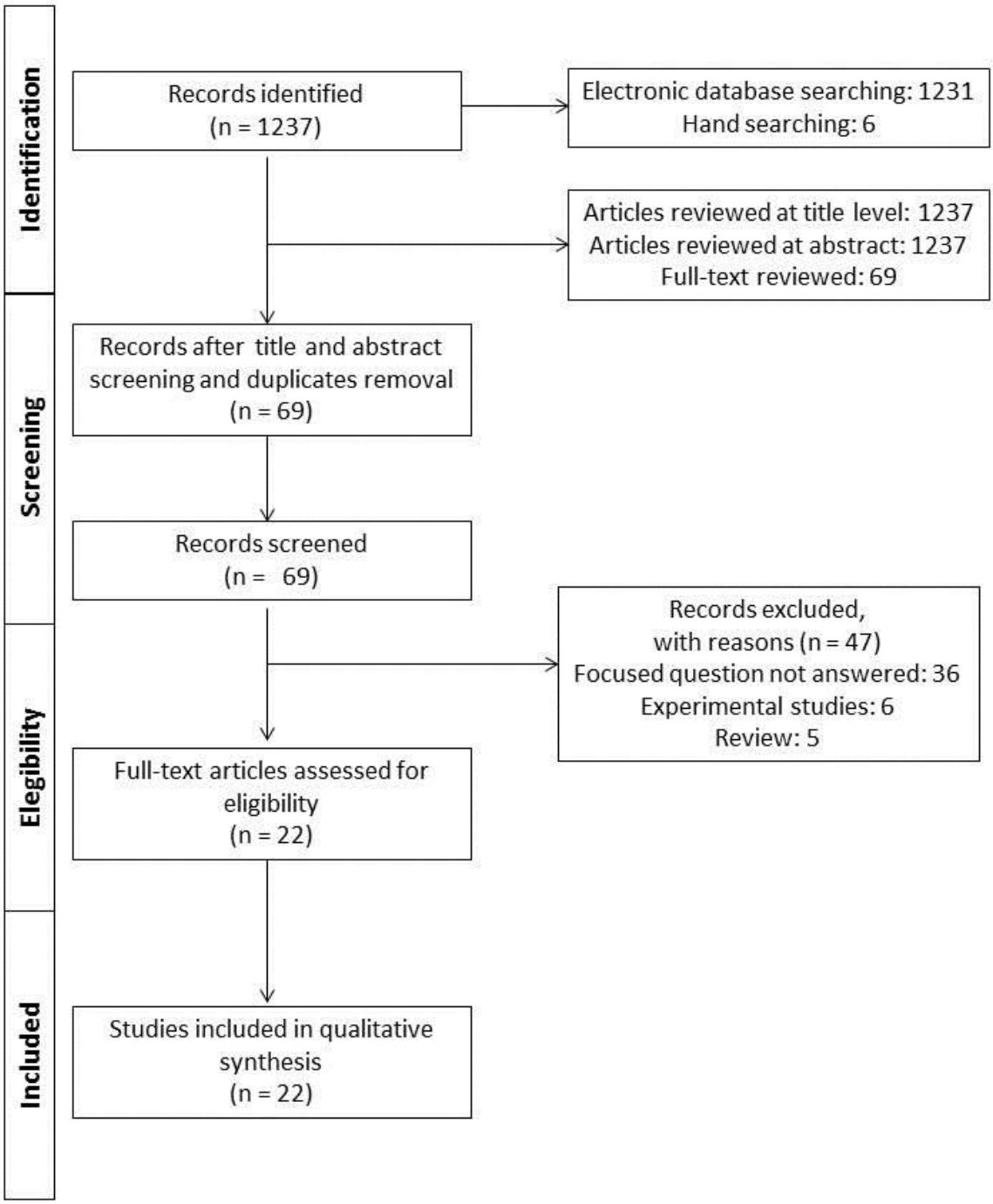

Figure 1: Article selection flow chart for the systematic review according to PRISMA guidelines

Table 1. General characteristics of included studies

Investigators (Region of study and year)	Study design	Number of patients	Mean age (age range in years)	Gender (F/M) (N=number)	Criteria for diagnosis of periodontitis	Confounding variables assessed
Studies with low level laser therapy						
Qadri et al. [5] (Sweden, 2005)	RCT (Split-mouth design)	17	53 (35-70)	10/7	CP; PD=4-6mm	Antibiotics; mobility class II or III; systemic disorders
Aykol et al. [6] (Turkey, 2011)	RCT (Parallel)	36 Control: 18 Test: 18	Control: 42.22±7.53 (31-53) Test: 43.56±6.70 (31-58)	14/22	CP; AAP criteria	Systemic diseases; periodontal treatment in the past 6 months; antibiotics; oral infections
Makhlouf et al. [7] (Egypt, 2012)	RCT (Split mouth)	16	(22-50)	12/4	CP; PD= 4-6mm in ≥3 teeth per quadrant	Systemic diseases; periodontal treatment in the past 6 months; smoking; pregnancy; medications
Calderín et al. [8] (Spain, 2013)	RCT (Parallel)	27 Control: 9 Test 1: 9 Test 2: 9	Control: 50.44±15.91 Test 1: 52.89±11.98 Test 2: 50.44±10.51	15/12	CP; PD= 4-6mm in ≥4 teeth per quadrant	Smoking; periodontal treatment in the past 12 months; systemic diseases; antibiotics and corticosteroid drugs; mouthrinses
Saglam et al. [9] (Turkey, 2014)	RCT (Parallel)	30 Control: 15	Control: 40.83±7.64	12/18	CP; PD≥5 mm in ≥2 teeth at each quadrant	Periodontal treatment in the past 12 months;

		Test: 15	(32-56) Test: 42.13±9.05 (32-57)			systemic diseases; pregnancy; smoking; chemotherapy; antibiotics, and anti-inflammatory drugs
Üstün et al. [10] (Turkey, 2014)	RCT (Split-mouth design)	19	40.23±10.18 (26-55)	12/7	CP; PD= 4 -7 mm on 2 anterior teeth in 2 quadrants	Systemic diseases; smoking; dental treatment in the past 6 months; antibiotics
Nguyen et al. [11] (USA, 2015)	RCT (Parallel)	22	61.8 (47-81)	9/13	CP; PD≥5mm and BOP in ≥1 sites	Systemic diseases; antibiotics and anti-inflammatory drugs; pregnancy
Gundogar et al. [12] (Turkey, 2016)	RCT (Split mouth)	25	40.44±8.69 (28-57)	16/9	CP; PD≥5 mm in ≥2 bilateral premolars	Smoking; periodontal treatment in the past 6 months; systemic diseases; antibiotics and corticosteroid drugs; pregnancy and lactancy
Koçak et al. [13] (Turkey, 2016)	RCT (Parallel)	60 T2DM Control: 30 Test: 30	(35-60)	30/30	CP; PD ≥5 mm in ≤8 sites	Systemic diseases other than T2DM; antibiotics and immunosuppressive drugs; periodontal treatment in the past 12 months; alcohol; pregnancy; smoking
Studies with high intensity laser therapy						

Liu et al. [14] (Taiwan, 1999)	RCT (Split-mouth design)	8	NA	NA	CP; PD= 4-6 mm, BOP and GI ≥2 in 1 to 2 sites of 3 adjacent single-root teeth in each of 4 quadrants	Periodontal treatment in the past 6 months
Lopes et al. [15] (Brazil, 2008)	RCT (Split-mouth design)	21	43 (31-55)	14/7	CP; PD= 5-9 mm and BOP in 4 sites	Periodontal treatment in the past 12 months; systematic diseases; antibiotics and anti-inflammatory drugs; smoking; pregnancy; contraceptives
Dominguez et al. [16] (Spain, 2010)	RCT (Parallel)	30	51±6 (33-65)	17/13	CP; PD= 4-6 mm in ≥4 teeth per quadrant.	Periodontal treatment in the past 12 months; antibiotics; smoking; systemic diseases; corticosteroid and anti-inflammatory drugs; mouthrinses
Qadri et al. [17] (Sweden, 2010)	RCT (Split-mouth design)	30	50 (26-70)	17/13	CP; PD= 4- 8 mm on ≥6 sites in each side of the mandible	Systemic diseases; antibiotics; mobility class II or III
Gomez et al. [18] (Spain, 2011)	RCT (Parallel)	30 Control: 15 Test: 15	51±6 (45-58)	15/15	CP;PD= 4-6 mm	Smoking; periodontal treatment in the past 12 months; antibiotics and corticosteroids drugs; mouthrinses; systemic diseases
Eltas et al. [19] (Turkey, 2012)	RCT (Split-mouth	20	46.1±8.3	10/10	CP; PD=4-6 mm in ≤3 teeth in at least 2	Periodontal treatment in the past 12 months;

	design)				quadrants	systemic diseases; antibiotics; pregnancy and lactancy
Studies with antimicrobial photodynamic therapy						
Ge et al. [20] (China, 2008)	RCT (Parallel)	58 Control: 20 Test 1: 18 Test 2: 20	43 (25-66) Control: 42 Test 1: 42 Test 2: 42	28/30	CP; ≥4 sites in 2 quadrants with PD≥5 mm	Systemic diseases; antibiotic; allergy to methylene blue
Lui et al. [21] (Hong Kong, 2011)	RCT (Split mouth-design)	24	50	14/10	CP; ≥2 bilateral sites with PD ≥5 mm, CAL of ≥3 mm, and radiographic signs of bone loss	Antibiotics, anti-inflammatory and immunosuppressive medications; pregnancy; systemic conditions; periodontal treatment
Souza et al. [22] (Brazil, 2013)	RCT (Split-mouth design)	15	NA (36-65)	9/6	CP; bilateral lower molars with class III furcation	Pregnancy and lactation; antibiotics, anti-inflammatory and hormonal therapy; systemic conditions; periodontal treatment
Pourabbas et al. [23] (Iran, 2014)	RCT (Split mouth-design)	22	46±8	12/10	CP; ≥30% of sites with attachment loss ≥3 mm, and ≥1 site per quadrant with BOP and PD≥4 mm	Pregnancy; smoking; antibiotics, anti-inflammatory and hormonal therapy; systemic conditions; allergy to toluidine blue; periodontal treatment
Queiroz et al. [24] (Brazil, 2015)	RCT (Split-mouth design)	20	46.05±6.38 (35-55)	11/9	CP; ≥2 bilateral sites with PD ≥5 mm	Aggressive periodontitis; pregnancy and lactation; anti-inflammatory medications; systemic

						conditions; periodontal treatment
Teymouri et al. [25] (Iran, 2016)	RCT (Split-mouth design)	12	(30-60)	NA	CP; ≥4 sites in each quadrant with PD of 4-6 mm	Pregnancy and lactation; medications; systemic conditions; periodontal treatment in the past 6 months.
da Cruz Andrade et al. [26] (Brazil, 2017)	RCT (Parallel)	28	(30-69)	14/14	CP; ≥4 teeth with PD of ≥4 mm	Pregnancy and lactation; antibiotics, anti-inflammatory and immunosuppressive therapy; smoking; T2DM; systemic conditions; periodontal treatment in the past 6 months.

RCT: randomized control trial F: female M: male PD: probing depth CP: chronic periodontitis GI: gingival index

NA: not available BOP: bleeding on probing GI: gingival index N: number CAL: clinical attachment loss

SRP: scaling and root planing AAP: American Academy of Periodontology T2DM: type 2 diabetes mellitus

Table 2. Periodontal parameters and pro-inflammatory cytokines profile among study groups

Investigators	Study groups	Follow-up (weeks)	Periodontal parameters	Measurement of cytokines level	Cytokines studied	Periodontal outcomes	Cytokines outcomes
Studies with low level laser therapy							
Qadri et al. [5]	Control: SRP + placebo Test: SRP+ LLLT with 2 wavelengths	Up to 6	PD, GI, PI	GCF: ELISA	Elastase IL-1β MMP-8	PD, GI, and PI levels were significantly lower in test sites compared with controls at follow-up	MMP-8 levels were lower in test sites compared with controls at follow-up
Aykol et al. [6]	Control: SRP Test: SRP+LLLT	Up to 24	PI, SBI, PD, CAL	GCF: ELISA	MMP-1 TIMP-1 TGF-β1 b-FGF	SBI, CAL and PD were significantly lower in test group compared with control at follow-up.	Comparable biomarkers reduction between groups at follow-up
Makhlouf et al. [7]	Control: SRP + placebo Test: SRP+LLLT	Up to 24	PI, GI, PD	GCF: ELISA	IL-1β	Comparable improvement in clinical parameters between the groups at follow-up	No significant difference in IL-1β levels between the groups at follow-up
Calderín et al. [8]	Control: SRP Test 1: SRP + single LT Test 2: SRP + repeated LLLT	Up to 8	PI, BOP, PD, CAL	GCF: ELISA	IL-1β TNF-α RANKL OPG	Comparable improvement in clinical parameters between the groups at follow- up	IL-1β, TNF-α and RANKL/OPG ratio were significantly lower in test 1 and test 2 groups compared with control at follow-up
Saglam et al. [9]	Control: SRP Test: SRP+LLLT	Up to 24	PI, GI, PD, CAL, BOP	GCF: ELISA	IL-1β IL-6 IL-8	PD, CAL, PI, GI and BOP were significantly lower in	TIMP-1 levels were significantly lower in test group

					MMP-1 MMP-8 TIMP-1	test group compared with control at follow-up	compared with control group at follow-up
Üstün et al. [10]	Control: SRP Test: SRP + LLLT	Up to 24	PD, CAL, PI, GI	GCF: flow cytometry	IL-1β	PD and CAL were significantly lower in test sites compared with controls at follow-up	IL-1β levels were significantly lower in test sites compared with controls at follow-up
Nguyen et al. [11]	Control: SRP Test: SRP + LLLT	Up to 12	PD, BOP, GR	GCF: ELISA	IL-1β	Comparable improvement in clinical parameters between the groups at follow- up	No significant difference in IL-1β levels between the groups at follow-up
Gundogar et al. [12]	Control: SRP Test 1: SRP + LLLT	Up to 24	PI, GI, PD, CAL	GCF: ELISA	IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, FGF, eotaxin, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF-BB, RANTES, TNF-α, VEGF	PD, CAL, PI, and GI were significantly lower in test group compared with control at follow-up	Comparable biomarkers reduction between groups at follow-up
Koçak et al. [13]	Control: SRP Test: SRP+LLLT	Up to 12	PI, GI, PD, CAL	GCF: ELISA	IL-1β IL-6 IL-8	Comparable improvement in clinical parameters	IL-6 levels and VCAM levels significantly lower

					ICAM VCAM	between the groups at follow-up	in test group compared with control at follow-up
Studies with high intensity laser therapy							
Liu et al. [14]	Control: SRP Test 1: HILT Test 2:HILT+SRP Test 3:SRP+HILT	Up to 12	GI	GCF: ELISA	IL-1β	Comparable GI reduction among the groups at follow-up.	IL-1β levels were significantly lower in test 3 group compared with other groups at follow-up
Lopes et al. [15]	Control: no treatment Test 1: SRP Test 2: SRP+HILT Test 3: HILT	Up to 4	PI, GI, PD, BOP, CAL, GR	GCF: ELISA	IL-1β	PI and GI levels were significantly lower in test 2 group compared with other groups at follow-up	No significant difference in IL-1β levels among the groups at follow-up
Dominguez et al. [16]	Control: SRP Test: SRP+HILT	Up to 8	PD, BOP, PI	GCF: ELISA	IL-1β TNF-α TAS	Comparable improvement in clinical parameters between the groups at follow- up	IL-1 β and TNF-α levels were significantly lower in test group compared with control at follow up
Qadri et al. [17]	Control: SRP Test: SRP + HILT	Up to 12	PD, GI, PI	GCF: ELISA	IL-1β IL-4 IL-6 IL-8 MMP-8	PD, PI and GI were significantly lower in test sites compared with controls at follow-up	IL-1β and MMP-8 levels were significantly lower in test sites compared with controls at follow-up
Gomez et al. [18]	Control: SRP Test: SRP+HILT	Up to 8	PD, BOP, PI	GCF: ELISA	IL-1β TNF-α TAS	Comparable improvement in clinical parameters between the groups at follow- up	TNF-α and IL-1β levels were significantly lower in test groups compared with

							control at follow-up
Eltas et al. [19]	Control: SRP Test: SRP+HILT	Up to 36	PI, GI, PD, CAL	GCF: ELISA	IL-1β MMP-8	GI, PD and CAL were significantly lower in test sites compared with controls at follow-up	IL-1β and MMP-8 values were lower (not statistically significant) in test sites compared with controls
Studies with antimicrobial photodynamic therapy							
Ge et al. [20]	Control: SRP Test 1: SRP + aPDT Test 2: SRP + aPDT baseline + aPDT 6 weeks later	Up to 12	CAL, PD, BOP	GCF: ELISA	IL-1β MMP-8	Improvements for both groups at follow up were comparable	IL-1 β concentration was significantly lower in both test groups compared to control at follow up. MMP-8 level was significantly lower in test 2 group compared to control at follow up.
Lui et al. [21]	Control: SRP Test: SRP + LLLT + aPDT + LLLT	Up to 4	BOP, PD, GR, PI	GCF: ELISA	IL-1β	BOP and PD were significantly lower for the test group compared to control at follow up	IL-1 β concentration for both groups at follow up were comparable
Souza et al. [22]	Control: SRP Test: SRP + aPDT	Up to 6	PD, PI, BOP	GCF: ELISA	TGF-β1	Improvements in PD for both groups at follow up were comparable	TGF-β1 expression was significantly lower for the test group as compared to control at follow up
Pourabbas et al. [23]	Control: SRP Test:	Up to 12	BOP, CAL, GR, PD	GCF: ELISA	IL-1β MMP-8	Improvements in PD, BOP, GR and CAL	TNF-α level was significantly lower

	SRP + aPDT				MMP-9 TNF-α	for both groups at follow up were comparable	for the test group as compared to control at follow up. IL-1β, MMP-8 and MMP-9 concentrations were comparable in both groups at follow up.
Queiroz et al. [24]	Control: Smokers + SRP Test: Smokers + SRP + aPDT	Up to 12	PI, BOP, GR, PD,CAL	GCF: ELISA	IL-1β MMP-8	Improvements in PD and CAL for both groups at follow up were comparable	IL-1β and MMP-8 concentration were significantly lower for the test group as compared to control at 1 week and 12 weeks follow up, respectively.
Teymouri et al. [25]	Control: SRP Test 1: aPDT + SRP Test 2: LLLT + SRP	Up to 6	BOP, CAL, PD	GCF: ELISA	IL-1β IL-17	Improvements in PD and CAL for all groups at follow up were comparable	IL-1β levels were comparable among groups after 6 weeks follow-up. Test groups presented lower IL-17 concentrations compared with control.

da Cruz Andrade et al. [26]	Control: SRP Test: SRP + aPDT 6 weeks later	Up to 52	PD, CAL, BOP, PI	GCF: ELISA	IL-1α IL-1β IL-4 IL-8 IL-10 IL-1ra FGF IFN-γ TNF-α VEGF	Improvements in PD, PI and CAL for both groups at follow up were comparable	IL-1α, IL-1β, IL-8, VEGF, IL-1ra, IFN-γ and IL-10 levels were significantly lower for the test group as compared to control at follow up. FGF, IL-4 and TNF-α concentrations were comparable in both groups at follow up.

SRP: scaling and root planing LLLT: low level laser therapy HILT: high intensity laser therapy

GI: gingival index GCF: gingival crevicular fluid SBI: sulcus bleeding index GR: gingival recession

MMP: matrix metalloproteinase BOP: bleeding on probing TAS: total anti-oxidative status TNF: tumor necrosis factor

ELISA: enzyme linked immunosorbent assay CP: chronic periodontitis TIMP: tissue inhibitor of metalloproteinase

ICAM: intercellular adhesion molecule VCAM: vascular cell adhesion molecule TGF: transforming growth factor

FGF: fibroblast growth factor CAD: coronary artery disease SH: systemically healthy OPG: osteoprotegerin

RANKL: receptor activator of nuclear factor kappa- β ligand G-CSF: granulocyte colony-stimulating factor PI: plaque index

GM-CSF: granulocyte-macrophage colony-stimulating factor IFN: Interferon IP: interferon gamma-induced protein

MCP-1: monocyte chemoattractant protein MIP: macrophage inflammatory protein PDGF: platelet-derived growth factor

RANTES: regulated on activation, normal T cell expressed and secreted VEGF: vascular endothelial growth factor

IL: interleukin aPDT: antimicrobial photodynamic therapy PD: probing depth CAL: clinical attachment level

Table 3. Laser parameters of included studies

Investigators	Type of laser	Wavelength (nm)	Power peak (W)	Power average (W)	Pulse repetition rate (Hz)	Optic fiber diameter (mm)	Energy per pulse (mJ)	Pulse width (ms)	Energy (J per tooth)	Energy density (J/cm^2)	Duration of application (seconds per tooth)	Number of applications (time interval)
Studies with low level laser therapy												
Qadri et al. [5]	Diode	635	NA	0.01	NA	NA	NA	NA	0.9	4.5	90	6 (weekly)
		830	NA	0.07	NA	NA	NA	NA	1.75	8.75	25	6 (weekly)
Aykol et al. [6]	Diode GaAlAs	808	NA	0.25	NA	NA	NA	NA	NA	4	10-20	1
Makhlouf et al. [7]	Diode	830	NA	0.1	NA	3	NA	NA	3	3	30	10 (Weeks 1,2,3,4,5)
Calderín et al. [8]	Diode	670	0.2	0.2	NA	NA	NA	NA	NA	NA	60	Test 1: 1 (Baseline) Test 2: 5 (Days 1, 2, 4, 7, 11)
Saglam et al. [9]	Diode AlGaInP	940	NA	1.5	NA	3	NA	20	NA	15	20	1
Üstün et al. [10]	Diode	810	2.5	1.25	20	3	NA	NA	NA	NA	80	1
Nguyen et al. [11]	Diode	940	NA	0.80	NA	NA	NA	NA	NA	NA	NA	1

Gundogar et al. [12]	Diode GaAlAs	980	NA	0.4	NA	10	NA	NA	NA	7.64	15	4 (Baseline, days 1, 3 and 7)
Koçak et al. [13]	Diode AlGaInP	940	NA	1.5	NA	3	NA	20	NA	15-20	20	1
Studies with high intensity laser therapy												
Liu et al. [14]	Nd:YAG	1064	NA	3	NA	0.4	150	NA	NA	NA	NA	Test 1 and 2: 1 (baseline) Test 3: 1 (6 weeks after SRP)
Lopes et al. [15]	Er:YAG	2940	NA	NA	10	0.5	100	0.25-0.5	NA	12.9	Test 2: 30 Test 3: 180-240	1
Dominguez et al. [16]	Er:YAG	2940	NA	NA	10	0.4	160	NA	NA	NA	NA	1
Qadri et al. [17]	Nd:YAG	1064	2.4	4	50	0.6	80	0.35	240-480	NA	60-120	1
Gomez et al. [18]	Nd:YAG	1064	NA	NA	10	0.2	75	2	NA	NA	60	1
Eltas et al. [19]	Nd:YAG	1064	NA	1	10	0.2	100	NA	NA	NA	120	1
Studies with antimicrobial photodynamic therapy												
Ge et al. [20]	Diode	675	NA	0.1-0.14	NA	0.6	NA	NA	NA	6	60	2 (42 days)
Lui et al. [21]	Diode	940	NA	5	NA	0.3	NA	NA	NA	NA	<30	1
Souza et al. [22]	Diode	660	NA	NA	NA	0.6	NA	NA	NA	NA	60	1
Pourabbas et al. [23]	Diode	638	NA	NA	NA	NA	NA	NA	NA	8-10	120	1

Queiroz et al. [24]	Diode	660	NA	0.060	NA	0.6	NA	NA	NA	16.72	60	1
Teymouri et al. [25]	Diode	NA	NA	NA	NA	NA	NA	NA	NA	NA	10	1
da Cruz Andrade et al. [26]	Diode	660	NA	0.04	NA	NA	NA	NA	NA	90	90	4 (baseline, 3, 6 and 9 months)

NA: not available GaAlAs: gallium-aluminum- arsenide AlGaInP: aluminum-gallium-indium-phosphide

Er: Erbium Nd: neodymium-doped YAG: yttrium aluminum garnet nm: nanometers W: watts Hz: hertz

mm: millimeters mJ: millijoules ms: milliseconds J: joules

Table 4. Quality assessment of included studies following CONSORT statement

Investigators	A (0-2)	B (0-2)	C (0-1)	D (0-1)	E (0-2)	F (0-2)	G (0-2)	Total score	Estimated risk of bias
Studies with low level laser therapy									
Qadri et al. [5]	0	1	1	1	2	1	2	8	High
Aykol et al. [6]	0	2	1	1	2	1	2	9	High
Makhlouf et al. [7]	0	2	1	1	2	2	2	10	High
Calderín et al. [8]	0	2	1	1	2	1	2	9	High
Saglam et al. [9]	2	1	1	1	2	2	2	11	Moderate
Üstün et al. [10]	2	2	1	1	2	2	2	12	Low
Nguyen et al. [11]	2	2	1	1	2	2	2	12	Low
Gundogar et al. [12]	0	2	1	1	2	1	2	9	High
Koçak et al. [13]	2	2	1	1	2	2	2	12	Low
Studies with high intensity laser therapy									
Liu et al. [14]	0	1	1	1	2	1	2	8	High
Lopes et al. [15]	2	2	1	1	2	2	2	12	Low
Dominguez et al. [16]	0	2	1	1	2	2	2	10	High
Qadri et al. [17]	2	2	1	1	2	2	1	11	Moderate
Gomez et al. [18]	2	2	1	1	2	2	2	12	Low
Eltas et al. [19]	0	1	1	1	2	1	2	8	High
Studies with antimicrobial photodynamic therapy									

Ge et al. [20]	0	2	1	1	2	2	2	10	High
Lui et al. [21]	0	1	1	1	2	1	2	8	High
Souza et al. [22]	2	2	1	1	2	1	2	11	Moderate
Pourabbas et al. [23]	2	1	1	1	2	1	2	10	High
Queiroz et al. [24]	2	2	1	1	2	1	2	11	Moderate
Teymouri et al. [25]	0	0	1	1	2	1	1	6	High
da Cruz Andrade et al. [26]	2	2	1	1	2	2	2	12	Low

(A) sample size calculation (minimum number of participants required to detect a significant difference among compared groups); (B) randomization and allocation concealment methods; (C) clear definition of inclusion and/or exclusion criteria; (D) complete follow up; (E) experimental and control groups comparable at study baseline; (F) presence of masking; and (G) appropriate statistical analysis