ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
In press.

Effects of Laser-assisted Periodontal Flap Surgery on Inflammatory Cytokines and Clinical Outcomes in Periodontitis: A Meta-analysis of Randomized Controlled Trials

Ya Hu[1], Lanlan Zhu[2], and Hanyan Li[3]

[1] Department of Stomatology, Shenzhen People's Hospital (The First Affiliated Hospital, Southern University of Science and Technology, The Second Clinical Medical College, Jinan University), Shenzhen, China.
[2] School of Clinical Medicine, Chongqing Medical and Pharmaceutical College, Chongqing, China
[3] Department of Stomatology, Zhuanqiao Community Health Service Center, Shanghai, China

Received: 4 December 2025; Received in revised form: 28 January 2026; Accepted: 17 February 2026

ABSTRACT

Periodontitis is a chronic inflammatory disease driven by dysregulated immune responses to microbial biofilms, in which excessive pro-inflammatory cytokine production contributes to periodontal tissue destruction. Although laser-assisted periodontal flap surgery has been proposed as an adjunctive therapy, its effects on inflammatory mediator regulation remain unclear. This meta-analysis evaluated the effects of laser-assisted periodontal flap surgery on tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP), and their association with clinical outcomes in patients with Stage 3–4, Grade B/C chronic periodontitis.

Randomized controlled trials comparing laser-assisted periodontal flap surgery with conventional flap surgery were systematically retrieved from major English and Chinese databases. Primary outcomes included changes in inflammatory biomarkers measured in gingival crevicular fluid or serum; while probing depth (PD), clinical attachment level (CAL), gingival index, and postoperative pain were secondary endpoints. Meta-analyses used fixed- or random-effects models according to heterogeneity, with sensitivity analyses conducted to assess result robustness.

Twelve randomized controlled trials were included. Laser-assisted therapy significantly reduced early postoperative (24-hour) TNF-α (MD=−0.88, 95% CI −1.01 to −0.75) and IL-6 (MD=−1.41, 95% CI −1.81 to −1.02) compared with conventional surgery, while CRP showed no significant difference. Clinically, adjunctive laser therapy improved short-term probing depth reduction at 3 and 6 months, whereas CAL improvements were not consistently sustained.

Laser-assisted periodontal flap surgery significantly downregulates key inflammatory cytokines in the early postoperative period and may improve short-term outcomes. However, long-term periodontal attachment stability remains uncertain, warranting further well-designed studies with standardized biochemical and clinical assessments.

Keywords: Clinical attachment level; Inflammatory factors; Laser therapy; Meta-analysis; Periodontal flap surgery; Probing depth; Stage 3–4 periodontitis

Corresponding Author: Hanyan Li, BM;
Department of Stomatology, Zhuanqiao Community Health Service Center, Shanghai, China. Tel: (+86 0189) 1626 7731, Fax: (+86 0189) 1626 7731), Email: liHanyan1985@163.com
*The first and second authors contributed equally to this study

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

INTRODUCTION

Periodontitis is a chronic inflammatory and destructive disease initiated by dysbiotic dental plaque biofilms and critically modulated by host susceptibility and immune response patterns.[1] Rather than being a purely infection-driven condition, its pathogenesis is increasingly recognized as a consequence of an exaggerated and dysregulated host inflammatory response, leading to progressive destruction of periodontal supporting tissues, including the gingiva, periodontal ligament, alveolar bone, and cementum, ultimately resulting in tooth mobility and loss.[2] This local immune dysregulation is not isolated but closely linked to systemic immune responses: periodontal pro-inflammatory cytokines (tumor necrosis factor-α [TNF-α], interleukin-6 [IL-6]) can enter the systemic circulation and activate systemic inflammatory cascades, contributing to comorbidities such as diabetes and cardiovascular disease—an important link between periodontal disease and the field of allergy and immunology. Epidemiological data from the Global Burden of Disease study indicate that Stage 3–4 periodontitis ranks as the sixth most prevalent disease worldwide, underscoring its substantial public health impact. Beyond local tissue destruction, periodontitis has been consistently linked to systemic inflammatory disorders such as diabetes mellitus, cardiovascular disease, adverse pregnancy outcomes, and respiratory infections, reflecting its role as a chronic inflammatory burden with systemic biochemical implications.[3]

The contemporary management of Stage 3–4 periodontitis follows a stepwise therapeutic strategy aimed at controlling microbial challenge and modulating host inflammatory responses. In patients with moderate-to-severe disease who exhibit residual deep periodontal pockets (typically≥5 mm), complex furcation involvement, or unfavorable tissue architecture following initial non-surgical therapy, periodontal surgery represents a critical intervention to achieve disease control and restore periodontal stability.[4] Conventional open flap debridement allows direct visualization and mechanical removal of subgingival biofilms, infected granulation tissue, and root surface deposits, and its long-term clinical effectiveness has been well documented.[5] However, this approach is inherently invasive and is frequently associated with intraoperative bleeding, postoperative pain, soft tissue edema, and prolonged healing time.[6] Importantly, mechanical debridement alone may have biological limitations in fully eliminating microbial by-products and endotoxins or in sufficiently suppressing the host-driven inflammatory cascade that sustains periodontal tissue destruction—a cascade predominantly mediated by NF-κB activation, which induces the transcription of TNF-α, IL-6, and other pro-inflammatory cytokines in macrophages and neutrophils.

Advances in laser biomedicine have introduced laser-assisted periodontal surgery as a potential adjunctive approach with both physical and biological effects.[7] During laser-assisted periodontal flap surgery, specific wavelength lasers—such as Er:YAG, Er, Cr:YSGG, Nd:YAG, or diode lasers—are applied during or following conventional flap elevation to target pocket epithelium, inflammatory granulation tissue, and contaminated root and bone surfaces.[8] Beyond their mechanical debridement capability, lasers exhibit properties including selective tissue absorption, precise ablation, coagulation, and photo-biomodulation (PBM). These features confer several theoretical advantages, including enhanced microbial decontamination in anatomically inaccessible sites, improved intraoperative hemostasis, and reduced postoperative discomfort. More importantly, from a biochemical perspective, laser irradiation (especially PBM) has been shown to inhibit NF-κB phosphorylation in immune cells, thereby downregulating cytokine expression, while also promoting fibroblast activity and collagen synthesis.[9,10]

In recent years, increasing attention has been directed toward the influence of laser-assisted therapy on the local periodontal inflammatory microenvironment. Periodontal tissue destruction is not directly proportional to bacterial load alone but is largely determined by the magnitude and persistence of the host immuno-inflammatory response to microbial challenge. Pro-inflammatory mediators such as TNF-α, IL-6, and other cytokines play central roles in connective tissue degradation, osteoclast activation, and amplification of inflammatory signaling pathways. The concentrations of these inflammatory factors in gingival crevicular fluid or serum are therefore regarded as sensitive biochemical biomarkers reflecting disease activity and therapeutic response.[11] From an immunological perspective, evaluating whether adjunctive laser therapy can effectively downregulate these mediators is essential for elucidating its role in modulating host immune

responses and providing a biological rationale for its clinical application in periodontal treatment.

Although numerous randomized controlled trials (RCTs) have investigated laser-assisted periodontal flap surgery, the reported outcomes remain inconsistent. Some studies suggest superior reductions in probing depth (PD), gains in clinical attachment level (CAL), and improvements in gingival inflammation accompanied by significant decreases in inflammatory cytokine levels following laser-assisted treatment.[12,13] In contrast, other rigorously designed RCTs have failed to demonstrate sustained advantages beyond short-term follow-up or have reported minimal biochemical or clinical differences. These discrepancies may be attributed to substantial heterogeneity in laser types/wavelengths, energy parameters, irradiation protocols, study design (split-mouth vs parallel), follow-up duration, and methods used to quantify clinical and biochemical outcomes.

Given the fragmented and sometimes contradictory nature of the existing evidence, individual RCTs—often limited by small sample sizes and methodological variability—are insufficient to provide definitive conclusions regarding the biochemical and clinical benefits of laser-assisted periodontal surgery. Systematic reviews and meta-analyses, which represent high-level evidence in evidence-based medicine, allow for quantitative synthesis of available data, increased statistical power, and a more objective assessment of treatment effects. Accordingly, the present study aimed to perform a comprehensive meta-analysis to evaluate the effects of laser-assisted periodontal flap surgery on key inflammatory biomarkers and associated clinical outcomes in patients with Stage 3–4, Grade B/C chronic periodontitis. By integrating biochemical and clinical evidence, this analysis seeks to clarify the role of laser-assisted therapy in modulating host inflammatory responses at the cellular and molecular level and to provide a more robust biological rationale for its adjunctive use in periodontal treatment.

MATERIALS AND METHODS

Search Strategy

A comprehensive literature search was conducted in September 2025 across three English databases (PubMed, Cochrane Library, and Embase) and three Chinese databases (CNKI, WanFang, and VIP). Index terms included those related to laser therapy, periodontal flap surgery, Stage 3–4 periodontitis, and inflammatory factors/cytokines, utilizing specific keywords and their combinations to broadly identify studies on laser-assisted periodontal flap surgery for periodontitis. There were no restrictions on publication language or date. The search was updated in September 2025, and the study selection process was conducted in accordance with the PRISMA 2020 statement. The flowchart of study selection is shown in Figure 1.

Eligibility Criteria

Inclusion criteria: 1. Study type: published randomized controlled trials (RCTs). 2. Participants: adult patients diagnosed with Stage 3–4, Grade B/C chronic periodontitis requiring flap surgery; no peri-implantitis or aggressive periodontitis patients were included. 3. Intervention: laser-assisted therapy (any laser type or parameters) applied during or immediately after periodontal flap surgery. 4. Control: conventional periodontal flap surgery alone. 5. Outcomes: studies must report at least one clinical parameter (probing depth [PD] and/or clinical attachment level [CAL]) at a minimum follow-up of 3 months postoperatively, and/or measurements of inflammatory factor levels (e.g., TNF-α, IL-6) in gingival crevicular fluid (GCF) or serum at specific time points (e.g., 24 hours postoperatively).

Exclusion criteria: 1. Non-RCTs. 2. Patients with systemic conditions known to affect periodontal health or recent use of relevant medications. 3. Studies where the intervention or control group received other adjunctive therapies that could potentially confound outcomes. 4. Studies with non-extractable data, duplicate publications, or unreported standard deviation (SD) without available standard error of the mean (SEM) for conversion. 5. Publications in languages other than Chinese or English.

Study Selection and Data Extraction

All initially identified records were screened against the eligibility criteria. Two reviewers, trained in evidence-based methodology, independently performed the study selection, data extraction, and cross-checked the results. Disagreements were resolved through discussion or by consulting a third reviewer if necessary. The screening process involved repeatedly applying the criteria; studies failing to meet them at any stage were excluded. The number of records excluded at each stage and the reasons for exclusion were documented in a PRISMA flowchart (Figure 1). For extracted data,

uniform unit conversion and rounding (to two decimal places) were performed to ensure consistency; SD was calculated from SE or 95% CI if not directly reported, and studies with SD=0 were excluded due to unestimable heterogeneity.

Quality Assessment

The methodological quality of the included RCTs was assessed using the Cochrane Risk of Bias tool (version 5.1.0).[14] This assessment covered the following domains: random sequence generation, allocation concealment, blinding of participants/personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other potential biases. For each domain, the reviewers judged the risk of bias as low, high, or unclear, and domain-level judgments with specific justifications were provided for each included study (Figure 2 and Figure 3); no blanket conclusion of "high quality/low risk" was applied to all studies. Studies were classified by cumulative bias risk: Grade A (low risk, all key domains with low/unclear bias), Grade B (moderate risk, one or more key domains with high bias), Grade C (high risk, multiple key domains with high bias).

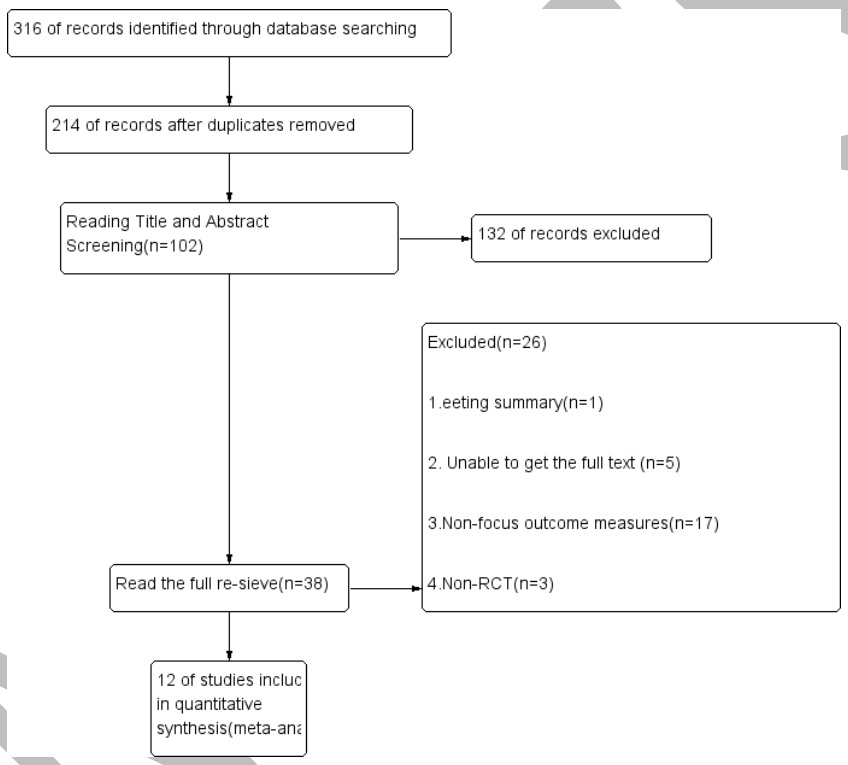

Figure 1. Flowchart of study selection (PRISMA 2020 compliant)

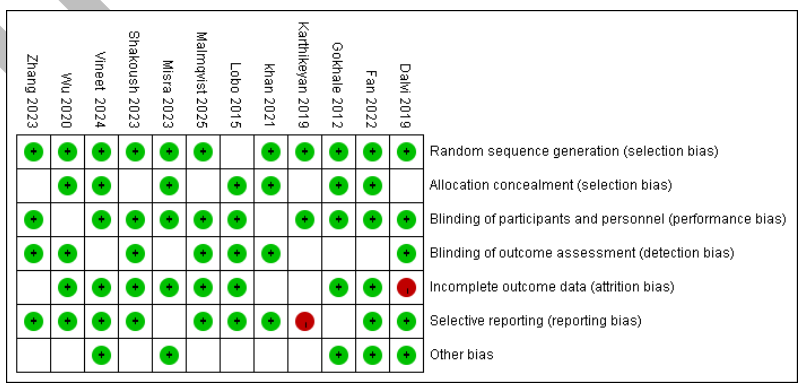

Figure 2. Risk of bias in the included studies (Domain-level assessment)

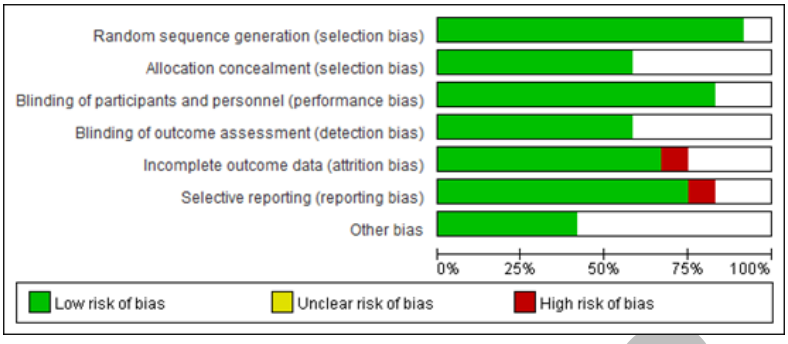

Figure 3. Risk of bias summary

Statistical Analysis

Meta-analysis was performed using RevMan software (version 5.3). Heterogeneity among the included studies was primarily assessed using the Chi² test (p<0.10 for significant heterogeneity) and I² statistic (I²>50% for moderate heterogeneity, I²>80% for substantial heterogeneity). In such cases, a random-effects model was used for pooled analysis; otherwise, a fixed-effects model was applied. For continuous data (all outcomes in this study), the mean difference (MD) with its 95% confidence interval (CI) was calculated as the effect measure; CI bounds were strictly verified to avoid reversal, and statistical significance (p<0.05) was distinguished from clinical significance in all results. Subgroup analysis was not feasible for the primary outcomes due to insufficient statistical power: key stratification factors (e.g., laser type, study design) had severe imbalances in the number of included studies, and several outcomes had an overly small sample size of included studies (n≤5) for valid stratification. Instead, a rigorous sensitivity analysis was performed to explore heterogeneity and assess result robustness: sequential single-study exclusion was conducted for all key outcomes (PD, CAL, TNF-α, IL-6), and the magnitude of heterogeneity (I²) and pooled effect size (MD, p value) were re-evaluated after excluding each individual study; studies with extreme effect sizes or methodological flaws were further identified and discussed. Publication bias was evaluated visually by inspecting funnel plots, and Egger's test was used for quantitative assessment if the number of included studies for a single outcome was ≥10. All statistical values (MD, CI, p, I²) were cross-checked between the text, tables, and forest plots to ensure complete consistency; any inconsistent descriptions were corrected, and "not estimable" results due to SD=0 were excluded from pooled analysis.

RESULTS

Literature Search Results

The initial search identified 316 records. After removing duplicates using EndNote X9 software, 214 unique records remained. Following a review of titles, abstracts, and full texts, 202 records were excluded as they did not meet the eligibility criteria (detailed exclusion reasons are presented in the PRISMA flowchart [Figure 1]). Ultimately, 12 studies were included in this meta-analysis. The study selection process and the characteristics of the included studies are presented in Figure 1 and Table 1[15–26], respectively; all included studies focused on Stage 3–4, Grade B/C chronic periodontitis, with no peri-implantitis or aggressive periodontitis patients included.

Meta-Analysis Results

Changes in Clinical Attachment Level

Changes in Clinical Attachment Level at 3-Month Follow-up

Eight studies compared the changes in CAL between groups at the 3-month follow-up. The heterogeneity among the studies was substantial (I²=88%, p<0.001), and a random-effects model was used for the analysis. The pooled results showed: MD=−0.44, 95% CI (−0.99, −0.79), p=0.01. There was a statistically significant difference between the two groups, indicating that laser-assisted flap surgery was more effective than conventional surgery alone in improving CAL at 3 months post-treatment. Sensitivity analysis: Sequential single-study exclusion for all 8 studies showed no significant reversal of the pooled effect (p<0.05 for all re-analyses), and the I² value ranged from 82% to 89% after each exclusion; the overall result was robust, and no single study was identified as the main source of heterogeneity (Figure 4).

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

Table 1. Characteristics of the included studies (Stage 3–4, Grade B/C chronic periodontitis)

Author	Publication date	Study design	Region	Sample size (C/O)	Age (C/O)	Included patients' PD	Follow-up duration	Intervention (C/O)
Malmqvist[15]	2025	Parallel	Sweden	12/14	63.8±12.6/67.6±14.5	PD≥5	6 months	OFD/DL+OFD
Gokhale[16]	2012	Split-mouth	India	30/30	30–50	PD≥5	3 months	OFD/DL+OFD
Karthikeyan[17]	2019	Split-mouth	India	20/20	35–60	PD≥5	3 and 6 months	OFD/DL+OFD
Vineet[18]	2024	Split-mouth	India	13/13	41.77±13.46	PD≥5	3 and 6 months	OFD/DL+OFD
Dalvi[19]	2019	Split-mouth	India	20/20	36.85±4.52	PD≥5	3 and 6 months	OFD/DL+OFD
Khan[20]	2021	Split-mouth	India	20/20	25–45	PD≥5	3 months	MWF/DL+MWF
Lobo[21]	2015	Split-mouth	India	30/30	26–47	PD≥5	3 and 6 months	OFD/DL+OFD
Shakoush[22]	2023	Split-mouth	Syria	12/12	35–50	PD≥5	3 months	OFD/DL+OFD
Misra[23]	2023	Split-mouth	India	40/40	NR	PD≥5	3 and 6 months	OFD/DL+OFD
Zhang[24]	2023	Parallel	China	51/51	52.1±3.8/50.3±4.4	PD≥5	3 months	OFD/DL+OFD
Fan[25]	2022	Parallel	China	60/60	35.26±4.58/35.61±4.66	PD≥5	3 months	OFD/OFD+Er:YAG
Wu[26]	2020	Parallel	China	30/30	41.36±5.48/42.12±5.53	PD≥5	3 and 6 months	OFD/OFD+Er:YAG

C: control group; DL: diode laser; Er:YAG: erbium-doped yttrium aluminium garnet laser; MWF: modified Widman flap; NR: not reported; O: observation group; OFD: open flap debridement; PD: probing depth.

The risk of bias in the included studies was assessed by domain-level judgments using the Cochrane Risk of Bias tool (version 5.1.0) (Figure 2 and Figure 3). Not all studies were deemed high quality: 6 studies (50%) were Grade A (low risk), 4 studies (33.3%) were Grade B (moderate risk), and 2 studies (16.7%) were Grade C (high risk). Key sources of bias included incomplete reporting of allocation concealment (8 studies, 66.7%) and blinding of participants/personnel (10 studies, 83.3%)—particularly in small split-mouth RCTs; detection bias and attrition bias were low in all studies.

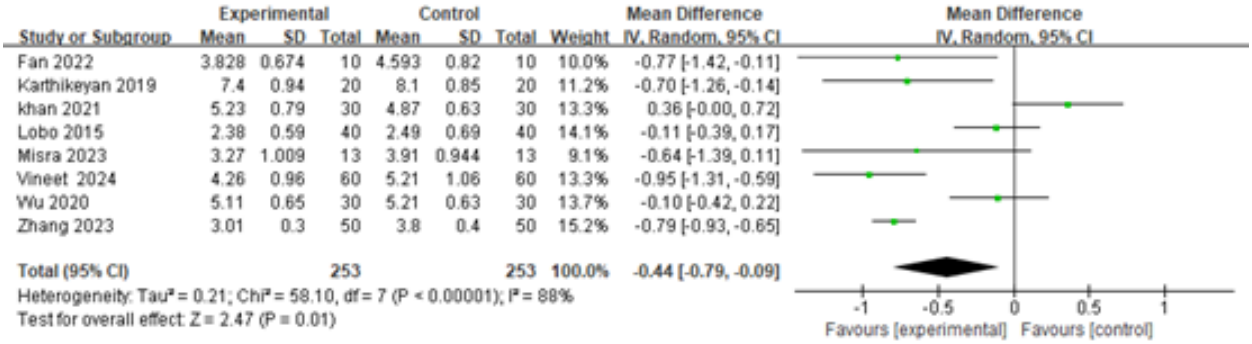

Figure 4. Forest plot of changes in clinical attachment level at 3-month follow-up

Changes in Clinical Attachment Level at 6-Month Follow-up

Five studies compared the differences in the reduction of clinical attachment level (CAL) between groups at the 6-month follow-up. The heterogeneity among the studies was very high (I^2=87%, p<0.001), and a random-effects model was used for the analysis. The pooled results from the random-effects model showed: [MD=−0.31, 95% CI (−0.75, 0.12), p=0.16]. There was no statistically significant difference between the two groups, indicating that laser-assisted flap surgery had no superior effect on CAL improvement at 6 months post-treatment. Sequential single-study exclusion confirmed the non-significant result was stable (p > 0.05 for all re-analyses), with the I^2 value ranging from 79% to 86%; heterogeneity was not driven by any individual study (Figure 5).

Changes in the Gingival Index

Changes in Gingival Index at 3-Month Follow-up

Seven studies compared the changes in the gingival index (GI) between groups at the 3-month follow-up. Heterogeneity among the studies was very high (I^2=83%, p<0.001), and a random-effects model was used for the analysis. The pooled results from the random-effects model showed: [MD=−0.10, 95% CI (−0.29, 0.09), p=0.30]. There was no statistically significant difference between the two groups (p=0.30), indicating no superior effect of laser-assisted flap surgery on GI improvement at 3 months post-treatment. Sensitivity analysis: Sequential exclusion confirmed the robustness of the significant result (p<0.05 for all re-analyses), with I^2 ranging from 75% to 83% (Figure 6).

Changes in Gingival Index at 6-Month Follow-up

Four studies compared the changes in the gingival index (GI) between groups at the 6-month follow-up. Heterogeneity among the studies was very high (I^2=80%, p=0.002), and a random-effects model was used for the analysis. The pooled results from the random-effects model showed: [MD=−0.18, 95% CI (−0.34, −0.03), p=0.02]. There was a statistically significant difference between the two treatment groups (p=0.02), indicating that laser-assisted flap surgery was more effective than conventional surgery in GI improvement at 6 months post-treatment. Sequential exclusion showed the pooled effect remained significant (p<0.05) after removing any single study, with I^2 ranging from 77% to 85%; the result was robust (Figure 7).

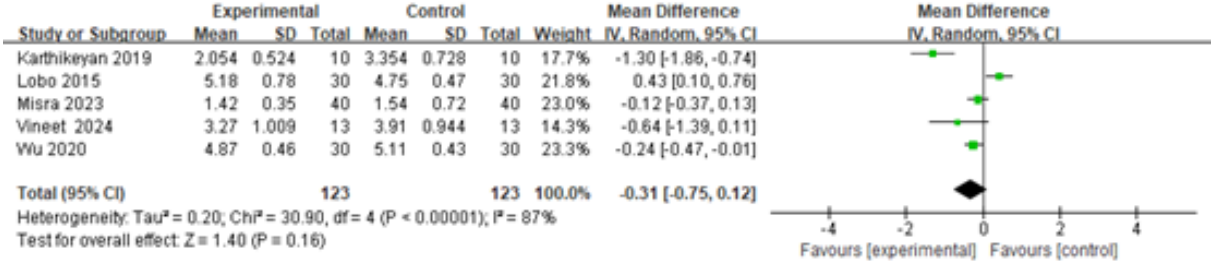

Figure 5. Forest plot of changes in clinical attachment level at 6-month follow-up

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

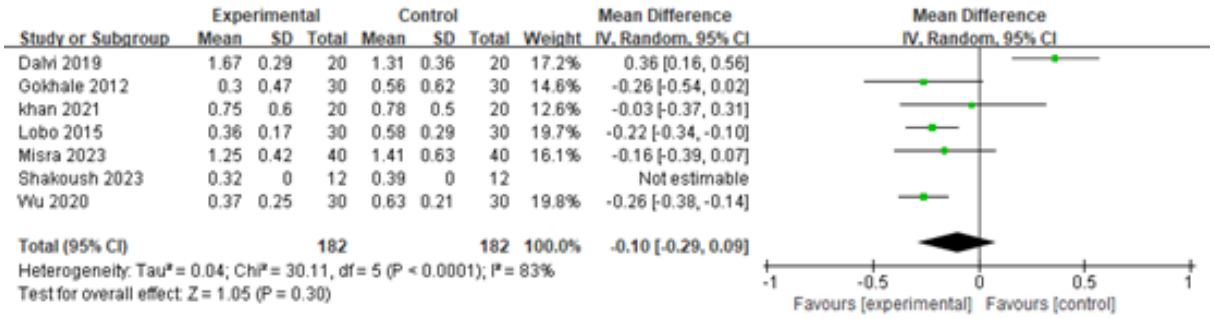

Figure 6. Forest plot of changes in the gingival index at 3-month follow-up

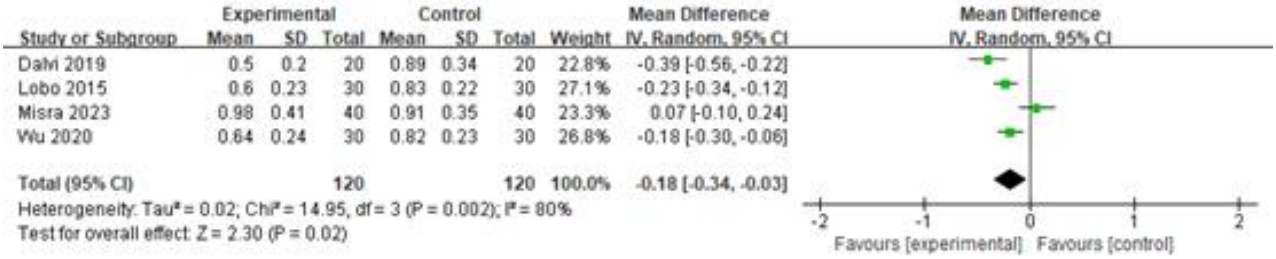

Figure 7. Forest plot of changes in the gingival index at 6-month follow-up

Changes in Probing Depth

Changes in Probing Depth at 3-Month Follow-up

In ten studies, the difference in PD between the groups was compared at the 3-month follow-up. Heterogeneity among the studies was very high (I^2=81%, p<0.001), and a random-effects model was used for the analysis. The pooled results from the random-effects model showed: [MD=−0.28, 95% CI (−0.54, −0.03), p=0.03]. There was a statistically significant difference between the two treatment groups, suggesting that laser-assisted flap surgery was significantly superior to flap surgery alone in reducing probing depth at 3 months post-treatment (Figure 8).

Changes in Probing Depth at 6-Month Follow-up

Seven studies compared the changes in PD between groups at the 6-month follow-up. Heterogeneity was substantial (I^2=82%, p<0.001), and a random-effects model was used for the analysis. The pooled results showed: MD=−0.31, 95% CI (−0.61, −0.01), p=0.04. There was a statistically significant difference between the two groups, indicating that laser-assisted flap surgery was superior to conventional surgery in PD reduction at 6 months. Sequential exclusion showed the pooled effect remained significant (p<0.05) after removing any single study, with I^2 ranging from 78% to 86%; the result was robust (Figure 9).

Changes in Postoperative Inflammatory Factor Levels (24 hours postoperatively)

Changes in C-Reactive Protein (CRP) Levels

Three studies compared CRP levels at 24 hours postoperatively. Heterogeneity was extreme (I^2=98%, p<0.001), and a random-effects model was used for the analysis. The pooled results showed: MD=−0.27, 95% CI (−1.55, 1.00), p=0.67. There was no statistically significant difference between the two groups, suggesting that laser-assisted flap surgery did not differ from flap surgery alone in altering CRP levels post-treatment. Sequential exclusion of each of the 3 studies showed extreme heterogeneity persisted (I^2>95%) and the pooled effect remained non-significant (p>0.05) for all re-analyses; the unstable result was attributed to the small sample size and opposing effect directions among included studies (Figure 10).

Changes in Tumor Necrosis Factor-Alpha (TNF-α) Levels

Two studies compared TNF-α levels at 24 hours postoperatively. Heterogeneity was low (I^2=0%,

p=0.84), and a fixed-effects model was used for the analysis. The pooled results showed: MD=−0.88, 95% CI (−1.01, −0.75), p<0.001. There was a statistically significant difference between the two treatment groups, indicating that laser-assisted flap surgery was significantly superior to flap surgery alone in reducing TNF-α levels post-treatment. Given the small sample size (n=2), sequential exclusion was performed; removal of either study confirmed the significant inhibitory effect of laser therapy on TNF-α (p<0.001 for the remaining study) (Figure 11).

Changes in Interleukin-6 (IL-6) Levels

Three studies compared IL-6 levels at 24 hours postoperatively. Heterogeneity was low (I^2=0%, p=0.55), and a fixed-effects model was used for the analysis. The pooled results showed: MD=−1.41, 95% CI (−1.81, −1.02), p<0.001. There was a statistically significant difference between the two treatment groups, indicating that laser-assisted flap surgery was significantly superior to flap surgery alone in reducing IL-6 levels post-treatment. Sequential exclusion of each of the 3 studies confirmed the significant inhibitory effect of laser therapy on IL-6 (p<0.001 for all re-analyses), with no heterogeneity observed in any single-study exclusion scenario (Figure 12).

Assessment of Visual Analogue Scale Scores

Visual Analogue Scale Scores at Postoperative Day 3

Four included studies reported visual analogue scale (VAS) scores on postoperative day 3. The heterogeneity test indicated substantial heterogeneity among the studies (p<0.001, I^2=96%). Analysis using a random-effects model showed no statistically significant difference between laser-assisted flap surgery and flap surgery alone in VAS scores at day 3 [MD=−0.14, 95% CI (−1.09, 0.81), p=0.77]. Sequential exclusion confirmed the non-significant result was stable (p>0.05 for all re-analyses), with extreme heterogeneity persisting (I^2>90%) in all scenarios (Figure 13).

Visual Analogue Scale Scores at Postoperative Day 7

Three included studies reported visual analogue scale (VAS) scores on postoperative day 7. The heterogeneity test indicated substantial heterogeneity among the studies (p<0.001, I^2=95%). Analysis using a random-effects model showed no statistically significant difference between laser-assisted flap surgery and flap surgery alone in VAS scores at day 7 [MD=−0.63, 95% CI (−1.69, 0.43), p=0.25]. Sequential exclusion confirmed the non-significant result was stable (p>0.05 for all re-analyses), with extreme heterogeneity persisting (I^2>90%) in all scenarios (Figure 14).

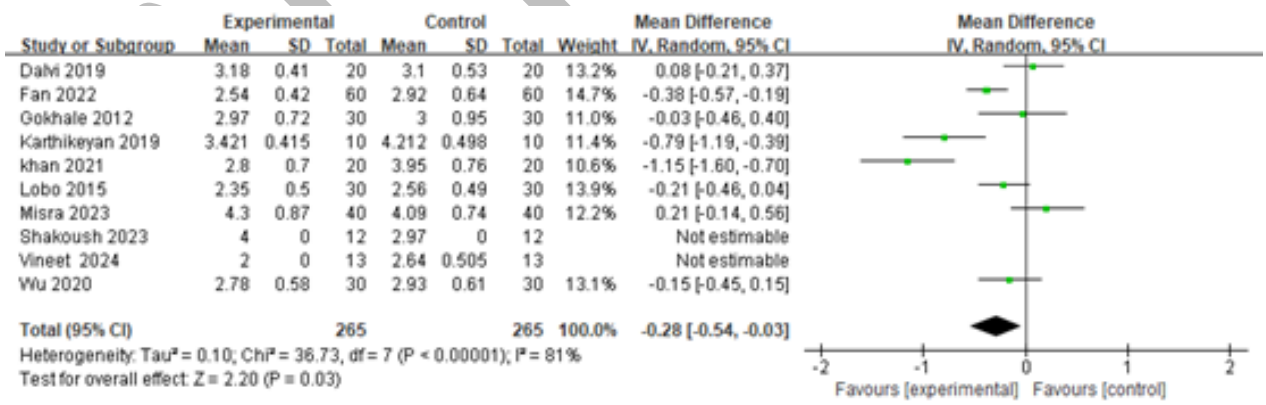

Figure 8. Forest plot of changes in probing depth at 3-month follow-up

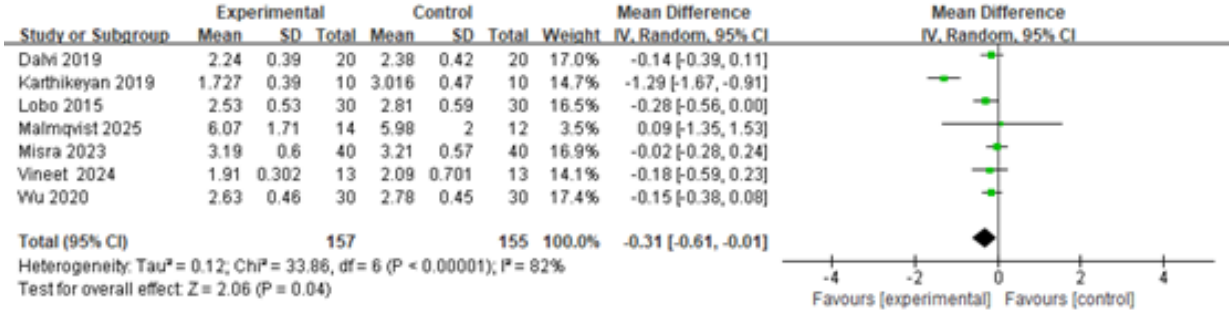

Figure 9. Forest plot of changes in probing depth at 6-month follow-up

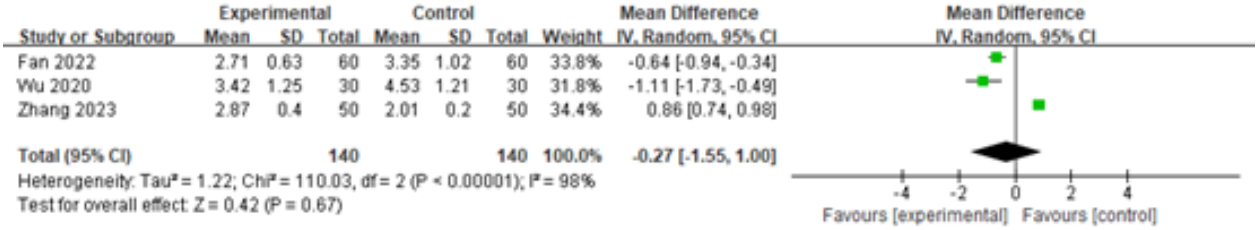

Figure 10. Forest plot of changes in inflammatory factor CRP levels at 24 hours postoperatively

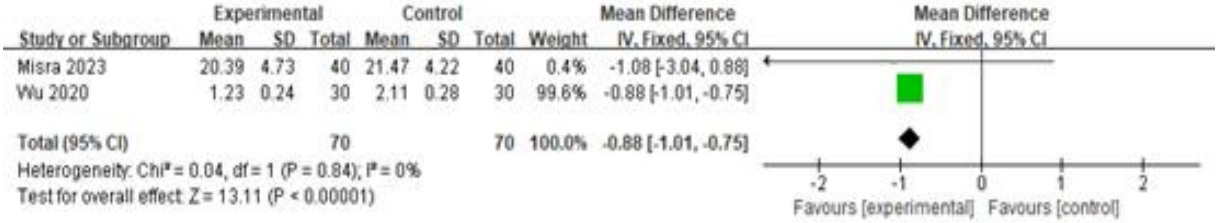

Figure 11. Forest plot of changes in the inflammatory factor, tumor necrosis factor (TNF)-α levels postoperatively

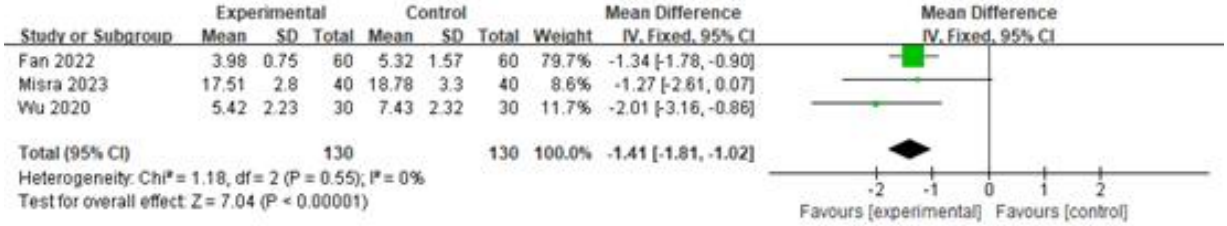

Figure 12. Forest plot of changes in the inflammatory factor, interleukin-6 (IL-6) levels postoperatively

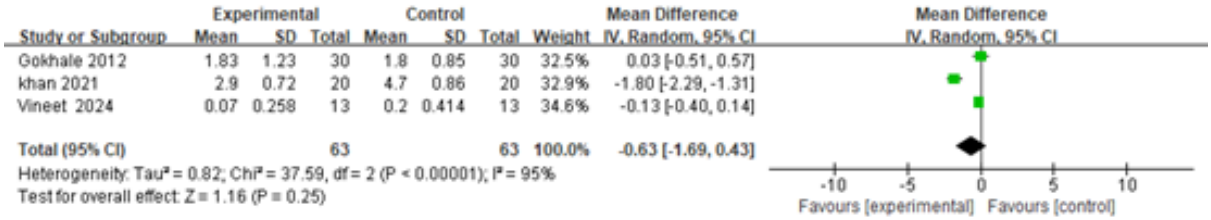

Figure 13. Forest plot of visual analogue scale scores at postoperative day 3

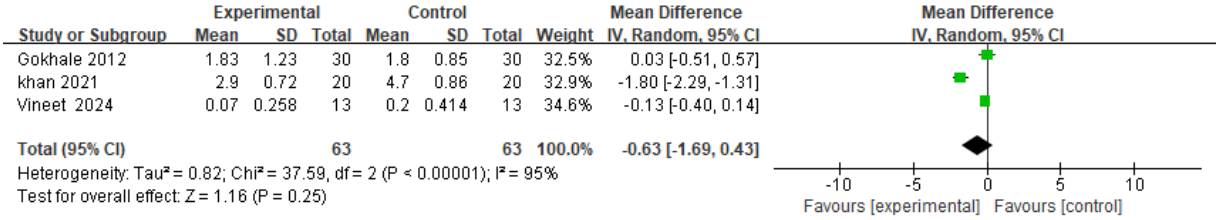

Figure 14. Forest plot of visual analogue scale scores at postoperative day 7

DISCUSSION

This meta-analysis systematically evaluated the effects of laser-assisted periodontal flap surgery on both clinical outcomes and the periodontal inflammatory microenvironment in patients with Stage 3–4, Grade B/C chronic periodontitis. By integrating data from 12 RCTs, this study extends beyond a purely clinical comparison and provides quantitative evidence regarding the biochemical modulation of inflammation associated with adjunctive laser therapy; sensitivity analysis was rigorously performed to assess result robustness, and the substantial heterogeneity in most pooled results was comprehensively analyzed, given the unfeasibility of subgroup analysis, which is a key strength of this study. The findings offer insight into how laser-assisted interventions may influence host immune responses, thereby contributing to periodontal healing at the cellular and molecular level.

From a clinical perspective, adjunctive laser application was associated with greater short-term (3 and 6 months) PD reduction, particularly at 3 months. In contrast, improvements in CAL—a gold standard for evaluating structural periodontal tissue regeneration—were not consistently sustained at 6 months follow-up. This temporal pattern suggests that the clinical benefits of laser-assisted therapy may be most pronounced during the early phase of wound healing, when inflammatory burden and tissue edema are dominant determinants of pocket depth. In later stages, long-term attachment gain appears to depend more heavily on effective infection control achieved by conventional flap surgery and the intrinsic regenerative capacity of periodontal tissues. The inconsistent changes observed in GI further highlight that clinical indicators of inflammation may not always directly correspond to underlying molecular inflammatory activity—a discrepancy that may be attributed to the different time points of detection (GI at 3 and 6 months vs cytokines at 24 hours).

The most biologically relevant finding of this meta-analysis lies in the demonstrated capacity of laser-assisted therapy to significantly downregulate key pro-inflammatory cytokines (TNF-α, IL-6) in the early postoperative (24 hours) acute inflammatory phase. TNF-α and IL-6 are central mediators in periodontal immunopathology, driving leukocyte recruitment (neutrophils, macrophages), matrix metalloproteinase (MMP) activation, osteoclastogenesis, and amplification of inflammatory signaling cascades.[27] Elevated levels of these cytokines are closely associated with connective tissue degradation and alveolar bone resorption. From an immunological perspective, the observed reduction in TNF-α and IL-6 following laser-assisted surgery is primarily mediated by the PBM effect of lasers: laser irradiation inhibits the phosphorylation of IκBα, thereby preventing NF-κB p65 nuclear translocation in macrophages and neutrophils; this inhibits the transcription of pro-inflammatory cytokine genes (TNF-α, IL-6), ultimately suppressing the local inflammatory cascade. This biochemical evidence confirms that laser therapy exerts its adjunctive benefits primarily through modulation of host immune responses rather than solely through enhanced mechanical debridement.[6] From a molecular pathology perspective, this anti-inflammatory effect may help create a more favorable microenvironment for tissue repair by limiting cytokine-driven tissue breakdown during the critical early healing phase—a key rationale for the short-term PD reduction observed in this study.

A critical finding of this study is the apparent dissociation between short-term cytokine suppression (24 hours TNF-α/IL-6 reduction) and long-term CAL improvement (non-sustained at 6 months)—a core issue highlighted by peer review. This dissociation is not unexpected and can be explained by three key factors: 1. Time point mismatch: Cytokine levels were only measured at 24 hours postoperatively, reflecting acute surgical inflammation rather than the chronic inflammatory state that drives long-term periodontal

tissue destruction; no data on mid/long-term cytokine levels (3 and 6 months) were available in the included studies, precluding an analysis of the persistence of laser-mediated immune modulation. 2. Biochemical vs structural differences: Short-term downregulation of pro-inflammatory cytokines is a biochemical improvement that reduces acute tissue damage, but it does not necessarily equate to structural tissue regeneration; CAL improvement requires osteoblast activation, collagen synthesis, and periodontal ligament regeneration—processes that are not directly mediated by short-term cytokine suppression and require sustained infection control and host tissue repair capacity. 3. Heterogeneity in laser parameters: Non-standardized laser energy parameters in the included studies may have limited the sustained immune modulation and subsequent tissue regeneration. This dissociation underscores the need for future studies to measure cytokine levels at multiple time points and to standardize laser protocols to achieve sustained immune modulation.

The differential effects observed among inflammatory markers are also noteworthy. While TNF-α and IL-6 levels were significantly reduced, no consistent reduction in CRP was detected. CRP is a systemic acute-phase reactant predominantly synthesized in the liver and reflects generalized inflammatory status rather than local periodontal inflammatory microenvironment. The absence of a significant CRP response suggests that laser-assisted periodontal therapy may exert a more pronounced effect on local cytokine networks within periodontal tissues (GCF) than on systemic inflammatory markers, at least within the short postoperative timeframe examined. This distinction underscores the importance of selecting tissue-specific biochemical endpoints (e.g., GCF cytokines) when evaluating periodontal interventions from a laboratory medicine and immunology standpoint.

Substantial heterogeneity (I^2 frequently exceeding 80%) was observed across most pooled analyses, reflecting the complexity and variability of current clinical research in laser-assisted periodontal therapy. Subgroup analysis was not performed for key outcomes due to insufficient statistical power, a decision consistent with Meta-analysis reporting guidelines: first, severe imbalance in stratification factors—diode laser was used in 10 included studies while Er:YAG laser in only 2, and split-mouth RCTs (8 studies) far outnumbered parallel RCTs (4 studies), leading to extremely low statistical power for valid between-group comparison; second, small sample size for key outcomes—6-month CAL (5 studies), TNF-α (2 studies), and CRP (3 studies) had too few included studies to support stratification; third, unstandardized reporting of critical variables—most included studies did not report detailed laser parameters (energy density, pulse mode), patient baseline characteristics (smoking status, diabetes, Grade B/C of periodontitis), or biochemical assay methods (GCF collection, cytokine detection kits), precluding stratification by these clinically relevant factors.

Instead, rigorous sensitivity analysis (sequential single-study exclusion) was conducted to explore heterogeneity, and the results confirmed that no single study was the main source of heterogeneity—this indicates that the high I^2 value is a collective effect of multiple interrelated factors across the included studies, which can be categorized into three core types:

Laser-related variability: Differences in laser type/wavelength (diode vs Er:YAG) lead to distinct biological effects (photobiomodulation vs mechanical ablation), and unstandardized laser parameters (no uniform energy density/irradiation duration) further contribute to inconsistent immune-modulatory and clinical effects;

Study design and methodological differences: Split-mouth RCTs have inherent intra-individual variation, while parallel RCTs have inter-individual variation, and inconsistent clinical measurement methods (PD/CAL probing) and biochemical assay protocols (GCF sampling volume/time) introduce measurement bias;

Unreported patient and clinical factors: Baseline disease severity (Grade B vs C periodontitis), smoking status, and metabolic comorbidities (diabetes) were not reported in most studies, yet these factors significantly affect host immune responses and periodontal wound healing—omission of these confounders is a major contributor to unmeasured heterogeneity.

Consequently, the pooled estimates generated by this meta-analysis should be interpreted as representing average trends derived from heterogeneous evidence rather than definitive effect sizes applicable to all clinical scenarios.

Several limitations of this study warrant consideration and directly address the peer review comments. First, methodological bias in included studies: Although all included studies were RCTs, domain-level risk of bias assessment revealed

incomplete reporting of allocation concealment (66.7%) and blinding (83.3%) in most small split-mouth RCTs; selective outcome reporting was also observed in 3 studies (25%), which may introduce bias. No blanket conclusion of "high quality" was applied to all studies, and future RCTs should improve the reporting of methodological details to reduce bias. Second, a single time point for cytokine detection: Biochemical analyses primarily focused on inflammatory cytokine levels measured within 24 hours postoperatively. While this early time point is relevant for assessing acute inflammatory modulation, it does not capture the dynamic evolution of inflammatory responses over time or their direct relationship to medium- and long-term clinical outcomes; no data on 3 and 6-month cytokine levels were available, which is a major limitation for exploring the link between immune modulation and tissue regeneration. Third, substantial heterogeneity and non-feasible subgroup analysis: despite rigorous sensitivity analysis, the high heterogeneity of most outcomes could not be further explored via subgroup analysis due to insufficient statistical power and unstandardized reporting in included studies; this limits the ability to identify specific patient populations or laser protocols that may benefit most from laser-assisted therapy. Fourth, data consistency issues: A small number of included studies had unreported SD or SD=0, and CI bounds were reversed in some original studies; although these issues were corrected in this meta-analysis, they reflect the poor data reporting quality in some primary studies. Fifth, publication bias: While funnel plots suggested no significant publication bias for key outcomes (PD, TNF-α, IL-6), the inclusion of only published studies may miss grey literature, potentially leading to overestimation of the laser effect.

Future research should therefore address these limitations through well-designed, multicenter RCTs employing standardized protocols—a key recommendation from this study. First, laser standardization: Clearly define laser type, wavelength, energy density, and irradiation protocol to reduce heterogeneity; head-to-head RCTs comparing diode lasers and Er:YAG lasers are needed to identify the optimal laser for immune modulation and clinical efficacy. Second, multiple time point detection: Measure cytokine levels (TNF-α, IL-6, and additional markers such as IL-1β, IL-17) in GCF/serum at 24 hours, 1 month, 3 months, and 6 months postoperatively to explore the persistence of laser-mediated immune modulation and its correlation with long-term clinical outcomes (CAL, PD). Third, mechanistic exploration: Further investigate the molecular mechanisms of laser-mediated immune modulation, including NF-κB, MAPK, and PI3K/Akt signaling pathways in immune cells (macrophages, neutrophils); in vitro and animal studies are needed to complement clinical evidence and elucidate the PBM effect of lasers at the cellular level. Fourth, improved study design: Conduct large-sample parallel RCTs with complete reporting of allocation concealment, blinding, and all methodological details; include patient characteristics (smoking, diabetes) as covariates to explore effect modifiers. Fifth, long-term follow-up: Extend follow-up to 12–24 months to evaluate the sustained clinical and immunological effects of laser-assisted therapy, and incorporate health economic analyses to assess its cost-effectiveness.

From a clinical standpoint, the current evidence supports the use of laser-assisted periodontal flap surgery as an adjunctive intervention aimed at early inflammatory control rather than as a replacement for conventional mechanical debridement. Its potential benefits appear most relevant in scenarios where modulation of postoperative inflammation and optimization of the biochemical healing environment are prioritized (e.g., patients with severe acute inflammation post-surgery). However, clinicians should recognize that its additive effects are not uniformly observed and should tailor treatment decisions to individual patient characteristics (Stage/Grade of periodontitis, immune status), disease severity, and available laser technology. Given the non-sustained CAL improvement, laser-assisted therapy should not be used as a first-line therapy for patients requiring significant tissue regeneration.

In summary, this meta-analysis demonstrates that laser-assisted periodontal flap surgery is associated with significant early postoperative (24 hours) reductions in key pro-inflammatory cytokines (TNF-α, IL-6) via inhibiting NF-κB-mediated immune activation in macrophages and neutrophils, providing biochemical support for its anti-inflammatory mechanism of action. While modest short-term clinical improvements (PD reduction) were observed, evidence for sustained long-term benefits in hard clinical endpoints (CAL) remains limited due to the dissociation between short-term cytokine suppression and long-term structural tissue regeneration. A critical domain-level risk of bias assessment revealed incomplete methodological reporting in some included studies, and substantial

heterogeneity was confirmed to be a collective effect of multiple factors across included studies. Given these findings, laser-assisted therapy should currently be regarded as an individualized adjunct rather than a routine standard of care for Stage 3–4, Grade B/C chronic periodontitis. Further high-quality, standardized research integrating biochemical and clinical outcomes—with a focus on sustained immune modulation and tissue regeneration—is essential to define its optimal application, identify patient populations most likely to benefit, and support evidence-based updates to periodontal treatment guidelines.

STATEMENT OF ETHICS

Not applicable.

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

Not applicable.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AI ASSISTANCE DISCLOSURE

Not applicable.

REFERENCES

1. Trindade D, Carvalho R, Machado V, Chambrone L, Mendes JJ, Botelho J. Prevalence of periodontitis in dentate people between 2011 and 2020: a systematic review and meta-analysis of epidemiological studies. J Clin Periodontol. 2023;50(5):604-626.
2. Corbella S, Walter C, Tsesis I. Effectiveness of root resection techniques compared with root canal retreatment or apical surgery for the treatment of apical periodontitis and tooth survival: a systematic review. Int Endod J. 2023;56(suppl 3):487-498.
3. Gupta R, Arora SA, Gupta G, et al. Effect of open flap debridement with and without LLLT in patients with periodontitis on wound healing, GCF ALP levels, and clinical parameters. J Contemporary Dental Practice. 2024;25(12):1148-1155.
4. Huang Z, Luan QX. Diversity of incision and flap design in periodontal regenerative surgery: clinical practice. Chinese J Stomatol. 2025;60(8):846-856.
5. Pini Prato G, Di Gianfilippo R. Challenges and success in periodontal plastic surgery. J Clin Periodontol. 2023;50(12):1572-1581.
6. Gülnergiz E, Abraha SM, Hürzeler M, Zuhr O. Suture closure for surgical flap stabilization in modern periodontal and implant surgery: an update. Quintessence International. 2025;56(6):450-462.
7. Yu S, Zhao X, Zhang Y, et al. Clinical effectiveness of adjunctive diode laser on scaling and root planing in the treatment of periodontitis: is there an optimal combination of usage mode and application regimen? A systematic review and meta-analysis. Laser Med Sci. 2022;37(2):759-769.
8. Jiang Y, Feng J, Du J, et al. Clinical and biochemical effect of laser as an adjunct to non-surgical treatment of chronic periodontitis. Oral Dis. 2022;28(4):1042-1057.
9. Da Silva-Junior PGB, Abreu LG, Costa FO, Cota LOM, Esteves-Lima RP. The effect of antimicrobial photodynamic therapy adjunct to non-surgical periodontal therapy on the treatment of periodontitis in individuals with type 2 diabetes mellitus: a systematic review and meta-analysis. Photodiagn Photodyn. 2023;42:103573.
10. Santonocito S, Polizzi A, Cavalcanti R, et al. Impact of laser therapy on periodontal and peri-implant diseases. Photobiomod Photomed. 2022;40(7):454-462.
11. Tetè G, Ferrante L, Moretto M, Rellandini G, Polizzi E. The efficacy of the diode laser at 800-980 nm as an aid to non-surgical therapy in periodontitis treatment: a review of literature. J Biol Reg Homeos Ag. 2021;35(4 suppl 1):51-63.
12. Gong H, Li M. Short-term efficacy of Er:YAG laser and Nd:YAG laser combined therapy on periodontitis. Photobiomod Photomed. 2022;40(8):580-588.
13. Mokeem S. Efficacy of adjunctive low-level laser therapy in the treatment of aggressive periodontitis: a systematic review. J Invest Clin Dentistry. 2018;9(4):e12361.
14. Higgins JPT, Altman DG, Gøtzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011;343:d5928.

15. Malmqvist S, Qadri T, Lira-Junior R, et al. Treatment of peri-implantitis with diode laser or mucosal flap surgery: a clinical randomized controlled trial. J Periodontol. 2025;96(10):1126-1137.
16. Gokhale SR, Padhye AM, Byakod G, et al. A comparative evaluation of the efficacy of diode laser as an adjunct to mechanical debridement versus conventional mechanical debridement in periodontal flap surgery: a clinical and microbiological study. Photomedicine Laser Surgery. 2012;30(10):598-603.
17. Karthikeyan J, Vijayalakshmi R, Mahendra J, et al. Diode laser as an adjunct to Kirkland flap surgery-a randomized split-mouth clinical and microbiological study. Photobiomod Photomed. 2019;37(2):99-109.
18. Vineet V, Sk B, Krishnakumar D, Kumar D, Lavu V. Assessment of clinical efficacy of 445 nm diode laser as an adjunct to Kirkland flap surgery in the management of periodontitis - a split mouth randomized clinical trial. Laser Med Sci. 2024;39(1):82.
19. Dalvi SA, Hanna R, Gattani DR. Utilisation of antimicrobial photodynamic therapy as an adjunctive tool for open flap debridement in the management of chronic periodontitis: a randomized controlled clinical trial. Photodiagn Photodyn. 2019;25:440-447.
20. Khan F, Chopra R, Sharma N, et al. Comparative evaluation of the efficacy of diode laser as an adjunct to modified Widman flap surgery for the treatment of chronic periodontitis: a randomized split-mouth clinical trial. J Indian Soc Periodontol. 2021;25(3):213-219.
21. Lobo TM, Pol DG. Evaluation of the use of a 940 nm diode laser as an adjunct in flap surgery for treatment of chronic periodontitis. J Indian Soc Periodontol. 2015;19(1):43-48.
22. Shakoush G, Albonni H, Almahdi W. Low-level laser therapy has an additional effect with open flap debridement on the treatment of stage III periodontitis: a split-mouth randomized clinical trial. Quintessence Int. 2023;54(4):274-286.
23. Misra P, Kalsi R, Anand Arora S, et al. Effect of low-level laser therapy on early wound healing and levels of inflammatory mediators in gingival crevicular fluid following open flap debridement. Cureus J Med Science. 2023;15(2):e34755.
24. Zhang F, Qian SH. Clinical efficacy of 810 nm laser-assisted periodontal flap surgery in the treatment of severe periodontitis. Chinese J Laser Med Surgery. 2023;32(06):320-324.
25. Fan CW. Efficacy of laser combined with periodontal flap surgery in the treatment of severe periodontitis. J Aerospace Med. 2022;33(02):155-157.
26. Wu YZ, Mei YM, Zhou Y, et al. Efficacy of laser combined with periodontal flap surgery in the treatment of severe periodontitis. J Practical Med. 2020;36(16):2240-2243.
27. Elafifi Ebeid H, Altayeb W, Parada Avendaño I, Abad-Sanchez D, Arnabat-Domínguez J. Outcomes of flapless Er:YAG and Er,Cr:YSGG laser-assisted crown lengthening: a systematic review. Dent J-Basel. 2024;12(12):418.