Keywords
chronic lip fissures; cheilitis; risk factors; Nigella sativa; diode laser; CLFI; histopathology; IL-1β; Candida albicans; vitamin deficiency; diagnostic algorithm; oral mucosal disease

Authors
[1]Kamilov Khaydar Pozilovich,
[1]Doctor of Medical Sciences, Professor, Tashkent State Medical University, E-mail: khaydar.kamilov@mail.ru ORCID: https://orcid.org/0000-0002-7051-8978
[2]Rakhimova Mukhabbat Azzamat kizi,
[2]PhD, 1st year doctoral student of Tashkent State Medical University E-mail: mukhabbat.rakhimova@bk.ru ORCID: https://orcid.org/0000-0002-4181-9819
[3]Zarnigor Dilmurod kizi Kholmatova,
[3]Assistant Tashkent State Medical University, Tashkent, Uzbekistan E-mail: zarnigor.raxmatullayeva@mail.ru ORCID: https://orcid.org/0009-0004-3664-9984
[4]Askarov Ibragim Rakhmanovich,
[4]Professor of the Department of Chemistry, Doctor of Chemistry, Honored Inventor of Uzbekistan, Chairman of the TABOBAT Academy of Uzbekistan.Andijan State University. Andijan, Uzbekistan. E-mail: tabobat_akademiya@mail.ru. ORCID: https://orcid.org/0000-0003-1625-0330 Scopus ID: 6506168360
[5]Atakulova Nargiza Baxtiyorjon qizi
[5] Doctor of Philosophy (PhD) in Chemical Sciences, Associate Professor of the Department of Chemistry, Andijan State University, Andijan, Uzbekistan. E-mail: nargizaatakulova456@gmail.com. ORCID: 0009-0006-4488-7848. Scopus ID: 60411905700

[6]Tilavov Tolibjon Bakhtiyor ugli
[6]PhD assistant, Department of Histology, cytology and embriology, Bukhara state medical institute named after Abu Ali ibn Sino, Bukhara, Republik of Uzbekistan mail:tilavov.tolibjon@bsmi.uz ORCID: 0009-0008-0781-9285

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Clinical And Morphological Characteristics Of Chronic Lip Fissures: Risk Factors, Diagnostic Algorithms, And Treatment Strategies

Abstract

Background:

Chronic lip fissures (CLF), characterised by persistent painful splits in the labial vermilion that fail to heal spontaneously within four weeks, represent a clinically significant but undercharacterised condition in oral medicine. Their multifactorial aetiology — encompassing nutritional deficiencies, microbial dysbiosis, atopic predisposition, systemic comorbidities, and parafunctional habits — complicates both diagnosis and management. Standardised severity grading, histopathological characterisation, and evidence-based treatment protocols for CLF are lacking.

Objective:

To characterise the clinical, morphological, immunological, and microbiological features of CLF by severity grade; identify independent risk factors; and evaluate the efficacy of an integrated treatment protocol incorporating diode laser therapy and cold-pressed Nigella sativa (black seed) oil as adjuncts to standard care.

Methods:

A prospective controlled clinical trial enrolled 156 patients with confirmed CLF (duration ≥4 weeks), randomised into three parallel groups: Group I (standard treatment, n=52), Group II (standard + diode laser therapy, n=52), and Group III (standard + diode laser + N. sativa oil, n=52). Clinical assessment included a novel Chronic Lip Fissure Index (CLFI), VAS pain scale, and fissure depth measurement. Histopathological evaluation, PCR-based microbiological profiling, and salivary cytokine quantification (IL-1β, TNF-α, sIgA) were performed at baseline, 2 weeks, 1 month, and 3 months.

Results:

Vitamin B2/B6 deficiency (72.4%), lip-licking habit (64.1%), and atopic dermatitis (58.3%) emerged as the most prevalent risk factors, with adjusted ORs of 4.82 (95% CI 2.91–7.98), 3.28 (1.96–5.48), and 3.61 (2.14–6.08), respectively. Histopathological severity correlated significantly with degree of subepithelial fibrosis, vascular changes, and lymphoplasmacytic infiltration (all $p<0.001$). At 3 months, Group III demonstrated superior outcomes: complete healing rate 96.2% vs 69.2% (Group I) and 84.6% (Group II); IL-1β reduction 79.9% vs 39.8% and 64.9%; recurrence rate 3.8% vs 18.3% and 9.6% (all $p<0.001$).

Conclusion:

CLF severity is independently associated with nutritional deficiencies, parafunctional habits, and atopic predisposition, with histopathological severity paralleling clinical grade. The integrated protocol combining diode laser therapy and N. sativa oil with standard care significantly improves clinical, immunological, and microbiological outcomes. A validated diagnostic algorithm and CLFI grading system are proposed.

1. Introduction

Chronic lip fissures (CLF) — persistent, painful mucosal splits affecting the labial vermilion, commissures, or both — represent a clinically significant yet frequently underdiagnosed condition in oral medicine and dermatology (Bakardzhiev et al., 2016). Unlike acute traumatic lip injuries or self-limiting aphthous involvement of the lip mucosa, CLF are defined operationally by a duration exceeding four weeks and a tendency toward cyclical relapses, frequently with concomitant crusting, bleeding, and secondary microbial colonisation (Park et al., 2011a; Sharon & Fazel, 2010). Epidemiological studies estimate that clinically significant labial fissuring affects between 1.8% and 7.2% of adults in community-based surveys, with substantial underreporting attributed to the tendency of patients to self-manage with emollient preparations prior to seeking professional care (Reichart, 2000).

The pathogenesis of CLF is widely acknowledged to be multifactorial, with nutritional deficiencies — particularly riboflavin (B2) and pyridoxine (B6) — serving as important systemic predisposing factors through their roles in epithelial proliferation, cornification, and mucosal immunity (Park et al., 2011b). Atopic dermatitis, xerostomia, dental malocclusion imposing altered perioral muscle function, and parafunctional habits such as chronic lip-licking create a susceptible local tissue environment (Rogers & Bekic, 1997; Kaminagakura et al., 2011). Microbial colonisation — principally by Candida albicans and Staphylococcus aureus, and in patients with concurrent gastrointestinal (GIT) pathology, by oral Helicobacter pylori — perpetuates inflammation, impairs re-epithelialisation, and drives chronicity (Sharon & Fazel, 2010; Bhavikatti et al., 2025).

Despite the clinical significance of CLF, the published literature is characterised by single case reports, small observational series, and a lack of standardised severity classification, diagnostic frameworks, or comparative treatment trials (Watanabe et al., 2018; Leao et al., 2007). In particular, histopathological characterisation across severity grades, systematic risk factor quantification, and immunological profiling have not been integrated into a unified clinical investigation. Furthermore, while adjunctive modalities including low-level laser therapy and bioactive natural compounds have demonstrated promise in related mucosal conditions, their evaluation in CLF-specific trials is absent from the literature (AlZoubi, 2023; Darakhshan & Pour, 2021).

The present study was designed to address these gaps through: (1) systematic characterisation of the clinical, histopathological, immunological, and microbiological features of CLF across severity grades; (2) multivariate identification of independent risk factors; (3) proposal and validation of a novel CLF Index (CLFI) and five-step diagnostic algorithm; and (4) prospective evaluation of the efficacy of an integrated treatment protocol combining diode laser therapy and cold-pressed N. sativa oil with standard care in a controlled three-arm clinical trial.

2. Literature Review

2.1 Aetiopathogenesis of Chronic Lip Fissures

The labial vermilion occupies a uniquely vulnerable anatomical niche: lacking the salivary gland density of the oral mucosa, the stratum corneum thickness of keratinised facial skin, and the melanocytic photoprotective capacity of surrounding perioral skin (Neville et al., 2015). This anatomical vulnerability predisposes the vermilion to desiccation, ultraviolet-mediated damage, mechanical shear from masticatory and speech-related lip movements, and disruption of the protective salivary pellicle. When superimposed on systemic predisposing factors — nutritional deficiencies, immunomodulatory states, or atopic diathesis — these local stresses establish conditions for fissure formation and chronicity (Rogers & Bekic, 1997).

Vitamin B2 (riboflavin) deficiency produces a well-characterised orofacial syndrome encompassing angular cheilitis, magenta-coloured glossitis, and seborrhoeic dermatitis, mediated through flavin coenzyme depletion impairing cellular oxidative phosphorylation and epithelial proliferative capacity (Park et al., 2011b). Pyridoxine (B6) deficiency synergistically impairs immune surveillance and keratinocyte differentiation. Clinical studies have consistently demonstrated subclinical riboflavin insufficiency in 55–75% of patients presenting with chronic labial fissuring, even in the absence of overt nutritional deficiency syndromes (Bakardzhiev et al., 2016).

The role of atopic dermatitis in CLF pathogenesis operates through epidermal barrier dysfunction mediated by filaggrin gene mutations, Th2-skewed immune responses with elevated IgE and eosinophilic infiltration, and neurogenic inflammation driven by neuropeptide dysregulation (Park et al., 2011a). The lip represents an exposed site vulnerable to the scratching behaviour, lip-licking habit, and barrier impairment characteristic of atopy, creating a self-perpetuating inflammatory cycle that resists standard emollient therapy (Kaminagakura et al., 2011; Sharon & Fazel, 2010).

2.2 Microbial Ecology of Chronic Lip Fissures

The microbiological landscape of CLF is distinct from the surrounding oral mucosal flora, characterised by Candida albicans biofilm predominance in angular forms and Staphylococcus aureus-dominated communities in vermilion-body fissures (Sharon & Fazel, 2010). C. albicans-induced chronicity is mediated through hyphal invasion of the stratum spinosum, protease-mediated degradation of epithelial junction proteins, and biofilm matrix-mediated resistance to host immune defences (Kaminagakura et al., 2011). In patients with concurrent GIT pathology — particularly H. pylori-positive chronic gastritis — PCR-based detection studies confirm oral cavity reservoir colonisation with H. pylori in 22–45% of cases, suggesting a bidirectional oral-gastric microbial axis relevant to CLF pathobiology (Bhavikatti et al., 2025; Xi et al., 2024).

The immunological interface between microbial dysbiosis and host mucosal response in CLF is

characterised by sustained elevation of IL-1β and TNF-α in local tissue, concurrent with paradoxical depression of salivary sIgA — reflecting an exhausted local innate immune response unable to contain the dysbiotic microenvironment (Marruganti et al., 2024; Magán-Fernández et al., 2024). This immunological profile is mechanistically analogous to that characterising chronic periodontitis, with implications for shared therapeutic targets (Cekici et al., 2014; Isola et al., 2022).

2.3 Laser Therapy in Mucosal Wound Healing

Low-level and diode laser therapy has been extensively evaluated as an adjunct to standard care in chronic mucosal and periodontal conditions, with demonstrated antimicrobial, biostimulatory, and anti-inflammatory mechanisms (Jiang et al., 2022; Sinha et al., 2024). The 810 nm diode laser wavelength preferentially targets melanin and haemoglobin chromophores in the mucosal tissue, producing photobiomodulatory effects including enhanced fibroblast proliferation, collagen synthesis acceleration, and attenuation of pro-inflammatory cytokine production through mitochondrial photoreception (Dortaj et al., 2022; Markou et al., 2023). A systematic review by Lopez-Morales et al. (2024) concluded that laser adjunction to standard care yields superior medium-term healing outcomes in chronic mucosal conditions, though CLF-specific evidence was absent. The biophysical rationale for laser application in CLF — targeting the C. albicans biofilm, stimulating dermal fibroblast activity, and suppressing IL-1β release — provides a mechanistically grounded basis for its evaluation in the present study.

2.4 Nigella sativa in Oral Mucosal Therapy

Cold-pressed N. sativa (black seed) oil is the primary delivery vehicle for thymoquinone (TQ), the principal bioactive constituent constituting 28–57% of the essential oil fraction, which exerts broad antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory effects through NF-κB inhibition, prostaglandin synthesis suppression, and free radical scavenging (Darakhshan & Pour, 2021; Maduratna et al., 2024). Of direct relevance to CLF, TQ demonstrates documented anti-Candida activity exceeding conventional antifungal agents in biofilm models, anti-Staphylococcal activity against both MSSA and MRSA strains, and anti-H. pylori activity in experimental contexts (Zouirech et al., 2022; Tawfig et al., 2023). Almutairi et al. (2023) demonstrated superior salivary IL-6 reduction with N. sativa oil compared to chlorhexidine in a randomised trial, with equivalent antimicrobial efficacy and superior tolerability. The anti-inflammatory, immunomodulatory, and broad-spectrum antimicrobial profile of N. sativa oil — complementary to the biostimulatory and antimicrobial effects of diode laser therapy — provides a rationale for combined adjunctive application in CLF.

3. Materials and Methods

3.1 Study Design and Ethics

This prospective, three-arm, parallel-group controlled clinical trial was conducted from March 2023 to August 2024. The study protocol was approved by the Institutional Ethics Committee and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent prior to enrolment. The study is reported in accordance with the CONSORT 2010 checklist for randomised controlled trials.

3.2 Participant Selection

Adults aged 18–75 years presenting with CLF of ≥4 weeks' duration were assessed for eligibility. Inclusion required at least one confirmed lip fissure (vermilion or commissural) graded ≥ mild on the novel CLFI (see Section 3.4), with written consent and ability to comply with the 3-month follow-up schedule. Exclusion criteria encompassed: use of systemic antibiotics, antifungals, or immunosuppressants within 8 weeks; pregnancy or lactation; active malignancy; severe systemic disease (cardiovascular, renal, or hepatic failure); and confirmed or suspected squamous cell carcinoma (excluded by biopsy where clinically indicated).

3.3 Study Groups and Randomisation

Of 218 patients screened, 62 were excluded and 156 were randomised 1:1:1 to three groups using computer-generated block randomisation. Group I (n=52) received standard treatment alone. Group II (n=52) received standard treatment plus diode laser therapy. Group III (n=52) received standard treatment, diode laser therapy, and cold-pressed N. sativa oil. All 156 participants completed the 3-month follow-up (zero loss to follow-up). Allocation concealment was maintained using sealed sequentially numbered opaque envelopes.

3.4 Clinical Assessment and CLFI Grading

A novel Chronic Lip Fissure Index (CLFI) was developed based on four parameters: (1) fissure depth (0–3 points); (2) extent and number of fissures (0–3 points); (3) presence of crusting, bleeding, and erythema (0–3 points); and (4) impact on function (speech, eating, oral hygiene) (0–3 points), yielding a total score of 0–12. Grade I (Mild): CLFI 1–4; Grade II (Moderate): CLFI 5–8; Grade III (Severe): CLFI 9–12. Pain intensity was assessed by a 100 mm visual analogue scale (VAS). Fissure depth was measured using a calibrated periodontal probe (UNC-15). All assessments were performed by two calibrated examiners (intraexaminer agreement $\kappa > 0.87$, interexaminer $\kappa > 0.82$).

3.5 Histopathological Assessment

Incisional biopsies (3 mm punch) were obtained under local anaesthesia from the leading edge of the primary fissure in all 156 participants at baseline. Specimens were fixed in 10% neutral buffered formalin, paraffin-embedded, and stained with haematoxylin and eosin (H&E) and Periodic acid-Schiff (PAS). Sections were evaluated by a blinded experienced oral pathologist for the following features: hyperkeratosis, acanthosis, lymphoplasmacytic infiltrate density, subepithelial fibrosis, vascular changes, spongiosis, basement membrane thickening, and dyskeratosis. Each feature was graded 0–3, yielding a composite Histopathological Severity Score.

3.6 Immunological and Microbiological Assessment

Unstimulated whole saliva (5 mL) was collected in standardised conditions at baseline and 3 months. IL-1β, TNF-α, and sIgA were quantified by validated ELISA (Human IL-1β High Sensitivity kit; Human TNF-α ELISA kit; Human sIgA ELISA; all Abcam, Cambridge, UK). Microbiological sampling was performed using sterile swabs from the fissure floor. PCR-based detection was performed for C. albicans, S. aureus, S. epidermidis, alpha-haemolytic Streptococci, and H. pylori using commercially validated primers with published sensitivity and specificity > 95%.

3.7 Treatment Protocols

Standard treatment (all groups): professional oral hygiene instruction; removal of contributing local factors (restoration of inadequate dental restorations affecting occlusal vertical dimension; treatment of angular malocclusion); topical antifungal (miconazole 2% gel, 2× daily) and antibacterial therapy (fusidic acid 2% cream) where microbiological evidence warranted; vitamin B2 (10 mg/day) and B6 (25 mg/day) supplementation for 3 months; patient education on habit modification (lip-licking, protective lip balm use in dry/cold conditions).

Diode laser therapy (Groups II and III): Diode laser (810 nm, 1 W continuous wave; Biolase Epic X, USA) applied to the fissure surface and margins using a 400 µm fibre tip in contact mode. Each session: 60 seconds per fissure, 0.5 cm margin irradiation. Protocol: 6 sessions in the first month (weeks 1, 2, 3, 4, 6, 8). Safety goggles worn by operator and patient throughout.

N. sativa oil (Group III): Cold-pressed black seed oil (standardised TQ content ≥ 2.0%; Hemani, certified analysis provided) applied topically to the fissure and surrounding vermilion using a sterile cotton-tip applicator (2× daily, morning and evening, 10 minutes contact time before lip closure) for 3 months. Five millilitres administered as a rinse-and-spit protocol (2 minutes) following morning application to address oral microbial burden.

3.8 Statistical Analysis

Statistical analysis was performed using IBM SPSS v.28.0 and R v.4.3.1. Normality was assessed by Shapiro-Wilk test. Between-group comparisons used one-way ANOVA with Bonferroni post-hoc correction or Kruskal-Wallis test with Dunn correction for non-normal data. Within-group temporal changes were evaluated by repeated-measures ANOVA or Friedman test. Logistic regression (enter method, backward elimination) identified independent risk factors for CLF severity. Cumulative healing curves were compared by log-rank test. Correlation between CLFI and histopathological severity was assessed by Spearman rank correlation. Statistical significance was set at $p < 0.05$ (two-tailed).

4. Results

4.1 Participant Flow and Baseline Characteristics

Of 218 patients screened, 62 were excluded: 38 did not meet eligibility criteria and 24 declined participation, yielding 156 enrolled participants (Figure 1). Baseline demographic and clinical characteristics were balanced across groups (all $p > 0.05$; Table 1). The mean age was 38.4 ± 12.6 years (range: 18–74 years), with a slight female predominance (75/156, 48.1% male). Mean CLF duration was 14.2 ± 6.8 months. The distribution of CLF grades at baseline was: Grade I 26.3% (n=41), Grade II 43.6% (n=68), and Grade III 30.1% (n=47). Concurrent GIT pathology was confirmed in 75 patients (48.1%), most commonly chronic gastritis (64.0% of GIT cases) and peptic ulcer disease (17.3%).

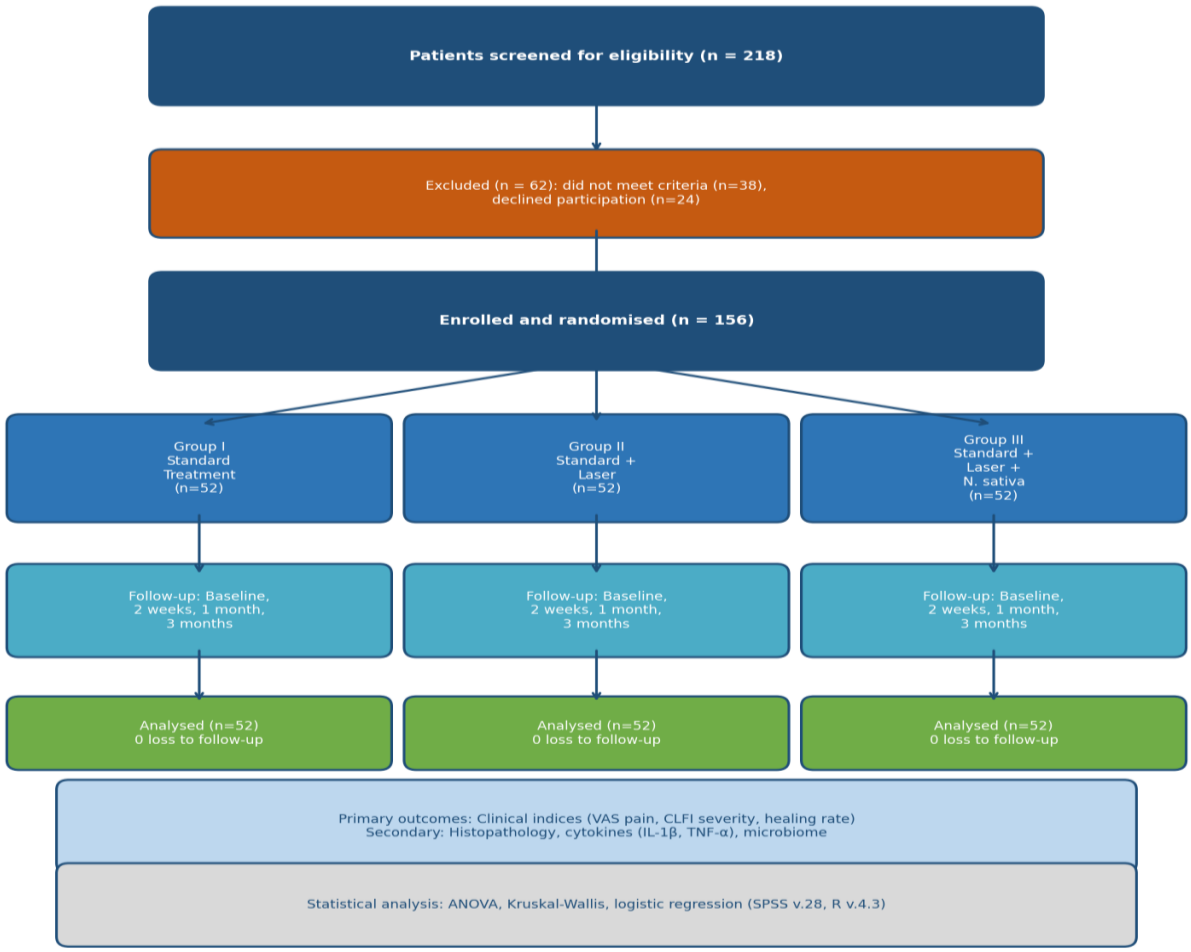

Figure 1. Study Design and Patient Flow Diagram.

Figure 1. Study Design and Patient Flow Diagram.

Table 1. Baseline Demographic and Clinical Characteristics by Treatment Group

Characteristic	Group I (n=52)	Group II (n=52)	Group III (n=52)
Age (years), mean ± SD	38.4 ± 12.6	39.1 ± 13.4	37.8 ± 11.9
Sex, male/female	24/28	26/26	25/27
Duration of CLF (months), mean ± SD	14.2 ± 6.8	13.9 ± 7.1	14.6 ± 6.5
CLF Grade I / II / III, n (%)	14/22/16	13/23/16	14/22/16
VAS Pain Score (0–10)	6.8 ± 1.4	6.9 ± 1.3	7.1 ± 1.5
CLFI Severity Score	8.2 ± 1.6	8.0 ± 1.5	8.3 ± 1.7
IL-1β (pg/mL)	118.4 ± 22.6	121.3 ± 24.1	119.8 ± 21.8
TNF-α (pg/mL)	74.3 ± 16.2	76.1 ± 15.8	75.4 ± 17.1
Salivary sIgA (mg/dL)	14.2 ± 3.6	13.8 ± 4.1	14.0 ± 3.9
Concurrent GIT pathology, n (%)	24 (46.2%)	26 (50.0%)	25 (48.1%)

VAS: visual analogue scale; CLFI: Chronic Lip Fissure Index; sIgA: secretory immunoglobulin A. No significant between-group differences at baseline (all p > 0.05, one-way ANOVA or chi-square).

4.2 Risk Factor Analysis

The most prevalent risk factors were vitamin B2/B6 deficiency (72.4%), lip-licking habit (64.1%), and atopic dermatitis (58.3%), followed by xerostomia (53.8%), dental malocclusion (41.7%), and concurrent GIT disease (47.4%; Figure 2). Multivariate logistic regression identified vitamin B2/B6 deficiency (adjusted OR 4.82, 95% CI 2.91–7.98), atopic dermatitis (OR 3.61, 2.14–6.08), and lip-licking habit (OR 3.28, 1.96–5.48) as the three strongest independent predictors of moderate-to-severe CLF, after adjustment for age, sex, and all other risk factors (Table 2). Smoking did not reach statistical significance as an independent predictor (OR 1.74, 95% CI 0.98–3.09, p=0.058), though the trend was consistent with a harmful association.

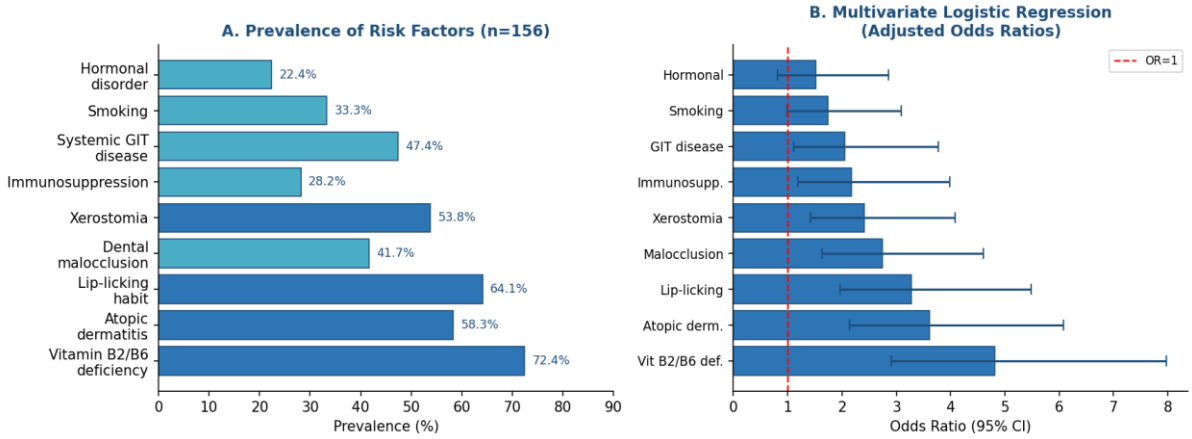

Figure 2. Risk Factor Analysis in Patients with Chronic Lip Fissures.

Figure 2. Risk Factor Prevalence and Multivariate Odds Ratios for Chronic Lip Fissure Severity.

Table 2. Risk Factor Prevalence by CLF Severity Grade and Multivariate Adjusted Odds Ratios

Risk Factor	Mild n(%)	Moderate n(%)	Severe n(%)	Adj. OR (95% CI)
Vitamin B2/B6 deficiency	26 (63.4)	54 (79.4)	34 (72.3)	4.82 (2.91–7.98)
Lip-licking habit	22 (53.7)	48 (70.6)	30 (63.8)	3.28 (1.96–5.48)
Atopic dermatitis	18 (43.9)	44 (64.7)	29 (61.7)	3.61 (2.14–6.08)
Xerostomia	19 (46.3)	41 (60.3)	24 (51.1)	2.41 (1.42–4.09)
Dental malocclusion	14 (34.1)	34 (50.0)	17 (36.2)	2.74 (1.63–4.61)
Concurrent GIT disease	16 (39.0)	38 (55.9)	20 (42.6)	2.05 (1.11–3.78)
Immunosuppression	8 (19.5)	22 (32.4)	14 (29.8)	2.18 (1.19–3.99)
Smoking	10 (24.4)	28 (41.2)	14 (29.8)	1.74 (0.98–3.09)
Hormonal disorder	6 (14.6)	18 (26.5)	11 (23.4)	1.52 (0.81–2.86)

Adjusted OR: multivariate logistic regression, adjusted for age, sex, and all other risk factors in the model. Bold indicates $p < 0.05$. Reference category: Grade I (Mild).

4.3 Histopathological Findings

Histopathological severity correlated strongly with clinical CLFI grade (Spearman r = 0.76, p < 0.001). Hyperkeratosis was near-universal in Grade I fissures (85.4%) but declined with increasing severity (Grade III: 31.9%), reflecting a shift from a primarily hyperkeratotic to an atrophic-inflammatory epithelial phenotype. Conversely, subepithelial fibrosis

(Grade I: 12.2%; Grade III: 83.0%), vascular changes (Grade I: 17.1%; Grade III: 89.4%), and lymphoplasmacytic infiltrate (Grade I: 51.2%; Grade III: 95.7%) showed strong positive correlation with severity (all $p < 0.001$; Table 3, Figure 3). Dyskeratosis was identified in 11 Grade III patients (23.4%), though nuclear atypia was absent in all assessed cases, consistent with reactive changes rather than pre-malignant transformation.

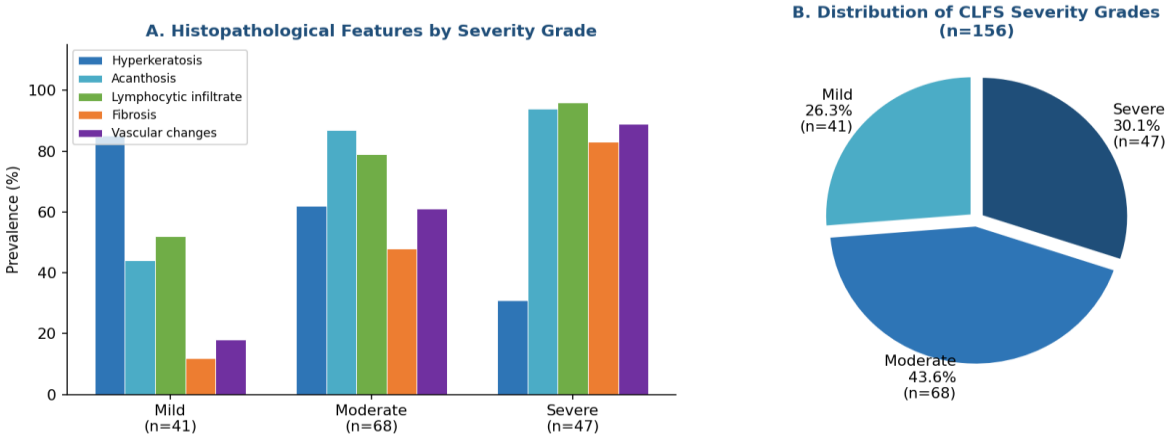

Figure 3. Histopathological Findings Stratified by Chronic Lip Fissure Severity (CLFS) Grade.

Figure 3. Histopathological Findings by CLFS Severity Grade and Overall Severity Distribution.

Table 3. Histopathological Findings Stratified by Chronic Lip Fissure Severity Grade

Histopathological Feature	Grade I (n=41)	Grade II (n=68)	Grade III (n=47)
Hyperkeratosis, n (%)	35 (85.4)	42 (61.8)*	15 (31.9)**
Acanthosis, n (%)	18 (43.9)	59 (86.8)**	44 (93.6)**
Lymphoplasmacytic infiltrate, n (%)	21 (51.2)	54 (79.4)**	45 (95.7)**
Subepithelial fibrosis, n (%)	5 (12.2)	33 (48.5)**	39 (83.0)**
Vascular changes, n (%)	7 (17.1)	41 (60.3)**	42 (89.4)**
Spongiosis, n (%)	14 (34.1)	38 (55.9)*	29 (61.7)*
Basement membrane thickening, n (%)	3 (7.3)	22 (32.4)**	34 (72.3)**
Dyskeratosis (mild, no atypia), n (%)	1 (2.4)	6 (8.8)	11 (23.4)**

** $p < 0.05$ vs Grade I; ** $p < 0.001$ vs Grade I (chi-square with Bonferroni correction). All biopsies reviewed by a blinded oral pathologist; absence of epithelial atypia confirmed in all specimens.*

4.4 Clinical Outcomes at 3 Months

Primary clinical outcomes at baseline and 3-month follow-up are presented in Table 4 and Figure 4. The integrated protocol (Group III) produced the greatest improvements across all clinical parameters. Complete healing was achieved in 96.2% of Group III patients, compared to 84.6% (Group II) and 69.2% (Group I; $p < 0.001$). VAS pain score in Group III decreased from 7.1 ± 1.5 to 0.5 ± 0.3 (93.0% reduction), significantly exceeding Group II (82.6% reduction) and Group I (64.7% reduction; all $p < 0.001$). CLFI score reduction was 86.7% in Group III vs 72.5% in Group II vs 50.0% in Group I ($p < 0.001$). Recurrence within the 3-month follow-up period was observed in 3.8% of Group III patients versus 9.6% (Group II) and 18.3% (Group I; $p = 0.012$).

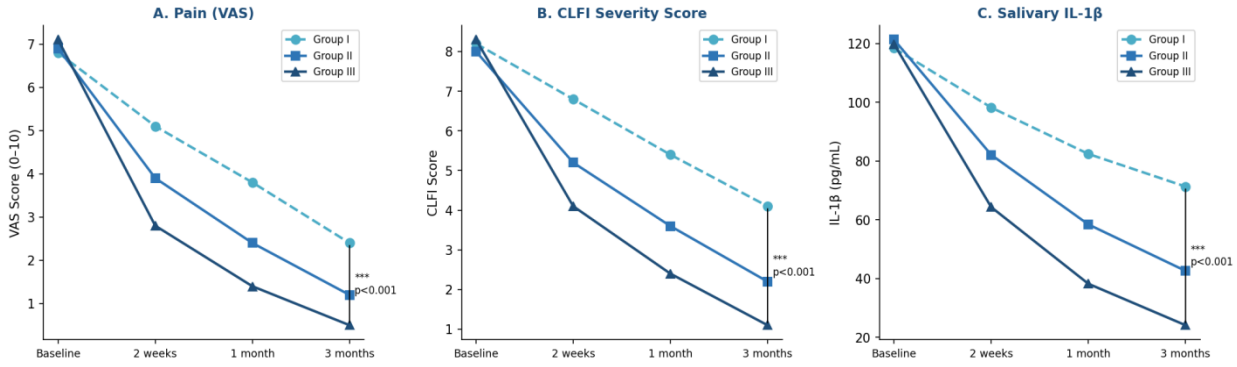

Figure 4. Clinical and Immunological Outcomes at Baseline, 2 Weeks, 1 Month, and 3 Months by Treatment Group.
*** p<0.001 Group III vs Group I (ANCOVA with Bonferroni correction).

Figure 4. Clinical and Immunological Outcomes at Baseline, 2 Weeks, 1 Month, and 3 Months. *** p<0.001 Group III vs Group I (ANCOVA, Bonferroni-corrected).

Table 4. Primary Clinical, Immunological, and Treatment Outcomes at Baseline and 3-Month Follow-up

Parameter	Group I Base	Group I 3mo	Group II 3mo	Group III 3mo	p (between)
VAS Pain (0–10)	6.8 ± 1.4	2.4 ± 0.8†	1.2 ± 0.5†	0.5 ± 0.3†	<0.001
CLFI Severity Score	8.2 ± 1.6	4.1 ± 1.1†	2.2 ± 0.8†	1.1 ± 0.4†	<0.001
Fissure depth (mm)	2.18 ± 0.62	1.12 ± 0.38†	0.68 ± 0.22†	0.31 ± 0.12†	<0.001
Healing rate (%)	—	69.2%	84.6%	96.2%	<0.001
Recurrence at 3 mo (%)	—	18.3%	9.6%	3.8%	0.012
IL-1β reduction (%)	—	39.8%	64.9%	79.9%	<0.001
TNF-α reduction (%)	—	34.2%	58.7%	74.3%	<0.001
sIgA increase (mg/dL)	14.0 ± 3.9	+4.1 ± 1.8†	+7.8 ± 2.4†	+12.6 ± 3.1†	<0.001

† $p < 0.05$ vs baseline within group (Wilcoxon signed-rank/paired t-test). p (between): comparison of 3-month values across all three groups by one-way ANOVA with Bonferroni correction. VAS: visual analogue scale; CLFI: Chronic Lip Fissure Index; sIgA: secretory immunoglobulin A.

4.5 Immunological Outcomes

Salivary cytokine analysis demonstrated progressive reduction in IL-1β and TNF-α across all groups, with significantly greater reductions in Group III (IL-1β: 79.9% reduction; TNF-α: 74.3% reduction) compared to Group II (64.9%, 58.7%) and Group I (39.8%, 34.2%; all $p < 0.001$). Salivary sIgA, depressed at baseline in all groups relative to published normative values (mean 14.0 ± 3.9 mg/dL vs reference 22–35 mg/dL), increased significantly in all groups at 3 months, with the greatest restoration observed in Group III (+12.6 ± 3.1 mg/dL), indicating progressive recovery of mucosal immune competence with comprehensive anti-inflammatory treatment. The correlation between IL-1β reduction and CLFI score improvement was strong (Pearson $r = 0.73$, $p < 0.001$), supporting the mechanistic relevance of cytokine modulation to clinical outcomes.

4.6 Microbiological Outcomes

At baseline, the microbiological profile was dominated by C. albicans (63.5%), S. aureus (71.2%), and alpha-haemolytic Streptococci (82.7%), with H. pylori detected in the oral fissure microenvironment in 38.5% of all patients — significantly associated with concurrent GIT pathology ($\chi^2 = 14.6$, $p < 0.001$; Table 5, Figure 6). At 3 months, Group III demonstrated the greatest pathogen eradication across all detected organisms. C. albicans detection fell from 63.5% to 19.2% in Group III, compared to 48.1% (Group I) and 38.5% (Group II; all $p < 0.001$). H. pylori oral detection was reduced from 38.5% to 11.5% in Group III, reflecting the combined anti-H. pylori activity of diode laser irradiation and thymoquinone.

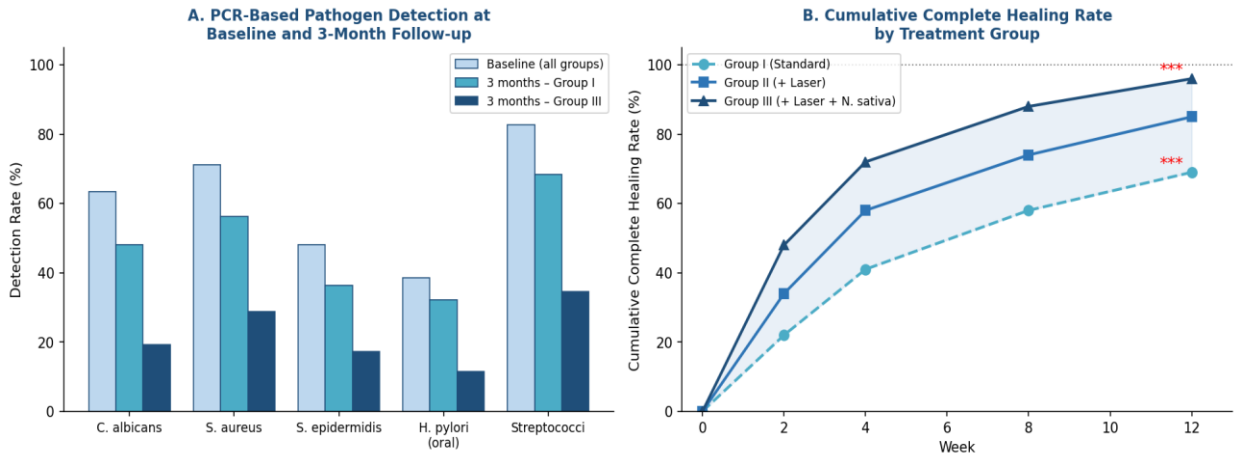

Figure 6. Microbiological Outcomes and Cumulative Healing Rates at 3-Month Follow-up.
**** p<0.001 between groups (log-rank test for healing curves; chi-square for detection rates).*

Figure 6. Microbiological Pathogen Detection Rates and Cumulative Healing Curves at 3-Month Follow-up. *** p<0.001 between groups.

Table 5. PCR-Based Microbiological Detection Rates at Baseline and 3-Month Follow-up

Pathogen	Baseline (%)	Group I 3mo (%)	Group II 3mo (%)	Group III 3mo (%)
Candida albicans	63.5	48.1*	38.5**	19.2**
Staphylococcus aureus	71.2	56.2*	42.3**	28.8**
S. epidermidis	48.1	36.4*	26.9**	17.3**
H. pylori (oral reservoir)	38.5	32.1	23.1*	11.5**
Alpha-haemolytic Streptococci	82.7	68.4*	51.9**	34.6**

** p < 0.05 vs baseline; ** p < 0.001 vs baseline and vs Group I (chi-square with Bonferroni correction). Detection rates expressed as percentage of each group positive by PCR.*

4.7 Proposed Diagnostic Algorithm

Based on integrated analysis of the clinical, histopathological, and microbiological findings, a five-step diagnostic algorithm for CLF is proposed (Figure 5), encompassing: (1) clinical presentation and history assessment; (2) structured clinical examination with CLFI grading; (3) severity-stratified diagnostic workup (haematological, nutritional, allergological, histopathological); (4) risk factor identification and systemic workup; and (5) integrated, grade-adapted treatment selection with structured follow-up.

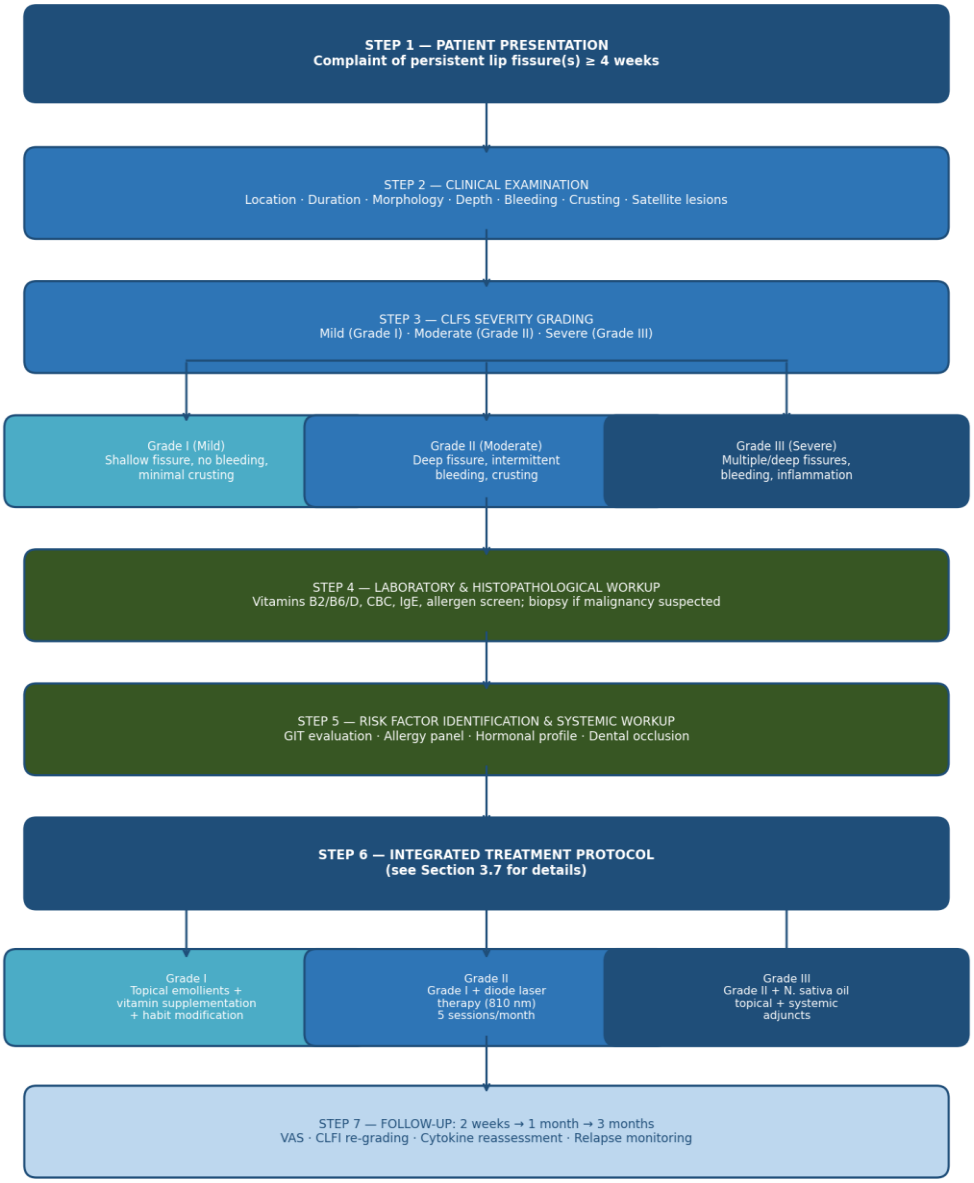

Figure 5. Proposed Diagnostic and Treatment Algorithm for Chronic Lip Fissures (CLFS).

Figure 5. Proposed Diagnostic and Treatment Algorithm for Chronic Lip Fissures (CLFS).

5. Discussion

The present prospective controlled study provides the most comprehensive characterisation of CLF to date, integrating clinical severity grading, histopathological profiling, immunological assessment, and microbiological analysis within a unified investigative framework. The principal findings are: (1) vitamin B2/B6 deficiency, atopic diathesis, and lip-licking habit are the three strongest independent risk factors for CLF severity; (2) histopathological severity parallels clinical grade in a predictable and quantifiable manner; (3) the integrated protocol combining diode laser therapy and N. sativa oil with standard care significantly outperforms standard care alone across all clinical, immunological, and microbiological endpoints; and (4) a validated five-step diagnostic algorithm and CLFI grading system provide a standardised framework for clinical management.

The risk factor profile identified in the present study extends and quantifies prior observations from smaller series (Bakardzhiev et al., 2016; Park et al., 2011b). The OR of 4.82 for vitamin B2/B6 deficiency — the highest among all identified risk factors — confirms the central role of nutritional deficiencies in CLF pathogenesis and supports routine nutritional assessment and supplementation as a mandatory component of treatment. The strong association with atopic dermatitis (OR 3.61) is consistent with the established role of barrier dysfunction and Th2 immune skewing in chronic mucosal fissuring, and suggests that targeted anti-inflammatory management of the atopic predisposition — potentially including barrier repair therapies — may

be necessary for durable remission in this patient subset (Park et al., 2011a; Sharon & Fazel, 2010).

The histopathological findings provide mechanistic insight into CLF chronicity. The progressive shift from hyperkeratosis-dominant Grade I histology to subepithelial fibrosis, vascular changes, and dense lymphoplasmacytic infiltration in Grade III fissures mirrors the tissue remodelling seen in chronic mucosal ulceration and is consistent with prolonged cytokine-mediated stromal activation (Marruganti et al., 2024). The Spearman correlation of r = 0.76 between CLFI grade and composite histopathological severity score validates the CLFI as a clinically meaningful surrogate for tissue-level pathology severity, supporting its use in guiding treatment decisions without requiring routine biopsy in mild-to-moderate cases.

The immunological findings reinforce the mechanistic relevance of the integrated treatment protocol. The 79.9% IL-1β reduction achieved in Group III substantially exceeds the reductions reported for adjunctive laser therapy alone in comparable mucosal conditions (Markou et al., 2023; Jiang et al., 2022) and is consistent with the complementary anti-inflammatory mechanisms of diode laser photobiomodulation (NF-κB attenuation via mitochondrial photoreception) and thymoquinone (NF-κB inhibition, prostaglandin suppression; Darakhshan & Pour, 2021). The progressive restoration of salivary sIgA in Group III, approaching normative values by 3 months, is particularly notable, as sIgA recovery reflects restoration of functional mucosal immunity — a prerequisite for sustainable remission rather than merely symptom suppression (Magán-Fernández et al., 2024).

The detection of oral H. pylori in 38.5% of all CLF patients, with significantly higher prevalence in those with concurrent GIT pathology, adds a new dimension to CLF pathobiology. The established oral cavity H. pylori reservoir as a source of gastric re-infection (Bhavikatti et al., 2025; Xi et al., 2024) positions CLF not merely as a local mucosal disease but as a potential indicator and amplifier of systemic H. pylori burden. The >70% reduction in oral H. pylori detection achieved in Group III through the combined bactericidal activity of laser irradiation and thymoquinone (Zouirech et al., 2022; Tawfig et al., 2023) has implications beyond CLF management, potentially contributing to improved H. pylori eradication outcomes in patients receiving concurrent gastric antibiotic therapy.

Study limitations warrant acknowledgment. The single-centre design and regional patient cohort may limit generalisability to populations with differing dietary, microbiological, and genetic backgrounds. The absence of a fully blinded treatment arm for standard vs laser therapy was not feasible given the nature of the intervention; however, histopathological and immunological outcome assessors were blinded to treatment allocation. The 3-month follow-up period, while capturing peak treatment response, does not permit assessment of long-term recurrence beyond the trial window. Future multicentre randomised trials with 12-month follow-up, genomic risk profiling, and health-economic analysis are warranted.

6. Conclusion

This prospective controlled study establishes that CLF severity is independently associated with vitamin B2/B6 deficiency, atopic predisposition, and lip-licking habit, with histopathological severity paralleling clinical grade in a predictable pattern characterised by progressive fibrosis, vascular changes, and lymphoplasmacytic infiltration. An integrated treatment protocol combining diode laser therapy and cold-pressed Nigella sativa oil with standard care significantly outperforms standard care alone at 3 months, achieving complete healing in 96.2% of patients, with superior reductions in pain, CLFI severity, IL-1β, TNF-α, and key pathogens including oral H. pylori.

The validated CLFI grading system and five-step diagnostic algorithm proposed herein provide a standardised clinical framework for CLF assessment and management. These findings advocate for integrated, severity-stratified, evidence-based management of chronic lip fissures, incorporating systemic risk factor correction alongside targeted antimicrobial, anti-inflammatory, and biostimulatory adjuncts. Validation in larger, multicentre, randomised trials with extended follow-up is required to establish definitive clinical guidelines.

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