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Original Article

Benefit of nanohydroxyapatite combined with triamcinolone for non-surgical treatment of severe periodontitis: a retrospective study

Long Chen[1*], Fujun Chen[2*], Fengming Xue[1], Lianghui Yang[1]

[1]Department of Stomatology, The Second Affiliated Hospital of Hainan Medical University, Haikou 570311, Hainan, China; [2]Department of Oral Implantology, Affiliated Stomatological Hospital of Guilin Medical College, Guilin 541004, Guangxi Zhuang Autonomous Region, China. [*]Equal contributors and co-first authors.

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Abstract: Objectives: To evaluate the possible benefit of combining nanohydroxyapatite (nHA) with triamcinolone (TR) in the non-surgical management of severe periodontitis and to assess the influence of baseline inflammation on treatment outcomes. Methods: A retrospective analysis was conducted on 120 patients who received one of the following local treatments: nHA+TR, nHA alone, TR alone, or conventional subgingival scaling. All patients were followed for 6 months. Clinical data - including probing depth (PD), clinical attachment level (CAL), periodontal pocket closure rate, bleeding on probing (BoP), plaque index (PI), gingival index (GI), and gingival recession (GR) - along with inflammatory biomarkers [interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP)] were evaluated over the follow-up period. Correlation, regression, and subgroup analyses were performed. Results: The nHA+TR group showed the greatest improvements in PD, CAL, and pocket closure rate, along with significant reductions in IL-1β, TNF-α, IL-6, and CRP (all $P < 0.01$). Changes in PD and CAL were strongly correlated with declines in inflammatory markers ($r > 0.80$, both $P < 0.001$). Multivariable analysis identified combination therapy and higher baseline PD, PI, and BoP as positive predictors of clinical improvement, whereas elevated baseline CRP and IL-1β were associated with poorer outcomes (all $P < 0.01$). Treatment with nHA+TR was significantly associated with clinical success - defined as a ≥ 2 mm reduction in PD and ≥ 1 mm gain in CAL (odds ratio = 48.49; all $P < 0.001$). In patients with CRP > 3 mg/L, combination therapy showed enhanced clinical benefits, with a significant interaction between treatment and baseline inflammation ($P_{interaction} = 0.032$). All interventions were well tolerated, with no serious adverse events reported. Conclusions: The combination of nHA and TR may enhance both clinical and inflammatory outcomes in the non-surgical treatment of severe periodontitis. Further randomized controlled trials are warranted to confirm these findings.

Keywords: Severe periodontitis, nano-hydroxyapatite, triamcinolone, inflammatory biomarkers, personalized treatment

Introduction

Periodontitis is a chronic, destructive inflammatory disease initiated by dental plaque biofilms. If left untreated, it can lead to progressive alveolar bone loss, destruction of periodontal supporting tissues, and ultimately tooth loss [1, 2]. According to the Global Burden of Disease Study, periodontitis is a leading cause of tooth loss in adults, with a global prevalence estimated between 20% and 50%, posing long-term threats to oral health, quality of life, and systemic health [3]. Advanced periodontitis (stage III-IV) is particularly severe, with an estimated prevalence of 11.2% among adults and marked geographic variation: for example, the prevalence in South Asia reaches 17.6%, while Africa bears a disproportionately high burden of aggressive periodontitis [4-6]. Increasing evidence suggests bidirectional associations between periodontitis and systemic conditions such as diabetes mellitus, cardiovascular disease, and rheumatoid arthritis, highlighting its complexity as a public health challenge [7].

Mechanistically, periodontitis is characterized by a biofilm-induced chronic inflammatory

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response involving immune cell activation and excessive release of inflammatory cytokines. Activation of the nuclear factor-kappa B (NF-κB) signaling pathway promotes the production of proinflammatory mediators such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and IL-6, which accelerate bone resorption and lead to irreversible tissue destruction [8, 9]. The current standard treatment - scaling and root planing (SRP) - mechanically debrides biofilms and reduces microbial burden. However, SRP often fails to eliminate pathogens in deep periodontal pockets, anatomically complex sites, or areas with intrabony defects, resulting in residual inflammation and potential for disease recurrence [1, 2]. Therefore, the development of adjunctive bioactive therapies that can modulate inflammation and promote bone regeneration alongside SRP has become a priority in periodontitis management [10].

In recent years, nanohydroxyapatite (nHA) has gained prominence in bone tissue engineering due to its biomimetic mineral composition. Structurally similar to the inorganic component of natural bone and dental tissues, nHA exhibits excellent biocompatibility, osteoconductivity, and osteoinductivity, supporting osteoblast adhesion, differentiation, and mineral deposition [11, 12]. In periodontal applications, nHA has been employed in guided bone regeneration (GBR) and treatment of intrabony defects, demonstrating significant improvements in probing depth (PD) and clinical attachment level (CAL) [13]. For example, one clinical study reported a bone fill rate of 63.39% with a combination of hydroxyapatite and platelet-rich fibrin (PRF), compared to only 15.96% in the SRP-only group [14].

Concurrently, glucocorticoids such as triamcinolone (TR) are widely used for their broad anti-inflammatory effects, including NF-κB pathway inhibition [15]. TR suppresses proinflammatory cytokine synthesis and leukocyte infiltration, thereby reducing tissue edema and destruction. Preliminary findings indicate that local TR application may attenuate periodontal soft tissue inflammation and enhance healing [16]. However, its use in non-surgical periodontal therapy remains limited, especially in combination with regenerative biomaterials. This presents an opportunity to explore a composite strategy that combines anti-inflammatory and osteogenic effects, offering a novel therapeutic approach for advanced periodontitis.

Based on this rationale, the present retrospective study is the first to assess the clinical efficacy of combining nHA and TR in the non-surgical treatment of stage III-IV periodontitis. We systematically compared four treatment modalities - nHA+TR, nHA alone, TR alone, and conventional SRP - across key clinical indicators (PD, CAL), radiographic bone regeneration, and inflammatory cytokine profiles. This study aims to investigate the potential synergistic effects of the combination therapy, assess its feasibility as a bioactive adjunct in non-surgical periodontal treatment, and provide preliminary data to support future clinical trials.

Materials and methods

Study design and participants

This retrospective observational study was conducted in the Department of Periodontology at a university-affiliated hospital from June 2023 to June 2024. The study protocol was approved by the Medical Ethics Committee of The Second Affiliated Hospital of Hainan Medical University (Approval No.: hyey2025-11). All patient data were anonymized prior to analysis, and no personally identifiable information was involved; therefore, the requirement for informed consent was waived.

A total of 120 patients with stage III-IV periodontitis who had completed non-surgical periodontal treatment and adjunctive topical therapy were included, based on electronic medical records. Patients were allocated to one of four groups (n = 30 each) according to the adjunctive topical therapy received after standard subgingival scaling and root planing (SRP): (1) combined nHA and TR, (2) nHA alone, (3) TR alone, (4) SRP alone (control group).

Sample size was determined based on preliminary clinical data and power analysis, assuming an intergroup difference in PD of 0.5 mm, with a standard deviation of 0.6 mm, significance level (α) of 0.05, and power (1-β) of 0.8. Allowing for a 15% possible dropout rate, a total of 120 patients were enrolled.

As a retrospective study, treatment selection was individualized and made during the initial

clinical consultation by attending periodontists, based on periodontal status, systemic health, and financial considerations. All treatment decisions were documented in the electronic medical records. No prospective randomization was performed. To minimize selection bias, strict exclusion criteria were applied (e.g., ongoing orthodontic treatment, uncontrolled systemic disease), and efforts were made to balance key baseline characteristics (age, sex, comorbidities, periodontal severity) across groups.

Inclusion criteria: Adults aged 25-70 years diagnosed with stage III or IV periodontitis according to the 2018 classification by the American Academy of Periodontology (AAP) and the European Federation of Periodontology (EFP). Eligible patients had at least two non-adjacent teeth with PD ≥ 6 mm and clinical attachment loss (CAL) ≥ 5 mm, were in generally good health, and had not received any periodontal therapy in the preceding 6 months.

Exclusion criteria: Patients were excluded if they had systemic diseases affecting periodontal health, were pregnant or lactating, were current smokers, had used antibiotics or non-steroidal anti-inflammatory drugs within the prior 3 months, had known allergies to study materials, or required immediate surgical periodontal intervention.

After excluding cases with incomplete data or loss to follow-up, 113 participants were included in the final analysis.

Grouping and treatments

Group allocation: Patients were retrospectively categorized into four groups based on the adjunctive subgingival topical gel applied following SRP. The choice of adjunctive therapy was made by certified periodontists (intermediate or senior level) based on clinical judgment and patient preference. All patients had provided informed consent for the original treatment, and complete documentation was maintained in the hospital's medical records.

The four treatment groups were as follows: Control group: Subgingival application of placebo gel after SRP. nHA group: Subgingival application of 5% nHA gel after SRP. TR group: Subgingival application of 0.1% TR gel after SRP. nHA+TR group: Subgingival application of a premixed 5% nHA + 0.1% TR gel after SRP.

Preparation of study medications: All study gels were prepared under sterile conditions by qualified pharmaceutical staff using carboxymethyl cellulose (CMC) as the carrier base and were visually indistinguishable.

nHA gel (5%): Prepared in-house using nHA with a particle size < 100 nm and > 99.5% purity (Sigma-Aldrich, USA), dispersed in a CMC matrix.

TR gel (0.1%): Contained 0.1% TR acetonide acetate (Sigma-Aldrich, USA) within a CMC base.

nHA+TR gel: Prepared by homogenizing 5% nHA and 0.1% TR in CMC using ultrasonic dispersion.

Placebo gel: CMC-based gel with identical appearance and texture but no active ingredients.

All formulations were packaged in coded containers and archived for quality assurance and clinical record-keeping.

Treatment procedures: At baseline, all participants underwent a standardized periodontal evaluation and treatment protocol. This included full-mouth clinical examination, biological sample collection (gingival crevicular fluid, subgingival plaque, and 10 mL peripheral blood), and radiographic imaging. Patients received comprehensive oral hygiene instruction, including the modified Bass brushing technique and interdental cleaning, and were provided with standardized oral hygiene products (Colgate Total® toothpaste and toothbrush).

All treatments were performed by a single, calibrated team following a standardized protocol to minimize inter-operator variability. The allocated topical therapy (nHA+TR, nHA, TR, or placebo) was documented in each patient's record. SRP was conducted using the full-mouth disinfection (FMD) approach under 2% lidocaine local anesthesia, with an average instrumentation time of 2-3 minutes per tooth.

In sites with PD ≥ 5 mm, 0.2 mL of the designated gel was delivered subgingivally and retained in situ for 5 minutes before gently

removing any excess. Patients were monitored for adverse reactions and oral hygiene compliance at 1, 3, and 6 months post-treatment. Only patients who completed the entire treatment protocol and follow-up assessments were included in the final analysis.

Outcome measures and data collection

The primary efficacy endpoints were changes in PD and CAL, as well as the proportion of periodontal pockets achieving closure (defined as PD ≤ 4 mm with no bleeding on probing [BoP]). PD and CAL were assessed at six sites per tooth (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, distolingual) using a UNC-15 periodontal probe (Hu-Friedy, USA) with a standardized probing force of 0.25 N and a precision of 1 mm. Pocket closure rate was calculated as: (Number of closed pockets/Total number of deep pockets at baseline) × 100%.

To ensure data integrity, all clinical measurements were independently verified by two calibrated examiners and entered into a secure electronic database.

Secondary outcome measures included gingival index (GI), BoP, plaque index (PI), and gingival recession (GR). GI and PI were evaluated using the Löe and Silness scoring system: Score 0: Healthy gingiva/No plaque. Score 1: Mild inflammation/Thin plaque. Score 2: Moderate inflammation/Moderate plaque. Score 3: Severe inflammation/Heavy plaque.

Each tooth was scored at four sites (buccal, lingual/palatal, mesial, distal), and mean PI was calculated as: PI = Total score/Number of sites examined.

BoP was recorded as a binary outcome (positive or negative), based on the presence of bleeding within 30 seconds of probing. The BoP percentage was calculated as: (Number of bleeding sites/Total number of sites examined) × 100%.

GR was measured as the vertical distance from the cementoenamel junction to the gingival margin, using a UNC-15 probe, and expressed in millimeters.

Gingival crevicular fluid (GCF) was collected from four non-adjacent sites with PD ≥ 6 mm using Periopaper strips (Oraflow Inc., USA), and volume was measured with a Periotron 8000. Concentrations of IL-1β, TNF-α, and IL-6 in GCF were determined using high-sensitivity ELISA kits (R&D Systems, USA). Serum C-reactive protein (CRP) levels were assessed from venous blood samples using a high-sensitivity immunoturbidimetric assay (hs-CRP, Roche Diagnostics, Switzerland). All biological samples were analyzed in a single laboratory using the same batch of reagents, with internal quality controls included in each assay to ensure reliability and reproducibility.

Statistical analysis

Statistical analyses were conducted using SPSS version 26.0 (IBM, USA), with a two-tailed significance threshold set at $\alpha = 0.05$. Continuous variables were presented as mean ± standard deviation (SD) for normally distributed data or as median with interquartile range (for non-normally distributed data); categorical variables were presented as frequencies and percentages. Data normality and homogeneity of variances were assessed using the Shapiro-Wilk and Levene's tests, respectively.

Intergroup comparisons of continuous variables were performed using one-way ANOVA or Kruskal-Wallis tests, while categorical variables were analyzed using the chi-square test or Fisher's exact test as appropriate. Significant baseline imbalances were adjusted for using covariate analysis in subsequent models. Within-group comparisons (baseline vs. 3 months, baseline vs. 6 months) were assessed using paired t-tests for normally distributed data.

Correlations between clinical and inflammatory data were evaluated using Pearson or Spearman correlation coefficients, and corresponding scatter plots were generated. To identify independent predictors of changes in PD and CAL, multivariable linear regression analysis was performed, adjusting for potential confounders including age, sex, body mass index (BMI), smoking status, baseline periodontal data, baseline inflammatory markers, tooth type, and PI. Results are reported as adjusted β-coefficients with corresponding 95% confidence intervals (CIs) and *P*-values. Collinearity diagnostics were performed to verify model validity.

Table 1. Comparison of baseline demographic and clinical characteristics

Variable	Total (n = 113)	Control (n = 28)	nHA (n = 29)	TR (n = 28)	nHA+TR (n = 28)	Statistic	P
Age	47.5 ± 8.3	46.8 ± 9.1	48.2 ± 7.9	47.6 ± 8.5	47.5 ± 8.0	47.5 ± 8.3	0.87
Sex (Male/Female)	54/59	13/15	14/15	13/15	14/14	$\chi^2 = 0.23$	0.92
BMI (kg/m^2)	24.3 ± 2.8	24.1 ± 3.0	24.5 ± 2.7	24.2 ± 2.9	24.4 ± 2.6	F = 0.19	0.903
PD (mm)	6.84 ± 0.45	6.86 ± 0.44	6.83 ± 0.59	6.83 ± 0.35	6.84 ± 0.38	F = 0.03	0.995
CAL (mm)	7.16 ± 0.62	7.20 ± 0.74	6.96 ± 0.60	7.11 ± 0.52	7.38 ± 0.57	F = 2.30	0.081
BoP (%)	86.04 ± 4.48	85.32 ± 4.28	86.86 ± 4.26	85.09 ± 4.69	86.87 ± 4.61	F = 1.31	0.274
PI	2.84 ± 0.33	2.81 ± 0.24	2.88 ± 0.43	2.78 ± 0.30	2.91 ± 0.29	F = 1.02	0.387
GI	2.56 ± 0.38	2.66 ± 0.31	2.62 ± 0.50	2.44 ± 0.31	2.50 ± 0.32	F = 2.03	0.114

nHA: nanohydroxyapatite; TR: triamcinolone; PD: probing depth; CAL: clinical attachment level; BoP: bleeding on probing; PI: plaque index; GI: gingival index.

To identify predictors of treatment success - defined as a reduction in PD ≥ 2 mm and CAL ≥ 1 mm at 6 months - logistic regression analysis was conducted. Treatment success was coded as a binary outcome (1 = success; 0 = failure). Covariates were selected using forward stepwise selection (method: forward likelihood ratio), with entry and removal criteria set at $P < 0.05$ and $P > 0.10$, respectively. Adjusted odds ratios (ORs) with 95% CIs and associated *P*-values are reported for the final model. Model performance was evaluated using classification accuracy, sensitivity, specificity, and receiver operating characteristic (ROC) curve analysis.

Subgroup analysis was performed to assess the influence of baseline systemic inflammation, defined by hs-CRP > 3 mg/L. Participants were dichotomized into high (hs-CRP > 3 mg/L, coded as 1) and low (hs-CRP ≤ 3 mg/L, coded as 0) inflammation groups. Interaction terms were included in regression models to test for differential treatment effects between subgroups.

Results

Comparison of baseline participant characteristics

No significant differences in baseline demographic or clinical characteristics were observed among the groups (all $P > 0.05$) (**Table 1**), indicating adequate baseline comparability and balanced group assignment.

Comparison of primary clinical data

All groups exhibited significant reductions in PD and CAL compared with baseline values (both $P < 0.01$), with marked intergroup differences observed. At 6 months, the mean PD in the nHA+TR group (3.51 ± 0.28 mm) was significantly lower than in the control group (4.95 ± 0.28 mm), the nHA group (4.04 ± 0.34 mm), and the TR group (4.90 ± 0.38 mm). The nHA group also demonstrated a significantly lower PD than the control and TR groups ($P < 0.001$) (**Figure 1A**).

A similar trend was observed for CAL improvement ($P < 0.01$) (**Figure 1B**). Pocket closure rates at 6 months were significantly higher in the nHA+TR group (74.30 ± 5.78%) compared to the controls (35.04 ± 5.48%), nHA (58.97 ± 5.26%), and TR (49.86 ± 5.48%) groups ($P < 0.01$). The nHA group also showed a significantly greater pocket closure rate than the control group ($P < 0.001$) (**Figure 1C**).

Comparison of secondary clinical data

All treatment groups showed significant reductions in BoP from baseline ($P < 0.001$) (**Figure 2A**). At 6 months, BoP in the nHA+TR group (37.93 ± 8.21%) was significantly lower than in the control group and also lower than in the nHA (26.92 ± 6.84%) and TR (29.83 ± 4.47%) groups ($P < 0.001$). BoP values in the nHA and TR groups were themselves significantly lower than those in the control group ($P < 0.001$).

Similar trends were observed for PI and GI (**Figure 2B** and **2C**), with all groups showing significant reductions (both $P < 0.001$). At 6 months, GI in the nHA+TR group was significantly lower than in both the control and TR groups ($P < 0.001$), but did not significantly differ from the nHA group ($P > 0.05$) (**Figure 2D**).

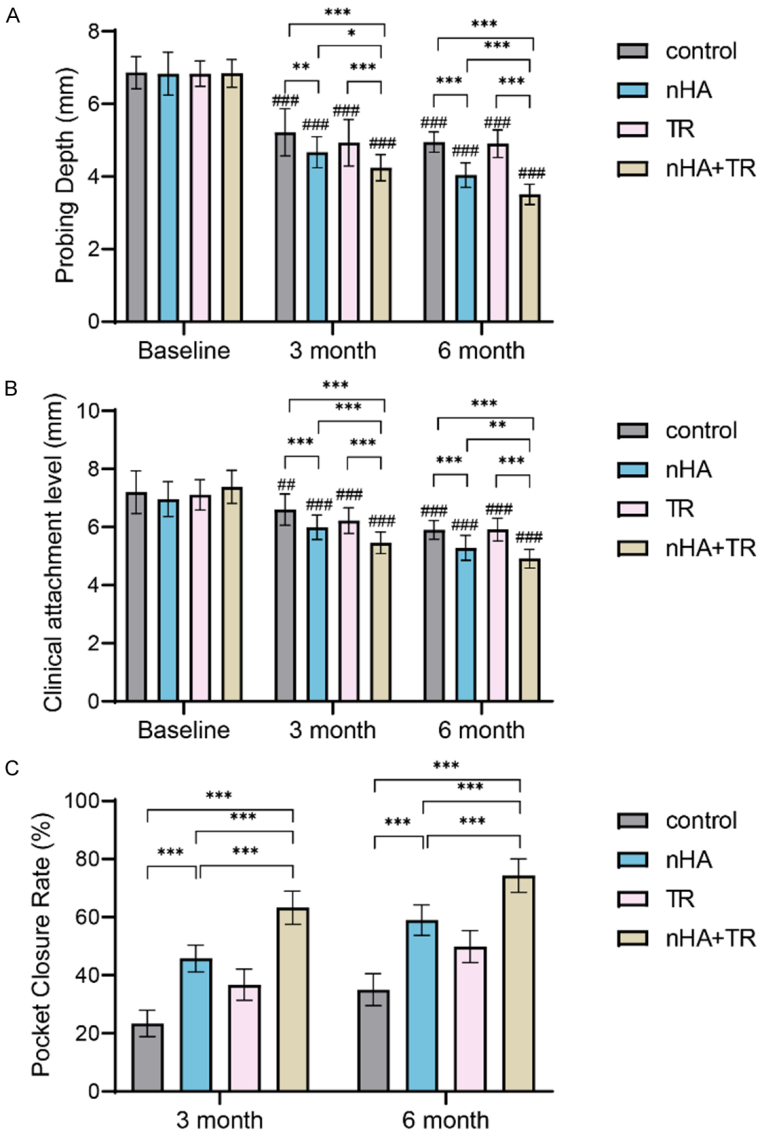

Figure 1. Changes in primary clinical outcomes over time. A: Changes in probing depth (PD) at baseline, 3 months, and 6 months. B: Changes in clinical attachment level (CAL) at baseline, 3 months, and 6 months. C: Pocket closure rate at 3 and 6 months. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control group (inter-group comparison); ##P < 0.01, ###P < 0.001 vs. baseline (intra-group comparison). nHA: nanohydroxyapatite; TR: triamcinolone; nHA+TR: nanohydroxyapatite plus triamcinolone.

While all groups showed slight increases in GR over time, no statistically significant differences were observed between groups (P > 0.05).

Comparison of inflammatory marker modulation and associations with clinical outcomes

To evaluate the anti-inflammatory effects of the treatments, we measured levels of key cytokines associated with periodontal tissue destruction - IL-1β, TNF-α, and IL-6 - along with the systemic marker CRP. These markers were chosen due to their established roles in periodontitis pathogenesis and correlation with disease severity.

At 6 months, all groups showed significant reductions in IL-1β, TNF-α, IL-6, and CRP levels (all P < 0.01). The most substantial decreases were observed in the nHA+TR group compared to either monotherapy group (all P < 0.01), highlighting the enhanced anti-inflammatory efficacy of the combined intervention (**Figure 3**).

To explore the clinical relevance of these findings, Pearson correlation analyses were conducted. Reductions in PD were strongly correlated with decreases in IL-1β (r = 0.80, P < 0.001), TNF-α (r = 0.88, P < 0.001), IL-6 (r = 0.88, P < 0.001), and CRP (r = 0.88, P < 0.001). Similarly, gains in CAL were significantly correlated with reductions in IL-1β (r = 0.80), TNF-α (r = 0.88), IL-6 (r = 0.88), and CRP (r = 0.87) (P < 0.001) (**Figure 4**).

These strong associations underscore that improvements in clinical periodontal data are closely linked to both local and systemic inflammatory suppression.

Regression analysis

Multivariable linear regression was performed to identify independent predictors of clinical improvement following non-surgical periodontal therapy. As shown in **Figure 5A**, the combined nHA+TR treatment was significantly associated with greater PD reduction (β = 1.09, P < 0.001). Baseline PD (β = 1.01, P < 0.001), PI (β = 4.38, P < 0.001), and BoP (β =

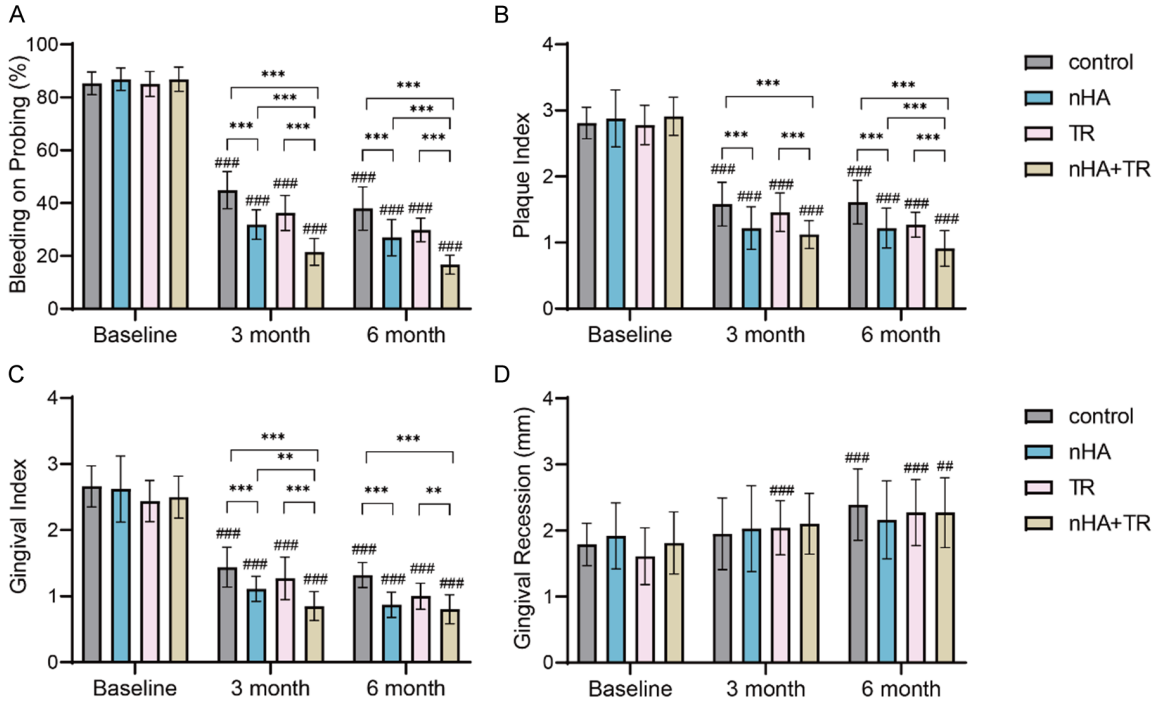

Figure 2. Changes in secondary clinical outcomes. A: Bleeding on probing (BoP) at baseline, 3 months, and 6 months. B: Plaque index (PI) at baseline, 3 months, and 6 months. C: Gingival index (GI) at baseline, 3 months, and 6 months. D: Gingival recession (GR) at baseline, 3 months, and 6 months. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control group (inter-group comparison); ##P < 0.01, ###P < 0.001 vs. baseline (intra-group comparison).

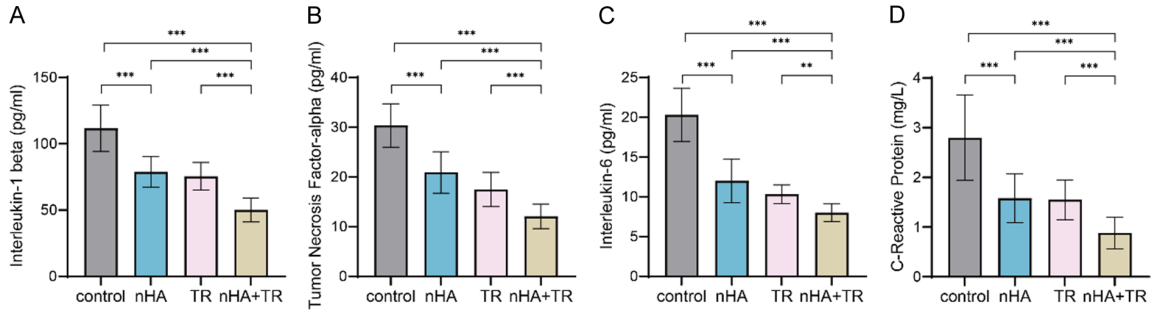

Figure 3. Inflammatory marker levels at 6 months. A: Gingival Crevicular Fluid (GCF) Interleukin-1 beta (IL-1β) levels. B: GCF Tumor Necrosis Factor-alpha (TNF-α) levels. C: GCF Interleukin-6 (IL-6) levels. D: Serum C-Reactive Protein (CRP) levels. **P < 0.01, ***P < 0.001.

0.23, P < 0.01) were also positive predictors. In contrast, elevated baseline serum CRP (β = -1.09, P < 0.001) and GCF IL-1β (β = -0.04, P < 0.01) were negatively associated with PD reduction.

Regarding CAL, the combination therapy again showed a significant positive effect (β = 1.33, P < 0.001) (**Figure 5C**). Additional independent predictors included baseline CAL (β = 1.08, P < 0.001), PI (β = 3.49, P < 0.001), and BoP (β = 0.16, P < 0.05), while higher CRP (β = -0.90, P < 0.001) and IL-1β (β = -0.03, P < 0.05) were significant negative predictors. Residuals were normally distributed, supporting the validity of both models (**Figure 5B** and **5D**). These findings underscore the influence of both baseline periodontal status and systemic inflammation on treatment response, reinforcing the potential value of adjunctive anti-inflammatory therapies in high-risk populations.

To further identify predictors of treatment success - defined as a ≥ 2 mm PD reduction and ≥

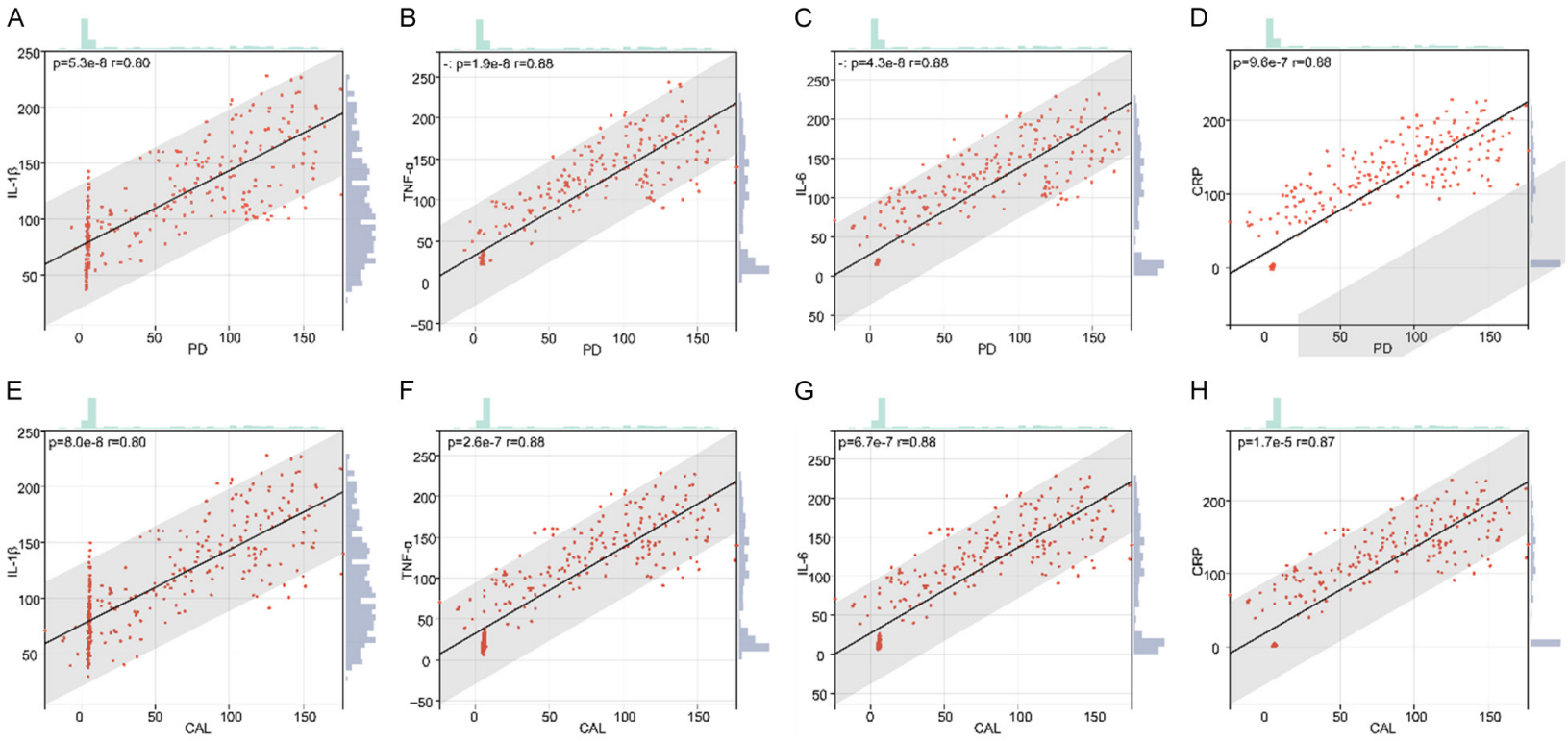

Figure 4. Correlations between clinical outcomes and inflammatory markers. A-D: Correlation of PD reduction with IL-1β, TNF-α, IL-6, and CRP. E-H: Correlation of CAL improvement with the same markers.

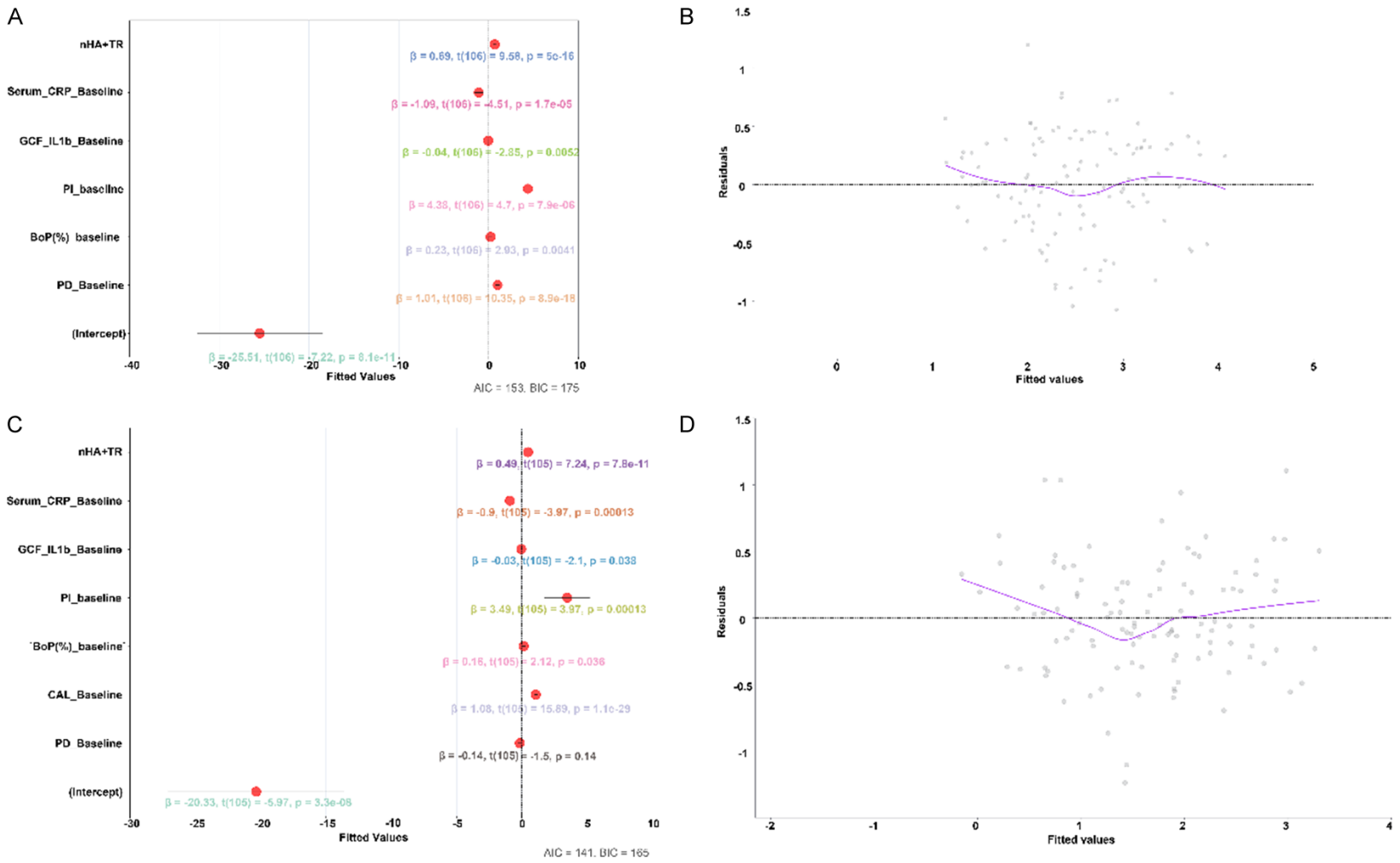

Figure 5. Multivariate linear regression analysis. A: Standardized coefficients (β) and 95% confidence interval (CI) for independent predictors of PD reduction. B: Residual plot for PD model. C: Coefficients for CAL gain predictors. D: Residual plot for CAL model. Significant predictors are shown with colored confidence intervals ($P < 0.05$).

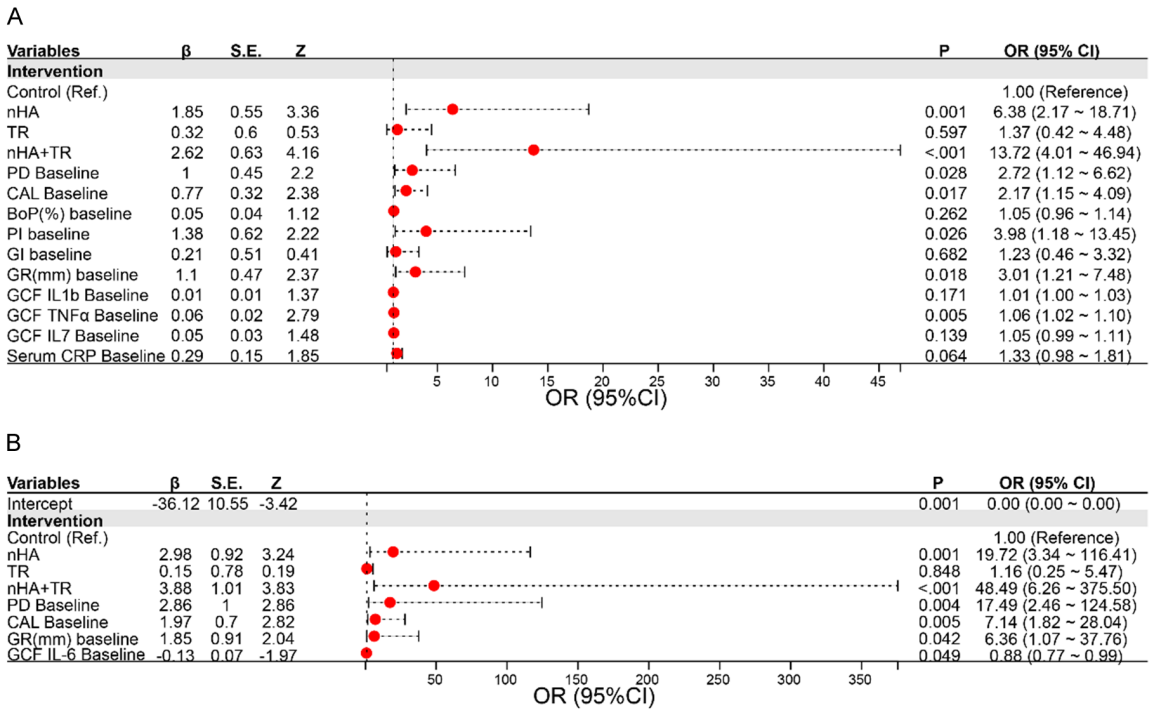

Figure 6. Logistic regression analysis of predictors for treatment success. A: Univariate analysis. B: Multivariate model adjusted for confounders. Red dots indicate odds ratios and 95% CIs.

1 mm CAL gain at 6 months - a logistic regression analysis was performed. Univariate analysis (**Figure 6A**) revealed significant associations between treatment success and multiple baseline variables, including clinical indices and inflammatory markers. Notably, nHA+TR therapy substantially increased the likelihood of clinical success compared to the control group (OR = 13.72; 95% CI: 4.01-46.94; P = 0.001). Other significant predictors included baseline PD (OR = 2.72, P = 0.028), CAL (OR = 2.17, P = 0.017), PI (OR = 3.98, P = 0.026), GR (OR = 3.01, P = 0.018), and TNF-α (OR = 1.06, P = 0.005).

After adjusting for potential confounders, nHA+TR remained a robust independent predictor of treatment success (OR = 48.49; 95% CI: 6.26-375.50; P < 0.001). Additional independent predictors retained in the final model included baseline PD, CAL, GR, and IL-6, indicating that more severe initial periodontal status combined with lower systemic inflammatory burden was associated with improved regenerative outcomes (**Figure 6B**).

Model performance was evaluated using ROC curve analysis (**Figure 7A**). The area under the curve (AUC) was 0.85 (95% CI: 0.81-0.88), indicating excellent discrimination. Calibration analysis (**Figure 7B**) demonstrated strong concordance between predicted probabilities and observed outcomes. At an optimal probability threshold of 0.127, the model achieved an accuracy of 72%, sensitivity of 70%, specificity of 84%, positive predictive value (PPV) of 96%, and negative predictive value (NPV) of 31%. These results support the robustness and clinical utility of the predictive model in identifying favorable responders to regenerative periodontal therapy (**Table 2**).

Subgroup analysis based on systemic inflammation

To evaluate whether systemic inflammation modulates treatment efficacy, subgroup analysis was conducted based on baseline hs-CRP levels. Participants with hs-CRP > 3 mg/L were classified as the high-inflammation group (n = 45), while those with hs-CRP ≤ 3 mg/L were categorized as the low-inflammation group.

In the high-inflammation group, patients treated with nHA+TR exhibited significantly greater PD reduction (3.7 ± 0.4 mm) compared to those

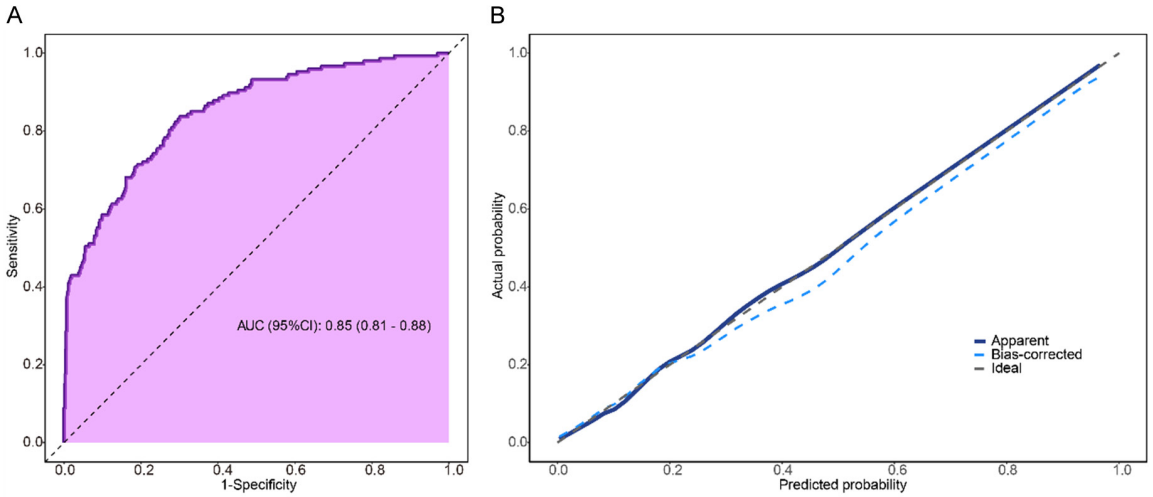

Figure 7. Evaluation of the logistic regression model. A: Receiver operating characteristic (ROC) curve demonstrating strong discriminatory power of the model. B: Calibration curve showing good agreement between predicted and observed outcomes. AUC: area under the curve.

Table 2. Confusion matrix of the predictive model

AUC (95% CI)	Accuracy (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Cut off
85% (81%-88%)	72% (69%-75%)	70% (67%-73%)	84% (78%-90%)	96% (95%-98%)	31% (27%-36%)	0.127

AUC: area under the curve; CI: confidence intervals; PPV: positive predictive value; NPV: negative predictive value.

in the low-inflammation group (3.0 ± 0.3 mm, P = 0.006). Moreover, IL-1β and TNF-α levels declined more substantially in the high-inflammation subgroup following combination therapy. Importantly, within this subgroup, nHA+TR significantly outperformed monotherapies and the control treatment (P < 0.01), a difference not observed in the low-inflammation cohort.

Interaction analysis revealed significant interaction terms between systemic inflammation and treatment modality for both PD reduction (P_interaction = 0.032) and cytokine reduction (P_interaction = 0.041), suggesting that the therapeutic benefit of combination therapy was amplified in patients with elevated systemic inflammation (**Table 3**).

Comparison of adverse events (AEs)

All treatment regimens were well tolerated over the 6-month follow-up period, with no serious AEs reported. The incidence of common local AEs - including gingival irritation, mild pain, swelling, bleeding, and tooth sensitivity - was comparable across groups (all P > 0.05) (**Table 4**).

Gingival irritation occurred in 10.71% of patients in both the control and nHA+TR groups, 7.14% in the nHA group, and 14.29% in the TR group. Mild pain was reported by 7.14% to 10.71% of participants across groups (all P > 0.05). Swelling and localized bleeding were infrequent (7.14% to 14.29%) and did not differ between groups (both P > 0.05). Tooth sensitivity was the most commonly reported AE, affecting 10.71% to 17.86% of patients, again without significant intergroup variation (P > 0.05).

Discussion

This retrospective study evaluated the clinical benefits of combining nHA with TR in the nonsurgical management of severe periodontitis. The results demonstrated that the combined therapy led to greater improvements in periodontal clinical characteristics and more pronounced reductions in both local and systemic inflammation. Notably, patients with elevated baseline inflammatory markers experienced significantly greater therapeutic responses, underscoring the potential utility of this combi-

Table 3. Subgroup analysis based on systemic inflammation (CRP)

Subgroup	Treatment	PD Reduction (mm, Mean ± SD)	%Δ IL-1β	%Δ TNF-α	P vs. Control	P vs. nHA	P vs. TR
Low Inflammation (CRP ≤ 3 mg/L, n = 68)	Control	2.0 ± 0.4	12.4%	14.1%	-	-	-
	nHA	2.7 ± 0.3	23.1%	25.6%	0.002	-	0.046
	TR	2.3 ± 0.4	19.5%	20.7%	0.020	0.081	-
	nHA+TR	3.0 ± 0.3	31.3%	33.7%	< 0.001	0.041	0.017
High Inflammation (CRP > 3 mg/L, n = 45)	Control	1.8 ± 0.5	9.8%	10.6%	-	-	-
	nHA	2.8 ± 0.4	26.5%	27.9%	< 0.001	-	0.012
	TR	2.2 ± 0.5	21.3%	22.5%	0.004	0.064	-
	nHA+TR	3.7 ± 0.4	41.8%	44.3%	< 0.001	0.007	0.003

CRP: C-reactive protein. %Δ = Percentage decrease from baseline. *P* values are adjusted for age, sex, BMI, and baseline PD using logistic regression with interaction terms.

Table 4. Comparison of adverse events

Variable	Control (n = 28)	nHA (n = 29)	TR (n = 28)	nHA+TR (n = 28)	P
Gingival irritation, n (%)	3 (10.71)	2 (7.14)	4 (14.29)	3 (10.71)	0.974
Mild pain, n (%)	2 (7.14)	3 (10.71)	2 (7.14)	3 (10.71)	0.990
Swelling, n (%)	2 (7.14)	2 (7.14)	3 (10.71)	2 (7.14)	0.993
Bleeding, n (%)	4 (14.29)	3 (10.71)	3 (10.71)	2 (7.14)	0.974
Tooth sensitivity, n (%)	4 (14.29)	5 (17.86)	4 (14.29)	3 (10.71)	0.982

nation therapy in inflammation-driven periodontal pathology. Collectively, these findings suggest that nHA+TR may serve as a promising adjunctive strategy to enhance the efficacy of conventional non-surgical periodontal treatment in patients with severe disease.

While all treatment groups achieved measurable clinical improvements, the nHA+TR group demonstrated superior outcomes in terms of PD reduction, CAL gain, and pocket closure rates. This enhanced efficacy likely reflects the complementary biological functions of the two agents. nHA, a bioactive material with osteoconductive and osteoinductive properties, supports periodontal regeneration by promoting osteogenesis and facilitating extracellular matrix formation [17]. TR, a corticosteroid with potent anti-inflammatory and immunosuppressive effects, suppresses inflammatory cascades and protects tissues from breakdown [18].

Importantly, nHA monotherapy also significantly outperformed the control group, consistent with previous randomized controlled trials reporting enhanced PD reduction and CAL gain with adjunctive nHA [19]. These regenerative effects are attributed to the nanoscale morphology of nHA, which mimics bone mineral composition, enhances surface bioactivity, promotes osteoblast adhesion and differentiation, and upregulates the expression of osteogenesis-related genes [20, 21].

The most compelling advantage of the combined nHA+TR therapy was its ability to suppress inflammation. Patients in this group exhibited significantly lower levels of IL-1β, TNF-α, IL-6, and serum CRP. These findings align with prior studies demonstrating that adjunctive anti-inflammatory therapies can significantly downregulate inflammatory cytokine expression in periodontitis patients [22]. IL-1β promotes matrix metalloproteinase activity while inhibiting tissue inhibitors of metalloproteinases, accelerating collagen degradation. TNF-α stimulates osteoclastogenesis and bone resorption, while IL-6 drives the acute-phase inflammatory response and contributes to tissue injury [23, 24]. Given their central role in periodontal pathogenesis, elevated cytokine levels are strongly associated with disease severity, and their suppression correlates with improved clinical outcomes [25].

In this study, Pearson correlation analysis confirmed that reductions in inflammatory markers were significantly associated with improvements in PD and CAL, highlighting the critical role of inflammation control in periodontal healing. TR likely exerts its anti-inflammatory effects through inhibition of NF-κB activation and suppression of proinflammatory mediator production [26]. Meanwhile, nHA may contribute to inflammation resolution through indirect mechanisms, including its intrinsic antibacterial activity and its capacity to promote tissue repair and regeneration [27]. Together, these agents exert a dual therapeutic effect - simultaneous suppression of inflammation and promotion of regeneration - potentially explaining the superior clinical outcomes observed in the nHA+TR group. The observed post-treatment decline in serum CRP further underscores the systemic relevance of periodontal inflammation and suggests that effective periodontal therapy may have broader implications for systemic health.

Multivariate regression identified baseline clinical parameters - including PD, CAL, PI, and BoP - along with systemic inflammatory status as significant predictors of treatment response. Elevated baseline inflammation, indicated by CRP > 3 mg/L, was independently associated with diminished clinical improvement, consistent with prior evidence that systemic inflammatory burden can hinder periodontal healing [28].

To further investigate this relationship, a predefined subgroup analysis was performed based on baseline serum high-sensitivity CRP (hs-CRP) levels. As a stable and clinically validated marker of systemic inflammation with well-established diagnostic thresholds, hs-CRP served as a reliable stratification variable [29]. The analysis revealed that patients with hs-CRP > 3 mg/L derived significantly greater clinical benefit from nHA+TR therapy. A significant interaction was observed between baseline inflammatory status and treatment response, indicating that systemic inflammation may influence therapeutic efficacy.

This finding mirrors observations in other chronic inflammatory conditions, where individuals with hs-CRP ≥ 3 mg/L demonstrate enhanced responsiveness to anti-inflammatory therapies [30]. Collectively, these results highlight the utility of personalized treatment approaches guided by systemic inflammatory profiling. In patients with elevated systemic inflammation, SRP alone may be insufficient to interrupt the cycle of tissue destruction. Instead, adjunctive strategies that integrate anti-inflammatory and regenerative functions - such as nHA+TR - may offer superior clinical benefits.

The findings of this study carry important clinical implications. First, the combined therapy of nHA and TR may result in superior improvements in PD and pocket closure during the non-surgical phase, thereby reducing the need for subsequent periodontal surgery. Previous studies have reported that the effectiveness of non-surgical interventions directly influences the necessity of surgical treatment [31].

Second, stratified treatment based on systemic inflammatory status suggests that this therapeutic approach may be particularly beneficial for high-risk individuals, aligning with the principles of precision medicine.

Third, the observed reductions in systemic inflammatory markers following periodontal treatment further emphasize the broader health benefits of maintaining periodontal health, supporting existing evidence that links periodontitis to systemic diseases [32].

Despite these clinically relevant findings, several limitations should be acknowledged. First, the retrospective study design introduces potential selection bias and limits the ability to establish causality.

Second, the follow-up period was limited to 6 months, which may be insufficient to fully assess the long-term stability of tissue regeneration, particularly in relation to bone reconstruction.

Third, cone-beam computed tomography (CBCT) or other advanced imaging modalities were not utilized to quantitatively evaluate bone regeneration, restricting the depth of imaging-based analyses.

In addition, although the overall sample size was adequate, the number of patients in certain subgroups - such as those with elevated systemic inflammation - was relatively small,

which may have reduced the statistical power of subgroup analyses.

Fourth, the predictive model was developed without validation using an external cohort, which may limit its generalizability and robustness across diverse populations.

Finally, this study did not include experimental assessments of inflammatory cell infiltration or mediator levels - such as tissue-based immunohistochemistry or cytokine profiling - which constrains our mechanistic understanding of how the combined application of nHA and TR modulates inflammation.

Future studies are warranted to address these limitations. Prospective, randomized controlled trials are needed to confirm the clinical efficacy and causative effects of nHA combined with TR.

Longer follow-up durations (≥ 12 months) are recommended to assess the durability of regenerative outcomes. Incorporating CBCT and other three-dimensional imaging techniques may allow for more accurate and quantitative evaluation of bone regeneration.

Further research should also explore the optimal dosing ratios and release kinetics of the nHA-TR combination. Expanding the study population - particularly to include individuals with systemic comorbidities such as diabetes mellitus - could facilitate a more comprehensive evaluation of treatment efficacy in varied clinical settings.

In addition, future predictive models should include external validation cohorts to enhance their clinical applicability and reliability. Mechanistic investigations should be strengthened by incorporating inflammatory cell analysis, immunohistochemical staining, and cytokine profiling (e.g., IL-1β, TNF-α) to elucidate the pathways involved in inflammation resolution and tissue regeneration, such as NF-κB signaling.

In conclusion, this retrospective study suggests that the combination of nano-hydroxyapatite and TR may serve as an effective adjunct to non-surgical periodontal therapy in patients with severe periodontitis. The combined therapy demonstrated favorable outcomes for improving periodontal clinical outcomes and attenuating both local and systemic inflammation.

Moreover, enhanced clinical benefits in patients with elevated baseline inflammation highlight the potential of this dual-modality approach for personalized treatment. Nevertheless, these findings should be validated in prospective, randomized studies to confirm their long-term efficacy and elucidate the underlying mechanisms of action.

Disclosure of conflict of interest

None.

Address correspondence to: Lianghui Yang, Department of Stomatology, The Second Affiliated Hospital of Hainan Medical University, No. 48 Baishuitang Road, Longhua District, Haikou 570311, Hainan, China. E-mail: ylh900502@163.com

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