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UNVEILING PHOSPHATASE SHIFTS FOLLOWING PERIODONTAL THERAPY IN NON-SMOKERS, FORMER AND CURRENT SMOKERS WITH CHRONIC PERIODONTITIS

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Abstract

Background: Chronic periodontitis is a complex inflammatory condition that is greatly affected by tobacco consumption. Salivary enzymes, including alkaline phosphatase (ALP) and acid phosphatase (ACP), act as useful biomarkers indicating periodontal tissue metabolism.

Aim: This study aimed to evaluate the effect of non-surgical periodontal therapy (NSPT) on clinical parameters and salivary levels of ALP and ACP in non-smokers, former smokers, and current smokers with chronic periodontitis.

Materials and Methods: Sixty patients aged 30–50 years with generalized moderate to severe periodontitis were divided equally into three groups: non-smokers, former smokers, and current smokers. At Baseline,15th day and 4weeks saliva samples were collected for ALP and ACP analysis. Clinical parameters, including plaque index, gingival index, probing pocket depth, and clinical attachment level, were recorded at baseline and 4 weeks.

Results: Non-smokers showed the greatest reduction in clinical indices and enzyme levels after therapy. Former smokers exhibited moderate improvement, with significant biochemical but limited clinical changes. Current smokers demonstrated minimal clinical improvement and inconsistent biochemical responses. **Conclusion:** NSPT improves periodontal health and reduces salivary enzyme activity, but smoking status strongly influences outcomes. Salivary ALP and ACP are reliable, non-invasive biomarkers for monitoring treatment response, highlighting the importance of smoking cessation in periodontal care.

Keywords: Chronic periodontitis; Smoking; Salivary biomarkers; Alkaline phosphatase; Acid phosphatase; Non-surgical periodontal therapy

INTRODUCTION:

Chronic periodontitis is a common inflammatory disease with multiple contributing factors, involving the supporting structures of the teeth such as the periodontal ligament, cementum, and alveolar bone. The condition is initiated predominantly by bacterial biofilms; however, its progression is significantly influenced by host immuno-inflammatory responses, systemic comorbidities, and lifestyle-related factors, particularly tobacco smoking. [1]

Smoking is a major modifiable risk factor for periodontitis, with studies showing that smokers are two to eight times more likely to experience clinical attachment loss and alveolar bone resorption compared to non-smokers. [2] The severity of periodontal breakdown correlates directly with the intensity and duration of tobacco exposure. [3]Mechanistically, tobacco smoking compromises immune competence by modulating lymphocyte activity, suppressing antibody synthesis, and impairing neutrophil chemotaxis, phagocytosis, and oxidative burst capacity. Additionally, there is dysregulated neutrophil enzyme release particularly elastase and collagenase contributing to connective tissue degradation. [4] Tobacco use is also associated with leukocytosis, dysfunctional T and B lymphocyte subsets, diminished cytokine production, and reduced serum immunoglobulin concentrations, except for IgE. [5]

In the quest for objective biomarkers of periodontal disease activity, two key intracellular lysosomal and membrane-bound enzymes alkaline phosphatase (ALP) and acid phosphatase (ACP)—have been widely investigated. [6]ALP, expressed in periodontal ligament fibroblasts, osteoblasts, and polymorphonuclear leukocytes, serves as a biomarker of both periodontal tissue catabolism and reparative bone formation. [7]Elevated ALP activity, triggered by bacterial endotoxins and proinflammatory cytokines such as interleukin (IL)-1β and tumour necrosis factor-alpha (TNF-α), reflects heightened bone turnover during active disease. [8]Conversely, ACP, predominantly localized in osteoclasts and inflamed soft tissues, is a surrogate indicator of osteoclastic bone resorption and lysosomal degradation. [9] Its expression is enhanced by cytokine-mediated activation of the nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) signaling cascades, with smoking further potentiating these pathways through oxidative stress and epigenetic modifications. [10]

Non-surgical periodontal therapy (NSPT), particularly scaling and root planing, remains the cornerstone of initial periodontal management, targeting microbial biofilm disruption and subgingival debridement. [11] In addition to clinical improvements such as reduced probing depths and inflammation, NSPT modulates host immune responses by downregulating pro-inflammatory mediators (e. g. , TNF-α, IL-1β) and upregulating anti-inflammatory cytokines (e. g. , IL-10), thus favoring periodontal wound healing. [12]

The present study aims to assess and compare salivary ALP and ACP levels at baseline, 15 days, and 4 weeks following NSPT in three cohorts: nonsmokers with chronic periodontitis, former smokers with chronic periodontitis, and current smokers with chronic periodontitis. Additionally, clinical parameters, including gingival index, plaque index, probing pocket depth, and clinical attachment level, will be recorded at baseline and 4 weeks to evaluate treatment outcomes.

MATERIALS AND METHOD

This longitudinal interventional study was conducted over 1. 5 years at NIMS Dental College & Hospital and NIMS Medical College & Hospital, Jaipur, Rajasthan. Ethical clearance was obtained from the Institutional Review Board of NIMS University, Jaipur (Approval No: IEC/P-243/2023). All procedures followed the Declaration of Helsinki (1964) and its amendments, and written informed consent was obtained from all participants. A total of 60 participants, both male and female, aged 30–50 years, were recruited from the Outpatient Department of Periodontology. They were randomly assigned into three equal groups, each comprising 20 individuals.

Group A consisted of non-smokers with chronic periodontitis, defined as individuals who had never smoked 100 or more cigarettes in their lifetime and were not currently smoking. Group B included

former smokers with chronic periodontitis, characterized by a history of smoking at least 100 cigarettes in their lifetime but who had quit prior to the study. Group C comprised current smokers with chronic periodontitis, defined as individuals with a lifetime history of smoking 100 or more cigarettes and who continued to smoke during the study period. Subjects aged 30–50 years with ≥20 permanent teeth (excluding third molars), systemically healthy, with generalized moderate to severe chronic periodontitis (≥4 qualifying sites in two quadrants, probing pocket depth >4 mm, clinical attachment level >3 mm), and no periodontal therapy or relevant medication in the past six months were included in the group. Exclusion criteria includes Inability to consent, systemic diseases (e. g. , diabetes, hypertension, thyroid disorders, endocrine diseases, osteoarthritis, osteosarcoma, renal failure), oral potentially malignant disorders, fixed prostheses or orthodontic appliances, pregnancy, or lactation. At baseline, written informed consent was obtained from each participant. A comprehensive clinical examination was carried out, recording bleeding on probing (BOP), probing pocket depth (PPD), plaque index (PI), gingival index (GI), and clinical attachment level (CAL). Periodontal measurements were recorded at six sites per tooth—mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual—using a UNC-15 probe, and the results were entered into each patient's record. All participants—Group A (non-smokers)[Fig1], Group B (former smokers)[Fig2], and Group C (current smokers)[Fig3] underwent full-mouth scaling and root planing at baseline, followed by a repeat intervention at the fourth week to reinforce periodontal therapy and support healing. For saliva collection, individuals were instructed to let saliva accumulate in the floor of the mouth before expectorating into a sterile container Using the spitting method, 2–2. 5 mL of unstimulated saliva was collected from each subject while seated with the head slightly tilted forward.

For biochemical analysis, 2–2. 5 mL of unstimulated saliva was collected via the spitting method, centrifuged at 3,000 rpm for 10 minutes [Fig4], and the supernatant separated. Alkaline phosphatase estimation was performed by mixing 20 µL of supernatant with 1,000 µL of ERBA Mannheim Alkaline phosphatase reagent (Mannheim, Germany)[Fig 5], and Acid phosphatase estimation by mixing 20 µL of supernatant with 1,000 µL of GPL Acid phosphatase reagent[Fig 5]. Enzyme activity was determined using an ERBA semi-autoanalyzer [Fig 6] at 405 nm. Following baseline sampling, all patients received non-surgical periodontal therapy. Follow-up visits were conducted at 15th day and 4th week , at which clinical parameters were reassessed and saliva samples collected for at baseline, 15th day and 4th week for Alkaline phosphatase and Acid phosphatase analysis. The assays utilized p-nitrophenyl phosphate as substrate, producing p-nitrophenol, which was quantified spectrophotometrically at 405 nm.

RESULT:

Table 1: Clinical parameters at baseline and 4th week and biochemical parameter at baseline, 15th day, 4th week following phase I periodontal therapy in Non- Smoker with chronic periodontitis (Group A)

Parameter	At baseline (Mean ± SD)	At 15th Day (Mean ± SD)	At 4 weeks (Mean ± SD)	p-value
Gingival Index (GI)	2. 0463 ± 0. 26137	–	1. 2474 ± 0. 21766	. 000
Plaque Index (PI)	2. 4516 ± 0. 35065	–	1. 2268 ± 0. 33800	. 000
Pocket Probing Depth (PPD)	5. 1705 ± 0. 84295	–	4. 0305 ± 0. 42588	. 000
Clinical Attachment Level (CAL)	5. 1874 ± 0. 66536	–	3. 9647 ± 0. 51021	. 000
Alkaline Phosphatase (ALP)	74. 4460±12. 30322	65. 3685±12. 26398	49. 5324±5. 53460	. 000
Acid Phosphatase (ACP)	10. 3690 ± 2. 45203	6. 9355 ± 1. 36269	6. 4182 ± 1. 17297	. 000

In Group A, significant improvements were observed in both clinical and biochemical parameters. Clinically, reductions were noted from baseline to 4 weeks in gingival index (2. 0463 to 1. 2474), plaque index (2. 4516 to 1. 2268), pocket probing depth (5. 1705 mm to 4. 0305 mm), and clinical attachment level (5. 1874 mm to 3. 9647 mm), all with p-values of . 000. Biochemically, alkaline phosphatase levels decreased from 74. 4460 to 49. 5324 and acid phosphatase from 10. 3690 to 6. 4182 by 4th week, also with highly significant p-values (. 000). These results indicate marked clinical and biochemical improvement over time in Group A.

Table 2: Clinical parameters at baseline and 4th week and biochemical parameter at baseline, 15th day, 4th week following phase I periodontal therapy in Former Smoker with chronic periodontitis (Group B)

Parameter	At baseline (Mean ± SD)	At 15th Day (Mean ± SD)	At 4 weeks (Mean ± SD)	p-value
Gingival Index (GI)	1. 9615 ± 0. 34307	–	1. 2780 ± 0. 18608	. 000
Plaque Index (PI)	2. 4610 ± 0. 41149	–	1. 9205 ± 2. 23567	. 297
Pocket Probing Depth (PPD)	5. 6065 ± 0. 75002	–	4. 7095 ± 0. 66751	. 003
Clinical Attachment Level (CAL)	5. 0800 ± 0. 80791	–	5. 0405 ± 0. 60492	. 003
Alkaline Phosphatase (ALP)	74. 4370± 8. 56425	68. 7895± 8. 36924	48. 9158± 8. 96725	. 000
Acid Phosphatase (ACP)	10. 4013± 1. 55416	8. 6505 ± 1. 81630	1. 6615 ± 1. 18411	. 000

In Group B, clinical and biochemical parameters showed variable improvement. The gingival index significantly decreased from 1. 9615 to 1. 2780 (p = . 000), and pocket probing depth reduced from 5. 6065 mm to 4. 7095 mm (p = . 003). Clinical attachment level showed a minor reduction (5. 0800 to 5. 0405 mm; p = . 003). However, the plaque index reduction was statistically non-significant (p = . 297). Biochemical markers showed significant decreases over time, with alkaline phosphatase levels dropping from 74. 4370 to 48. 8195 and acid phosphatase from 10. 4013 to 6. 1615 (p = . 000). These findings suggest moderate clinical improvement and marked biochemical changes in Group B.

Table 3: Clinical parameters at baseline and 4 week and biochemical parameter at baseline, 15th day, 4th week following phase I periodontal therapy in Current Smoker with chronic periodontitis (Group C)

Parameter	At baseline (Mean ± SD)	At 15th Day (Mean ± SD)	At 4 weeks (Mean ± SD)	p-value
Gingival Index (GI)	1. 8635 ± 0. 37710	–	1. 6560 ± 0. 17316	. 000
Plaque Index (PI)	2. 5560 ± 0. 41570	–	2. 3795 ± 0. 31590	. 000
Pocket Probing Depth (PPD)	5. 6015 ± 0. 63407	–	5. 5780 ± 0. 28988	. 004
Clinical Attachment Level (CAL)	5. 2880 ± 0. 77919	–	5. 1940 ± 0. 53986	. 001
Alkaline Phosphatase (ALP)	80. 6225 ±4. 95746	79. 0780 ±1. 44396	80. 2435 ±9. 04815	. 000
Acid Phosphatase (ACP)	12. 1545 ±2. 14305	8. 3955 ± 2. 45594	8. 8463 ± 1. 86375	. 000

In Group C, minimal clinical improvements were observed. The gingival index slightly decreased from 1. 8635 to 1. 6560, and plaque index from 2. 5560 to 2. 3795, both with statistically significant p-values (. 000). Pocket probing depth and clinical attachment level showed negligible reductions (5. 6015 to 5. 5780 mm and 5. 2880 to 5. 1940 mm respectively), though statistically significant (p = . 004 and . 001). Biochemical changes were inconsistent: alkaline phosphatase showed minimal fluctuation (80. 6225 to 80. 2435), while acid phosphatase decreased from 12. 1545 to 8. 8463, both with p = . 000. Overall, Group C exhibited minor clinical changes and inconsistent biochemical alterations over the study period.

Group A showed the greatest clinical and biochemical improvement, with significant reductions across all parameters. Group B had moderate improvement, especially in clinical parameters, though plaque index change was non-significant. Group C showed minimal changes. Overall, Group A was the most effective, followed by Group B, with Group C least effective.

DISCUSSION: This study evaluated the influence of non-surgical periodontal therapy (NSPT) on clinical outcomes and salivary enzyme activity in non-smokers, former smokers, and current smokers with chronic periodontitis. NSPT improved plaque accumulation, gingival health, probing depth, and clinical attachment across all groups; however, outcomes varied with smoking status. Kinane et al. (1) and Johnson and Hill (2) observed that smokers are more susceptible to attachment loss and bone resorption, while Leite et al. (3) demonstrated a dose–response relationship between smoking intensity and periodontitis severity. Ryder (4) and Palmer et al. (10) reported that tobacco impairs host defences by reducing neutrophil activity, suppressing antibody production, and enhancing pro-inflammatory cytokine release, which may explain the inconsistent reduction of salivary alkaline phosphatase (ALP) and acid phosphatase (ACP) in smokers.

The significant decline of ALP and ACP in non-smokers, and to a lesser extent in former smokers, reflects their role as biomarkers of periodontal activity, consistent with Yamalik and Çağlayan (7), Perinetti et al. (8), and Sari et al. (9). Partial improvement in former smokers supports the findings of Calsina et al. (5), indicating that smoking cessation partially restores healing potential.

Limitations include the small sample size, short follow-up, and restricted biomarker panel. Larger, multicentre studies are needed to validate these findings. Clinically, structured smoking cessation programmes, biomarker-based monitoring, and customised maintenance should be incorporated into periodontal care.

CONCLUSION: The findings of this study indicate that non-surgical periodontal therapy (NSPT) leads to measurable improvements in both clinical outcomes and salivary enzyme activity in individuals with chronic periodontitis, though the extent of these benefits is influenced by smoking status. Participants who had never smoked achieved the most substantial reductions in gingival inflammation, probing depth, attachment loss, and salivary levels of alkaline phosphatase (ALP) and acid phosphatase (ACP), reflecting an optimal healing response. Former smokers showed moderate improvement, suggesting that some recovery of periodontal healing potential occurs after quitting tobacco. In contrast, current smokers experienced only slight changes in clinical and biochemical measures, highlighting the continued negative effect of smoking on periodontal repair processes. The results confirm the usefulness of salivary ALP and ACP as convenient, non-invasive biomarkers for tracking periodontal disease activity and treatment progress. They also reinforce the importance of integrating structured smoking cessation programs and customized maintenance schedules into periodontal care—particularly for individuals with a history of smoking—to maximize therapeutic success and support long-term oral health stability.

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CONFLICT OF INTEREST
The authors declare that they have no commercial or financial relationships that could be construed as a potential conflict of interest. Neither author has received salaries, equipment, supplies, travel reimbursement, consulting fees, shares, patents, or royalties from organizations that could be influenced by the publication of this research.

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Tables

Table 1. Clinical parameters at baseline and 4th week and biochemical parameters at baseline, 15th day, and 4th week following non-surgical periodontal therapy in non-smokers with chronic periodontitis[Group A]

Abbreviations: GI = Gingival Index; PI = Plaque Index; PPD = Probing Pocket Depth; CAL = Clinical Attachment Level; ALP = Alkaline Phosphatase; ACP = Acid Phosphatase; SD = Standard Deviation.

Table 2. Clinical parameters at baseline and 4th week and biochemical parameters at baseline, 15th day, and 4th week following non-surgical periodontal therapy in former smokers with chronic periodontitis[Group B]

Abbreviations: GI = Gingival Index; PI = Plaque Index; PPD = Probing Pocket Depth; CAL = Clinical Attachment Level; ALP = Alkaline Phosphatase; ACP = Acid Phosphatase; SD = Standard Deviation.

Table 3. Clinical parameters at baseline and 4th week and biochemical parameters at baseline, 15th day, and 4th week following non-surgical periodontal therapy in current smokers with chronic periodontitis[Group C]

Abbreviations: GI = Gingival Index; PI = Plaque Index; PPD = Probing Pocket Depth; CAL = Clinical Attachment Level; ALP = Alkaline Phosphatase; ACP = Acid Phosphatase; SD = Standard Deviation.

Figure Legends

Figure 1. Group A (non-smokers with chronic periodontitis): pre-operative and post-operative clinical photographs showing scaling and root planing.

Figure 2. Group B (former smokers with chronic periodontitis): pre-operative and post-operative clinical photographs showing scaling and root planing.

Figure 3. Group C (current smokers with chronic periodontitis): pre-operative and post-operative clinical photographs showing scaling and root planing.

Figure 4. Biochemical analysis procedure: 2–2. 5 mL of unstimulated saliva collected, centrifuged at 3,000 rpm for 10 minutes

Figure 5. For alkaline phosphatase estimation, 20 µL of supernatant was mixed with 1,000 µL of ERBA Mannheim Alkaline Phosphatase reagent (Mannheim, Germany). For acid phosphatase estimation, 20 µL of supernatant was mixed with 1,000 µL of GPL Acid Phosphatase reagent.

Figure 6. Enzyme activity determination using an ERBA semi-autoanalyser

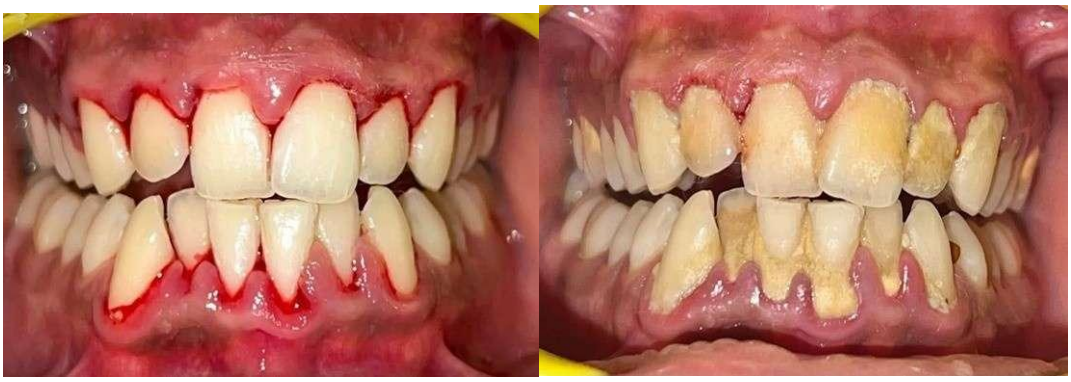

Figure 1. Group A (non-smokers with chronic periodontitis): pre-operative and post-operative clinical photographs showing scaling and root planing.

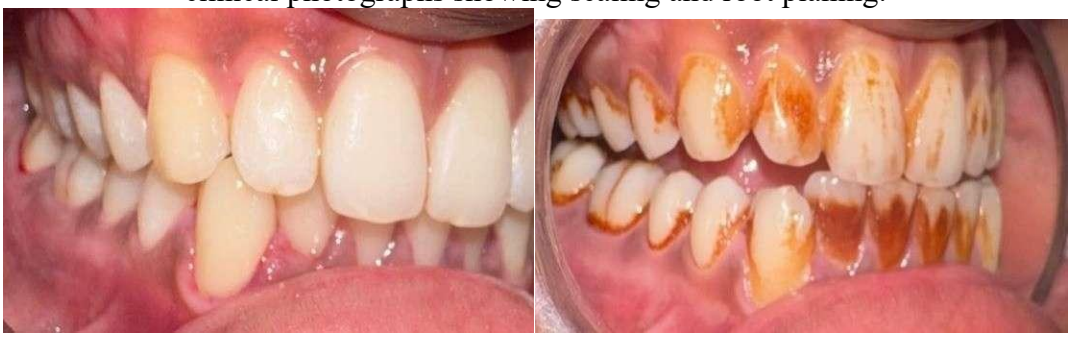

Figure 2. Group B (former smokers with chronic periodontitis): pre-operative and post-operative clinical photographs showing scaling and root planing.

Figure 3. Group C (current smokers with chronic periodontitis): pre-operative and post-operative clinical photographs showing scaling and root planing.

Figure 4. Biochemical analysis procedure: 2–2. 5 mL of unstimulated saliva collected, centrifuged at 3,000 rpm for 10 minutes

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