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Non-invasive biomarker-based evaluation of periapical bone healing using salivary and urinary deoxypyridinoline: A prospective cohort study

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Abstract:

Radiographic assessment of periapical healing after non-surgical root canal treatment (NSRCT) may not reflect early biological changes and requires repeated radiation exposure, highlighting the need for reliable non-invasive biomarkers. Therefore, it is of interest to evaluate the salivary and urinary deoxypyridinoline (DPD) levels in 35 patients with periapical lesions and 40 healthy controls, correlating biomarker changes with cone-beam computed tomography (CBCT)-based healing over 3 months. Salivary and urinary DPD concentrations were measured by ELISA at baseline, 1 month and 3 months, while lesion volume was assessed volumetrically using CBCT. Both salivary and urinary DPD levels declined significantly over time ($p < 0.001$), showed a moderate positive correlation ($r = 0.52$, $p = 0.001$) and 97.1% of patients showed radiographic healing, although DPD changes were not significantly associated with lesion volume reduction ($p = 0.20$, $p = 0.242$). Thus, data shows that salivary DPD may serve as a non-invasive adjunctive biomarker for monitoring periapical bone healing following NSRCT.

Keywords: Bone biomarker, cone-beam computed tomography (CBCT), deoxypyridinoline, periapical healing, root canal treatment, saliva

Background:

Periapical lesions are inflammatory conditions arising from pulpal infection that result in localised destruction of periapical bone and represent a common therapeutic challenge in endodontics. Root canal treatment aims to eliminate intracanal infection and facilitate regeneration of periapical tissues, thereby promoting bone healing [1, 2]. Assessment of healing has traditionally relied on radiographic methods, including periapical radiographs and cone-beam computed tomography (CBCT), which remain the current standard for evaluating periapical changes [3]. However, radiographic findings often lag behind the biological events occurring during tissue repair and repeated imaging raises concerns regarding cumulative radiation exposure and patient safety [4, 5]. Bone remodelling is a dynamic process involving coordinated osteoclastic resorption and osteoblastic formation [6]. Deoxypyridinoline (DPD), a mature collagen cross-link released during degradation of type I collagen, is a well-established biomarker of bone resorption and has been extensively used in the assessment of systemic bone disorders [7, 8]. Its application as a marker of localised periapical bone remodelling, however, remains insufficiently explored. Saliva has emerged as a promising diagnostic medium because of its non-invasive, cost-effective and patient-friendly nature, making it particularly suited to repeated longitudinal assessment [9]. Although urinary DPD has showed utility in evaluating systemic bone metabolism [10], evidence regarding salivary DPD as a biomarker of periapical healing remains limited. Therefore, it is of interest to evaluate the temporal changes in salivary and urinary deoxypyridinoline (DPD) levels following non-surgical root canal treatment and their correlation with cone-beam computed tomography (CBCT)-based periapical healing.

Materials and Methods:

This prospective cohort study was conducted at the Department of Dentistry, All India Institute of Medical Sciences, Kalyani, India. The study enrolled two parallel groups: an intervention

cohort of patients with radiographically confirmed periapical lesions undergoing non-surgical root canal treatment (NSRCT) and a concurrent healthy control group. Participants were recruited consecutively from the outpatient clinic and followed longitudinally over a 3-month period, with biomarker assessments at baseline, 1 month and 3 months post-treatment. The healthy controls provided cross-sectional baseline reference values only. Ethical approval was obtained from the Institutional Ethics Committee of AIIMS Kalyani (IEC/AIIMS/Kalyani/certificate/2025/011) and written informed consent was obtained from all participants. The manuscript was prepared in accordance with the STROBE guidelines for reporting observational cohort studies. The sample size was determined a priori as 80 participants (40 per group) to provide 80% statistical power to detect significant changes in DPD levels at a two-tailed alpha of 0.05, with a 15–20% anticipated dropout rate. Inclusion criteria for the intervention group comprised age 18–50 years, a single radiographically confirmed periapical lesion and willingness to comply with follow-up. Healthy controls were recruited from accompanying persons and hospital staff with no clinical or radiographic evidence of pulpal, periapical or periodontal disease. Exclusion criteria for both groups included systemic disorders affecting bone metabolism, pregnancy or lactation, active periodontal disease, current or recent tobacco or alcohol use and use of medications known to influence bone turnover (bisphosphonates, corticosteroids, hormone replacement therapy). Of the 40 patients enrolled in the intervention group, 35 completed all follow-up assessments and were included in the final analysis; all 40 controls completed baseline assessment. Periapical lesions were diagnosed on the basis of clinical signs and symptoms consistent with pulpal necrosis or irreversible pulpitis and radiographic evidence of periapical pathology confirmed on CBCT, defined as a periapical radiolucency with loss of lamina dura and widening of the periodontal ligament space of ≥ 1 mm in at least two orthogonal planes. Lesion volume was calculated using the ellipsoid formula $V = (\pi/6) \times L$

× W × H, where L, W and H represent the maximum lesion dimensions in the axial, coronal and sagittal planes respectively, measured by two blinded, calibrated evaluators with discrepancies resolved by consensus. Unstimulated whole saliva (5 mL) and midstream urine samples were collected at baseline before treatment, with repeat collections at 1 and 3 months post-treatment in the intervention group. Samples were centrifuged and stored at -80 °C until analysis. Salivary and urinary DPD concentrations were quantified using a commercially available enzyme-linked immunosorbent assay kit (Human Deoxyypyridinoline ELISA Kit; Chongqing Biospes Co., Ltd., China) according to the manufacturer's instructions, with optical density measured at 405 nm and concentrations expressed as nmol/L. Raw nmol/L values were retained rather than creatinine-normalised values to allow direct comparison between salivary and urinary DPD on the same immunoassay platform. Sample collection was standardised by time of day to minimise diurnal variation and samples were processed in the same analytical batch where possible to reduce inter-assay variability. Root canal therapy was performed by experienced endodontists using standardised clinical protocols, with treatment considered complete on placement of the coronal restoration. CBCT imaging was performed pre-operatively and at 3-month follow-up using standardised parameters following ALARA principles. Radiographic healing was assessed by quantitative volumetric measurement of lesion dimensions in three planes by blinded evaluators. Statistical analysis was performed using STATA version 19 (StataCorp LLC, College Station, TX, USA). Normality was assessed using the Shapiro-Wilk test. Continuous variables were expressed as mean ± standard deviation and categorical variables as frequencies and percentages. Baseline differences between groups were assessed using the independent samples t-test or Mann-Whitney U test as appropriate. Temporal changes in DPD across the three time points were evaluated using repeated-measures analysis of variance with Bonferroni-adjusted post-hoc comparisons. The association between salivary and urinary DPD was assessed

using Pearson's or Spearman's correlation coefficient and the relationship between DPD change and CBCT lesion volume reduction was assessed using Spearman's correlation. Linear regression was performed to evaluate the predictive value of salivary DPD for radiographic healing. A two-tailed p-value of < 0.05 was considered statistically significant.

Results:

A total of 80 participants were enrolled between February 2025 and August 2025 (40 patients with radiographically confirmed periapical lesions and 40 healthy controls). Thirty-five patients in the intervention group completed all follow-up assessments and were included in the final analysis (Table 1). Baseline salivary and urinary DPD levels were significantly lower in the periapical lesion group than in healthy controls (Table 2). Mean baseline salivary DPD was 89.62 ± 16.69 nmol/L in the intervention group versus 106.37 ± 10.42 nmol/L in controls (p = 0.023) and baseline urinary DPD was 79.34 ± 15.96 versus 115.30 ± 3.90 nmol/L (p < 0.001). Repeated-measures analysis showed significant progressive reductions in both salivary DPD (F = 242.29, p < 0.001) and urinary DPD (F = 229.23, p < 0.001) across the three time points (Table 3). Bonferroni-adjusted pairwise comparisons confirmed significant decreases between all consecutive time points for both biomarkers (Table 4). A significant moderate positive correlation was observed between salivary and urinary DPD concentrations (r = 0.52, p = 0.001), indicating concordance between the two biomarkers. No significant correlation was observed between changes in salivary DPD and CBCT-assessed lesion volume reduction (ρ = 0.20, p = 0.242) and linear regression did not demonstrate a significant predictive relationship (R² = 0.078, p = 0.104) (Table 5). Radiographic healing was achieved in 34 of 35 patients (97.1%), with mean periapical lesion volume decreasing from 55.10 ± 67.54 mm³ at baseline to 6.37 ± 9.07 mm³ at 3 months (Table 6). Receiver operating characteristic analysis could not be meaningfully performed owing to the near-absence of variation in the healing outcome.

Table 1: Descriptive statistics of the study cohort (n = 35 intervention; n = 40 controls)

Variable	Intervention group (Mean ± SD)	Control group (Mean ± SD)
Baseline salivary DPD (nmol/L)	89.62 ± 16.69	106.37 ± 10.42
Salivary DPD at 1 month (nmol/L)	47.69 ± 15.58	N/A
Salivary DPD at 3 months (nmol/L)	29.64 ± 13.25	N/A
Baseline urinary DPD (nmol/L)	79.34 ± 15.96	115.30 ± 3.90
Urinary DPD at 1 month (nmol/L)	47.41 ± 9.67	N/A
Urinary DPD at 3 months (nmol/L)	27.48 ± 9.52	N/A
Baseline lesion volume (mm³)	55.10 ± 67.54	N/A
Lesion volume at 3 months (mm³)	6.37 ± 9.07	N/A

Table 2: Baseline biomarker comparison between study groups

Variable	Periapical lesion group (n = 35) Mean ± SD	Healthy controls (n = 40) Mean ± SD	p-value
Salivary DPD (baseline) (nmol/L)	89.62 ± 16.69	106.37 ± 10.42	0.023*
Urinary DPD (baseline) (nmol/L)	79.34 ± 15.96	115.30 ± 3.90	<0.001*
Baseline lesion volume (mm³)	55.10 ± 67.54	-	-

*Statistically significant (p < 0.05); independent samples t-test

Table 3: Longitudinal changes in salivary and urinary DPD following root canal treatment (n = 35)

Time point	Salivary DPD (nmol/L) Mean ± SD	Urinary DPD (nmol/L) Mean ± SD
Baseline	89.62 ± 16.69	79.34 ± 15.96
1 month	47.69 ± 15.58	47.41 ± 9.67

3 months	29.64 ± 13.25	27.48 ± 9.52
Repeated-measures ANOVA (F)	242.29	229.23
p-value	<0.001*	<0.001*

*Statistically significant (p < 0.05); repeated-measures ANOVA

Table 4: Pairwise comparison of biomarker changes over time (Bonferroni-adjusted)

Comparison	Mean difference (nmol/L)	95% CI	p-value
Salivary DPD			
Baseline vs 1 month	41.93	33.06–50.80	<0.001*
Baseline vs 3 months	59.98	51.11–68.85	<0.001*
1 month vs 3 months	18.05	9.18–26.92	<0.001*
Urinary DPD			
Baseline vs 1 month	31.93	24.89–38.97	<0.001*
Baseline vs 3 months	51.86	44.82–58.89	<0.001*
1 month vs 3 months	19.93	12.89–26.96	<0.001*

*Statistically significant (p < 0.05)

Table 5: Correlation and regression analyses

Analysis	Coefficient	p-value
Salivary DPD vs urinary DPD (Pearson)	r = 0.52	0.001*
Salivary DPD vs CBCT lesion healing (Spearman)	ρ = 0.20	0.242 (NS)
Linear regression (salivary DPD predicting lesion healing)	R ² = 0.078	0.104 (NS)

*Statistically significant (p < 0.05); NS: Not Significant

Table 6: CBCT-assessed lesion volume changes and healing rate following treatment

Variable	Mean ± SD / n (%)
Baseline lesion volume (mm ³)	55.10 ± 67.54
3-month lesion volume (mm ³)	6.37 ± 9.07
Radiographic healing rate	34/35 (97.1%)

Discussion:

The principal finding of this prospective cohort study was a statistically significant and progressive reduction in both salivary and urinary DPD concentrations following NSRCT, with levels declining substantially from baseline through 1 and 3 months post-treatment. Salivary DPD decreased from 89.62 ± 16.69 nmol/L at baseline to 29.64 ± 13.25 nmol/L at 3 months, representing a cumulative reduction of approximately 67% and urinary DPD showed a parallel decline of comparable magnitude. A moderate positive correlation was observed between salivary and urinary DPD at baseline (r = 0.52, p = 0.001) and radiographic healing was confirmed in 34 of 35 participants (97.1%) at 3 months. No statistically significant association was identified, however, between changes in salivary DPD and CBCT-assessed periapical lesion volume reduction. DPD is a mature collagen cross-link formed during the post-translational modification of type I collagen and is released into biological fluids during osteoclastic degradation of mineralised bone matrix, providing a specific index of bone resorptive activity [11]. The progressive reduction in DPD concentrations observed across all time points following NSRCT is consistent with progressive attenuation of osteoclast-mediated bone resorption at the periapex, plausibly explained by elimination of the microbial stimulus that sustains periapical inflammation. The pathogenesis of periapical bone loss is mediated through microbially driven upregulation of the receptor activator of nuclear factor-κB ligand (RANKL)/osteoprotegerin axis, which promotes osteoclastogenesis and uncoupled bone resorption

[12]. Effective root canal debridement and obturation reduce the intracanal microbial load, thereby diminishing the inflammatory cytokine milieu responsible for sustained RANKL expression. The sustained reduction in DPD concentrations confirmed by repeated-measures ANOVA in the present study (F = 242.29; p < 0.001 for salivary DPD) is consistent with this biological sequence. A secondary objective was to evaluate the potential of salivary DPD as a non-invasive surrogate for urinary DPD. The moderate positive correlation identified between the two biomarkers, together with their parallel longitudinal trajectories, provides preliminary evidence that salivary DPD reflects systemic bone resorption activity in patients with periapical disease. The biological basis for salivary DPD involves both transudation from the systemic circulation via the salivary glands and a local contribution from periodontal and periapical tissue turnover [13]. Gudac *et al.* identified saliva as a diagnostically relevant medium for assessing oral disease activity given its accessibility and suitability for repeated longitudinal sampling [14], while Flórez-Moreno *et al.* showed that salivary DPD concentrations increased significantly during orthodontic tooth movement, mirroring periods of mechanically induced bone resorption [15]. The present findings extend this evidence to the endodontic context, where the inverse relationship - a progressive decline in salivary DPD following removal of the infectious stimulus - provides complementary support for the responsiveness of this biomarker to changes in periapical bone activity. Compared with urinary collection, saliva offers practical advantages including non-invasive chair-side collection, patient acceptability and suitability for repeated sampling during routine endodontic review. An unexpected observation was that baseline salivary and urinary DPD concentrations were significantly lower in the periapical lesion group than in healthy controls. Periapical lesions are anatomically circumscribed inflammatory processes involving a relatively small volume of alveolar bone and the net contribution of localised periapical osteoclastic activity to the overall systemic DPD pool may be small relative to physiological skeletal remodelling in healthy individuals [16]. Chronically established periapical lesions may also exhibit a dampened osteoclastic drive compared with actively progressing acute lesions, with a lower local RANKL/OPG ratio that does not translate to measurable elevation of systemic resorption markers [17]. Although participants with known systemic bone disorders were excluded, subclinical differences in bone metabolism between groups cannot be excluded in the absence of bone mineral density assessment or a comprehensive panel of systemic bone turnover markers. This finding warrants prospective investigation in larger cohorts. The absence of a statistically significant correlation between changes in salivary DPD and CBCT-assessed lesion volume reduction at 3 months should not be construed as evidence against the utility of salivary DPD as a monitoring tool. Biochemical markers and radiographic imaging

measure fundamentally different aspects of healing: DPD reflects the cellular event of osteoclastic collagen degradation in real time, whereas CBCT volumetric assessment captures macroscopic changes in bone architecture, which are the cumulative result of both resorption and subsequent osteoblastic formation and mineralisation [18]. Meaningful radiographic evidence of periapical bone regeneration may require 6–12 months or longer; a 3-month follow-up, although sufficient to capture the early molecular response, may be insufficient to observe strong biomarker–radiographic concordance. The near-universal radiographic healing rate of 97.1% additionally restricted the variance available for correlation analysis and precluded receiver operating characteristic analysis. The study has several limitations. The sample size of 35 participants in the intervention arm limited statistical power for regression analyses and precluded multivariable adjustment for potential confounders, including age, sex and tooth type. The 3-month follow-up period is shorter than the minimum 1-year follow-up recommended by current European Society of Endodontology quality guidelines for definitive assessment of periapical healing. The single-centre tertiary academic setting and the restrictive eligibility criteria limit generalisability to community endodontic practice and to patients with systemic comorbidities, tobacco use or multiple or retreatment lesions. Only DPD was evaluated as a bone marker; a broader panel including markers of bone formation such as bone-specific alkaline phosphatase and osteocalcin would provide a more complete assessment of the bone remodelling cycle.

Conclusion:

Non-surgical root canal treatment resulted in a significant progressive reduction in salivary and urinary DPD levels over 3 months, with substantial radiographic healing on CBCT. Salivary and urinary DPD showed moderate concordance, supporting salivary DPD as a potential non-invasive adjunctive biomarker for monitoring periapical healing. The lack of short-term correlation between DPD changes and volumetric CBCT healing highlights the need for larger, longer-term studies to validate its clinical utility.

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Conflict of interest:

The authors declare no conflict of interest.

Data availability:

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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