Original Article

# Baseline serum 25-hydroxyvitamin D levels as a predictor of periapical healing and clinical success after primary root canal treatment: A prospective, observational, and cohort study

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## Abstract

**Background:** Systemic host factors, such as serum 25-hydroxyvitamin D(25[OH]D), may influence periapical healing after root canal treatment (RCT); however, prospective clinical evidence remains limited.

**Aim:** To evaluate whether baseline serum 25(OH)D levels predict periapical healing and 12-month clinical success following a primary RCT.

**Materials and Methods:** Ninety systemically healthy adults (18–50 years old) requiring primary RCT of single-rooted teeth were enrolled. Participants were categorized as Vitamin D–deficient (<20 ng/mL) or sufficient (≥20 ng/mL). The lesion size, periapical index (PAI), pain (Visual Analog Scale), and clinical success were assessed at 6 and 12 months.

**Statistical Analysis:** This comprised repeated-measures tests, independent group comparisons, receiver operating characteristic (ROC) analysis, and multivariable logistic regression adjusted for baseline lesion size, with $P < 0.05$ indicating significance.

**Results:** Both groups demonstrated significant improvement over time ($P < 0.05$). At 12 months, the sufficient group demonstrated greater lesion reduction, lower PAI and pain scores, and higher clinical success (93.3% vs. 60.0%; $P < 0.001$). Vitamin D sufficiency independently predicted success (adjusted odds ratio, 6.62; $P = 0.009$). ROC analysis identified 20.4 ng/mL as the optimal predictive cutoff.

**Conclusion:** Baseline serum 25(OH)D sufficiency was associated with improved periapical healing and clinical success after primary RCT.

**Keywords:** Endodontic; periapical healing; predictive; root canal treatment; serum 25-hydroxyvitamin D

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## INTRODUCTION

Apical periodontitis (AP) is an inflammatory disease of the periradicular tissues caused primarily by microbial infection



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of the root canal system, with a global prevalence of 52% at the individual level and 5% at the tooth level.[1,2] Although modern endodontic procedures have improved success rates, approximately 12% of patients experience persistent postoperative symptoms or delayed periapical healing despite technically adequate root canal treatment (RCT). This suggests that host-related systemic and immunological factors may influence healing responses beyond the local microbial control.[3]

Vitamin D is a pleiotropic secosteroid hormone involved in immune modulation, cytokine regulation, antimicrobial peptide expression, and bone remodeling.[4] Vitamin D deficiency is common, especially among South Asian communities, and has been implicated in chronic inflammation, delayed wound healing, musculoskeletal pain, and disrupted bone metabolism.[5] In the oral cavity, low serum Vitamin D levels have been linked to the progression of periodontal disease, alveolar bone loss, and delayed osseous healing.[6,7]

However, there is limited evidence regarding its role in postendodontic healing. A randomized controlled trial by Maraş *et al*. reported a faster periapical lesion reduction in patients whose serum Vitamin D levels were maintained within the optimal range.[8] However, prospective clinical studies evaluating the baseline serum 25-hydroxyvitamin D(25[OH]D) status as a predictor of post-RCT healing remain scarce.

Therefore, this prospective, observational, and cohort study aimed to assess the association between baseline serum 25(OH)D levels and periapical healing following a primary RCT. Postoperative pain, radiographic lesion size, periapical index (PAI) scores at 6 and 12 months, and 12-month clinical success were evaluated. The null hypothesis stated that baseline serum 25(OH)D levels were not associated with healing outcomes after the primary RCT.

## MATERIALS AND METHODS

This prospective, observational, and cohort study was conducted at the department of conservative dentistry and endodontics. This study adhered to the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.[9] Ethical clearance was obtained from the Institutional Ethics Committee, and all procedures were performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all the participants before enrolment. As this investigation was an observational cohort study without any therapeutic intervention or randomization, prospective clinical trial registration was not required.

Patients aged 18–50 years who required primary RCT of a single-rooted permanent tooth (incisors, canines, or premolars) were screened. The inclusion criteria were systemically healthy individuals diagnosed with symptomatic AP or symptomatic irreversible pulpitis, with radiographic evidence of periapical involvement.[10] Teeth were required to have adequate periodontal support (probing depth ≤4 mm; mobility ≤Grade I) and sufficient coronal structure for final restoration.

Symptomatic irreversible pulpitis was diagnosed based on a history of spontaneous or lingering pain to cold stimulation, exaggerated response to pulp sensibility testing, and absence of periapical radiolucency. Symptomatic AP was diagnosed when teeth exhibited tenderness to percussion or biting, with radiographic evidence of periapical radiolucency and/or widening of the periodontal ligament space according to the American Association of Endodontists and European Society of Endodontology guidelines.[11,12] Cold pulp sensibility testing was performed using Endo-Ice (Coltene Whaledent, Switzerland).[13] Two calibrated endodontists independently confirmed the diagnoses and disagreements were resolved by the consensus.

Exclusion criteria included previous RCT or retreatment, root fracture, resorption, advanced periodontal disease, pregnancy or lactation, systemic diseases affecting bone metabolism or pain perception (such as uncontrolled diabetes mellitus and chronic kidney disease), long-term corticosteroid therapy, Vitamin D or calcium supplementation, medications altering Vitamin D metabolism, and inability to complete follow-up. The sample size estimation was performed using Heinrich Heine University Düsseldorf, Germany. Assuming clinical success rates of 60% in the Vitamin D–deficient group and 85% in the sufficient group, with $\alpha = 0.05$ and power = 80%, a minimum of 80 patients was required.

Consecutive recruitment was performed by screening all patients who presented to the outpatient endodontic clinic during the study period and required primary RCT. Every patient meeting the eligibility criteria was invited to participate until the required sample size was achieved, thereby minimizing selection bias, and their serum 25(OH)D levels were assessed at baseline. Enrolment continued until the required sample size for both Vitamin D-deficient and Vitamin D-sufficient groups was achieved. Baseline demographic and clinical variables, including age, sex, body mass index, smoking status, sun exposure, and dietary patterns, were recorded. Preoperative pain intensity was assessed using a Visual Analog Scale (VAS) with a preoperative pain score of ≥5.[14] Clinical examination included the evaluation of percussion tenderness, palpation tenderness, swelling, and sinus tract.

Radiographs were standardized using the long-cone paralleling technique with a positioning device (Rinn XCP

holder) to ensure reproducible angulation. The exposure parameters were kept constant for all radiographs, and the same digital radiographic unit was used throughout the study. Follow-up radiographs were obtained using the same positioning device and angulation as the baseline images. Periapical status was assessed using the PAI, a five-point ordinal scale that reflects the increasing severity of AP.[15] Those with a preoperative PAI score of >3 were included.[16] Two blinded calibrated examiners independently evaluated the radiographs, and consensus scoring was applied when necessary.

Prior to the initiation of endodontic therapy, 3–5 mL of venous blood was collected under the aseptic conditions. Serum was separated by centrifugation and analyzed for 25(OH)D levels using a chemiluminescent immunoassay analyzer (ARCHITECT i2000SR, Abbott Diagnostics, USA). The calibration and quality control procedures were performed according to the manufacturer's recommendations. Using well-documented criteria, the study participants were divided into Vitamin D-deficient (<20 ng/mL) and Vitamin D-sufficient (≥20 ng/mL) groups.[17,18] The doctor performing the procedure did not know the patient's Vitamin D serum status during the surgery. Serum 25(OH)D levels were assessed at baseline and at 6 and 12 months to monitor the stability of the Vitamin D status during the follow-up period.

All RCTs were performed in a single visit by an experienced endodontist using a standardized protocol. Local anesthesia was administered using 2% lidocaine with 1:80,000 adrenaline. Rubber dam isolation was achieved and the operative field was disinfected with 2% chlorhexidine. Working length was determined using an electronic apex locator (Root ZX II; J. Morita Corp., Japan) and confirmed radiographically. Cleaning and shaping were performed using a rotary nickel–titanium system (ProTaper Gold; Dentsply Sirona, Switzerland). The irrigation protocol was standardized for all the procedures. Each canal received approximately 10–15 mL of 3% sodium hypochlorite during instrumentation, followed by a final irrigation sequence consisting of 5 mL of 17% ethylenediaminetetraacetic acid, 5 mL sodium hypochlorite, and 5 mL sterile saline.

Obturation was completed using gutta-percha cones and an epoxy resin-based sealer (AH Plus; Dentsply Sirona, Germany). Postobturation radiographs verified obturation quality. Access cavities were restored using a nanohybrid composite resin (Filtek Z350 × T; 3M ESPE, USA) with an etch-and-rinse bonding agent (Adper Single Bond 2; 3M ESPE, USA).

The patients were reviewed at 6 and 12 months. Pain intensity during the follow-up represented postoperative periapical pain associated with the treated tooth and was measured using a 10-cm VAS, where 0 indicated no pain and 10 indicated worst imaginable pain. Clinical signs (tenderness, swelling, and sinus tract swelling) were evaluated. Standardized periapical radiographs were obtained to measure the lesion size and reassess the PAI score. The primary outcome was 12-month clinical success, defined as the absence of clinical signs and symptoms combined with radiographic healing (PAI ≤2 and/or a substantial reduction in lesion size). The secondary outcomes included the changes in pain intensity, lesion size, and PAI scores over time and between-group comparisons.

### Statistical analysis

Data were analyzed using the SPSS software version 26.0 (IBM Corp., Armonk, NY, USA). Normality was assessed using the Shapiro–Wilk test. The continuous variables are expressed as mean ± standard deviation or median (interquartile range); categorical variables are expressed as frequencies and percentages. Within-group comparisons were performed using the repeated-measures analysis of variance or Friedman test. Between-group comparisons were performed using the independent *t*-test, Mann–Whitney *U*-test, or Chi-square or Fisher's exact test as appropriate, with Bonferroni correction for multiple comparisons (adjusted $\alpha = 0.0125$). Receiver operating characteristic (ROC) curve analysis was used to determine the optimal 25(OH)D cutoff using the Youden index. Multivariable logistic regression identified the independent predictors of 12-month clinical success after adjusting for baseline lesion size. Statistical significance was set at $P < 0.05$.

## RESULTS

Ninety patients (45 Vitamin D-deficient and 45 Vitamin D-sufficient) completed the 12-month follow-up and were included in the final analysis. The serum 25(OH) D levels remained stable within the groups throughout the follow-up period, with no significant intragroup changes ($P > 0.05$). Baseline demographic and lifestyle variables, including body mass index, smoking status, and sun exposure duration, were comparable between the groups [Supplementary Table 1]. No significant differences were observed in terms of age, sex distribution, diagnosis, initial lesion size, baseline PAI score, or preoperative pain intensity. As expected, baseline serum 25(OH) D levels differed significantly between the deficient (16.2 ± 1.3 ng/mL) and sufficient (35.8 ± 4.9 ng/mL) groups ($P < 0.001$).

Within-group analysis demonstrated significant improvements in lesion size, PAI score, and pain intensity over 12 months in both the groups [Supplementary Table 2]. In the sufficient group, lesion size showed a pronounced and sustained reduction ($P < 0.001$), accompanied by significant decreases in the PAI and pain scores (both $P < 0.001$). The deficient group also exhibited statistically significant improvements; however, the magnitude of the

change was comparatively smaller (lesion size, $P = 0.006$; PAI and pain, $P < 0.001$).

Between-group comparisons revealed no significant differences at 6 months after Bonferroni correction [Table 1]. However, at 12 months, the sufficient group demonstrated significantly superior outcomes. The residual lesion size was smaller ($P < 0.001$), the reduction in lesion size was greater ($P = 0.016$), and the PAI scores were significantly lower ($P = 0.024$). The pain scores were also lower in the sufficient group ($P = 0.048$), although the difference was borderline after correction. Notably, the 12-month clinical success rate was significantly higher in the sufficient group (93.3%) than in the deficient group (60.0%) ($P < 0.001$).

ROC analysis showed good discriminatory ability of the baseline serum 25(OH) D level for predicting 12-month clinical success (area under the curve = 0.845; 95% confidence interval [CI] 0.71–0.93; $P < 0.001$) [Figure 1]. The optimal cutoff value was 20.4 ng/mL, closely approximating the conventional deficiency threshold. Patients with levels ≥20.4 ng/mL had a relative risk of success of 2.44 (95% CI 1.46–4.07) and an odds ratio (OR) of 15.3 (95% CI 4.1–56.7), with a sensitivity of 78.3% and specificity of 81.0% [Supplementary Table 3].

Multivariable logistic regression confirmed that Vitamin D sufficiency independently predicted 12-month clinical success (adjusted OR = 6.62; 95% CI 1.62–27.10; $P = 0.009$) [Table 2]. Baseline lesion size was not an independent predictor ($P = 0.281$). The model demonstrated a good fit (Nagelkerke $R^2 = 0.38$; Hosmer–Lemeshow $P = 0.64$). Accordingly, the null hypothesis is rejected.

## DISCUSSION

The present study demonstrates that baseline serum 25(OH)D levels significantly influence periapical healing following a primary RCT. The main measure of effectiveness was clinical success at 12 months, which was determined as the lack of clinical signs and symptoms and radiographic healing. Importantly, this association remained independent of baseline lesion size, identifying Vitamin D as a modifiable host-related factor that may affect endodontic outcomes beyond local variables, such as microbial control and obturation quality.

The biologically reasonable basis for this association is that Vitamin D has several functions. It boosts innate immunity by producing antimicrobial peptides, such as cathelicidin, modulates the activity of macrophages, and regulates cytokine balance, thereby leading to controlled and resolved inflammation.[19] Furthermore, Vitamin D plays a role in the differentiation of osteoblasts and formation of mineralized tissues, which are crucial for the healing of periapical bone.[20] The more pronounced intergroup differences observed at 12 months were consistent with its role in chronic tissue repair and bone remodeling rather than acute inflammatory modulation.[21] Similar findings have been reported in periodontal research, where deficiency impairs alveolar bone healing and prolongs inflammatory resolution.[22,23] Retrospective data also linked low serum 25(OH)D levels with a higher prevalence of extensive chronic periapical lesions.[24]

While previous randomized trials have shown enhanced lesion reduction with Vitamin D supplementation, this prospective study established the baseline serum 25(OH)D status as a predictive biomarker.[8] This is

**Table 1: Between-group comparison of outcomes at 6 and 12 months**

| Outcome | Time (months) | Vitamin D-deficient ($n$=45) | Vitamin D-sufficient ($n$=45) | Test statistic | $P$ | Adjusted $P$ |
|---|---|---|---|---|---|---|
| Lesion size (mm)[a] | 6 | 2.23±0.62 | 1.73±0.55 | 2.35 | 0.026 | 0.084 |
| | 12 | 1.72±0.62 | 0.94±0.47 | 3.87 | 0.001 | <0.001* |
| Reduction in lesion size (mm)[a] | 6 | 0.04±0.49 | 0.36±0.37 | 2.02 | 0.053 | 0.244 |
| | 12 | 0.55±0.53 | 1.15±0.51 | 3.17 | 0.004 | 0.016* |
| PAI scorec | 6 | 2 (1–4) | 1 (1–2) | 70 | 0.047 | 0.156 |
| | 12 | 1 (0–4) | 0 (0–1) | 51 | 0.006 | 0.024* |
| Pain score (VAS)[c] | 6 | 4 (2–5) | 2 (1–3) | 52 | 0.008 | 0.032* |
| | 12 | 2 (0–2) | 0 (0–1) | 55 | 0.012 | 0.048* |
| Clinical success (%)[b] | 6 | 24/45 (53.3) | 36/45 (80.0) | 2.40 | 0.121 | 0.484 |
| | 12 | 27/45 (60.0) | 42/45 (93.3) | 14.6 | 0.001 | <0.001* |

*Statistically significant after adjustment, [a]Independent samples *t*-test, [b]Chi-square test, [c]Mann–Whitney *U*-test. Bonferroni correction was applied for multiple secondary comparisons. Data are presented as mean±SD or median (IQR). SD: Standard deviation, IQR: Interquartile range, PAI: Periapical index, VAS: Visual Analog Scale

**Table 2: Multivariable logistic regression analysis for 12-months clinical success**

| Predictor | $B$ | SE | Wald $\chi^2$ | $P$ | Adjusted OR | 95% CI for OR |
|---|---|---|---|---|---|---|
| Vitamin D sufficiency (≥20 ng/mL) | 1.89 | 0.72 | 6.90 | 0.009* | 6.62 | 1.62–27.10 |
| Baseline lesion size (mm) | −0.41 | 0.38 | 1.16 | 0.281 | 0.66 | 0.31–1.39 |
| Constant | −2.73 | 1.01 | 7.31 | 0.007 | - | - |

*Statistically significant at $P$<0.05. Hosmer–Lemeshow test $P$=0.64, overall model accuracy=81.1%. Nagelkerke $R^2$=0.38. SE: Standard error, OR: Odds ratio, CI: Confidence interval

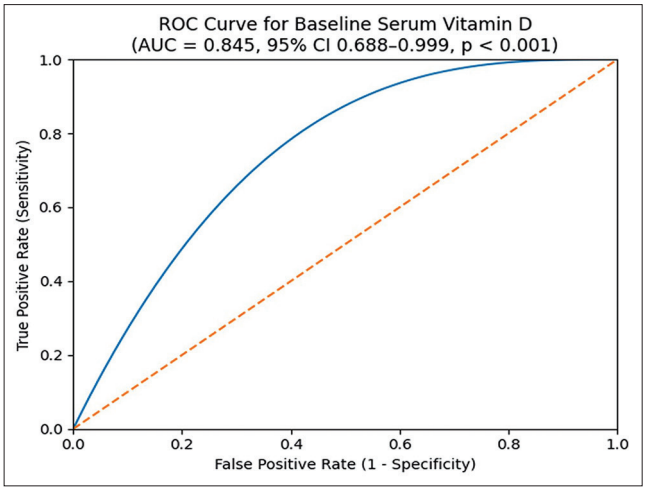


**Figure 1:** Receiver operating characteristic curve for baseline serum 25-hydroxyvitamin D levels in predicting 12-month clinical success. ROC: Receiver operating characteristic

particularly relevant in the regions with a high deficiency prevalence, such as South Asia.[5] The cutoff value derived, which is very close to the typical deficiency threshold, makes it more clinically relevant. Hence, these results concur with the idea that successful endodontic therapy is dependent not only on technical skills but also on the systemic health of the patient. Persistent apical pathology may reflect an impaired reparative environment, and Vitamin D deficiency may contribute to incomplete healing in a subset of patients.

Clinically, preoperative assessment of serum 25(OH)D levels may help identify patients at higher risk of delayed healing. Targeted Vitamin D supplementation in patients identified with deficiency prior to treatment may represent a simple adjunctive strategy to improve host immune response and periapical healing outcomes. The study's caveats consist of its observational nature, the possibility of residual confounding, the fact that it was carried out at a single center, and the fact that it only dealt with single-rooted teeth. Future multicenter randomized trials are warranted to determine whether correction of Vitamin D deficiency before RCT enhances the long-term healing outcomes.

## CONCLUSION

This prospective cohort study indicated that baseline serum 25(OH)D sufficiency independently predicted improved periapical healing and clinical success after primary RCT in single-rooted teeth. Adequate Vitamin D levels are associated with superior radiographic resolution and fewer residual symptoms at 12 months. These findings suggest that serum 25(OH)D level is a modifiable host factor that influences endodontic prognosis. Preoperative screening and correction of deficiency may enhance treatment predictability and clinical outcomes.

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## Conflicts of interest

There are no conflicts of interest.

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## Supplementary Table 1: Baseline demographic and clinical characteristics of the study groups

| Variable | Vitamin D-deficient ($n=45$) | Vitamin D-sufficient ($n=45$) | Test statistic | *P* |
|---|---|---|---|---|
| Age (years)[a] | 32.7±7.2 | 34.1±6.8 | 0.55 | 0.587 |
| Sex (male/female)[b] | 28 /17 | 23 /22 | 1.13 | 0.287 |
| BMI (kg/m$^2$)[a] | 24.8±3.4 | 24.1±3.1 | 0.96 | 0.339 |
| Smoking status (yes/no)[b] | 11/34 | 9/36 | 0.29 | 0.592 |
| Sun exposure (>30 min/day)[b] | 16 | 19 | 0.41 | 0.522 |
| Diagnosis (AP/CP)[b] | 23 /22 | 20 /25 | 0.40 | 0.526 |
| Initial lesion size (mm)[a] | 2.27±0.61 | 2.09±0.57 | 0.83 | 0.414 |
| Initial PAI score[c] | 4 (3–5) | 4 (3–5) | 101.5 | 0.631 |
| Baseline pain score (VAS 0–10)[c] | 8 (7–9) | 8 (7–9) | 109.5 | 0.903 |
| Baseline serum 25(OH) D (ng/mL)[a] | 16.2±1.3 | 35.8±4.9 | 14.8 | 0.001* |

*Statistically significant at $P<0.05$, [a]Independent samples *t*-test, [b]Chi-square test, [c]Mann–Whitney *U*-test. Data presented as mean±SD or median (IQR). AP: Symptomatic apical periodontitis, CP: Chronic apical periodontitis with pulp involvement, PAI: Periapical index, VAS: Visual Analog Scale, SD: Standard deviation, IQR: Interquartile range, BMI: Body mass index, 25(OH) D: 25-hydroxyvitamin D

## Supplementary Table 2: Intragroup changes in clinical and radiographic parameters over time

| Group | Parameter | Baseline | 6 months | 12 months | Test statistic | *P* |
|---|---|---|---|---|---|---|
| Vitamin D-deficient | Lesion size (mm) | 2.27±0.61 | 2.23±0.62 | 1.72±0.62 | $F=3.4$ | 0.041* |
| | PAI score | 4 (3–5) | 2 (1–4) | 1 (0–4) | $\chi^2=21.8$‡ | 0.001* |
| | Pain score (VAS) | 8 (7–9) | 4 (2–5) | 2 (0–2) | $\chi^2=32.1$‡ | 0.001* |
| Vitamin D-sufficient | Lesion size (mm) | 2.09±0.57 | 1.73±0.55 | 0.94±0.47 | $F=20.6$ | 0.001* |
| | PAI score | 4 (3–5) | 1 (1–2) | 0 (0–1) | $\chi^2=29.4$‡ | 0.001* |
| | Pain score (VAS) | 8 (7–9) | 2 (1–3) | 0 (0–1) | $\chi^2=34.2$‡ | 0.001* |

*Statistically significant at $P<0.05$, †Friedman test (‡) was used for ordinal/non-normally distributed variables. Repeated-measures ANOVA used for normally distributed variables. Data presented as mean±SD or median (IQR). SD: Standard deviation, IQR: Interquartile range, PAI: Periapical index, VAS: Visual Analog Scale

## Supplementary Table 3: Diagnostic performance of baseline serum 25-hydroxyvitamin D levels cutoff (≥20.4 ng/mL) for predicting 12-months clinical success

| Baseline serum 25(OH)D levels | Success | Failure | Total | Relative risk (95% CI) | OR (95% CI) |
|---|---|---|---|---|---|
| ≥20.4 ng/mL | 54 | 4 | 58 | 2.44 | 15.3 |
| <20.4 ng/mL | 15 | 17 | 32 | (1.46–4.07) | (4.1–56.7) |
| Total | 69 | 21 | 90 | | |

Sensitivity=78.3%, Specificity=81.0%, Positive predictive value=93.1%, Overall accuracy=78.9%. OR: Odds ratio, CI: Confidence interval, 25(OH)D: 25-hydroxyvitamin D