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Evaluation of salivary bone turnover markers and vitamin D as non-invasive, early predictive indicators of bone health in pre-, peri-, and post-menopausal women

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Abstract

Background Menopause adversely affects bone health, often going unnoticed. Salivary biomarkers may provide a non-invasive approach to assess bone turnover and oral health changes across menopausal stages.

Aim To evaluate salivary bone turnover markers—alkaline phosphatase (ALP), osteocalcin, and vitamin D3—in pre-, peri, and postmenopausal women, and explore their association with oral health.

Methods A cross-sectional study was conducted among 150 women: premenopausal ($n=50$), perimenopausal ($n=50$), and postmenopausal ($n=50$). Salivary ALP, osteocalcin, and vitamin D3 were measured using Enzyme linked immuno sorbent assay (ELISA). Oral health status was assessed through hard tissue changes (decayed, missing, calculus and mobility) and soft tissue changes. Salivary calcium and phosphorus levels were also estimated.

Results Postmenopausal women showed significantly lower salivary osteocalcin (685.16 ± 248.91; 855.87 ± 351.39; cohens d = 0.56;$p<0.05$)and vitamin D3 (137.35 ± 58.34;110 ± 45.6; cohens d = 0.52;$p<0.01$) compared to perimenopausal women, while ALP levels did not differ significantly among peri (10.54 ± 2.58) and postmenopausal group(8.95 ± 3.94; cohens d = 0.48;$p=0.09$). Salivary calcium and phosphorus were reduced in peri- and postmenopausal women($p<0.001$). Poor oral health correlated positively with duration of menopause($r=0.7$;$p=0.0038$), whereas vitamin D3 showed a negative correlation(r=-0.6; $p=0.0038$).

Conclusion Salivary vitamin D3 and osteocalcin, along with oral tissue changes, vary significantly with menopausal stage, while ALP remains unchanged. These findings highlight the potential of salivary biomarkers as early, non-invasive indicators for detecting bone loss and mitigating postmenopausal health consequences.

Keywords Menopause, Osteocalcin, Osteoporosis, Periodontitis, Salivary biomarkers, VitaminD3

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Introduction

Menopause marks the transition of a woman from reproductive to the non-reproductive phase in life. Peri menopause denotes the phase transition from reproductive phase to menopause [1].Menopause in general affects the quality of life in women owing to its extensive effects on systemic health in general and bone health in particular [2].With the increase in life expectancy, number of post-menopausal women across the world is expected to be 1.2 billion by 2030 [3]. The approximate age of menopause in Indian women is 46 years [4].

The relatively early menopausal age in Indian women increases the risk of osteoporosis and associated bone fragility and fractures [5–7] as menopause decreases the bone mineral density due to the lack of estrogen.

Bone turnover markers are widely used to evaluate the risk of fractures [8, 9] and management of metabolic bone disorder such as osteoporosis and other systemic diseases affecting bone health. Markers of bone turnover can be divided into those indicating bone formation, such as osteocalcin, alkaline phosphatase, and procollagen peptides, and those indicating bone resorption, including collagen breakdown products and specific enzymes associated with bone degradation [10].

Furthermore, menopause is associated with systemic hormonal changes that adversely affect the periodontal tissues [11]. Reduced estrogen levels contribute to decreased bone mineral density, alterations in collagen turnover, and impaired host immune response, all of which increase the risk of periodontal disease progression, resulting in the destruction of the tooth supporting tissues, eventually leading to alveolar bone loss and tooth loss [12, 13]. Tooth loss among peri- and postmenopausal women is often reflective of underlying osteoporosis [14].

While, the mechanistic relationship between osteoporosis and periodontitis is well established [15], the relationship between systemic bone loss associated with osteoporosis and alveolar bone loss still remains contentious.

As women spend nearly one third of their lives post menopause, it is particularly important to study bone health and take timely intervention measures to minimize the harm of postmenopausal osteoporosis to human health.

Available literature discusses the relationship between menopause and serum bone turnover markers in assessing systemic bone loss [16, 17].

While the role of serum bone turnover markers in menopause-related bone loss is well established, limited evidence exists regarding their salivary counterparts. The potential of saliva as a non-invasive medium to reflect systemic bone changes and their association with oral health during menopausal transition remains underexplored.

Considering this gap in the existing literature this study aimed to explore whether salivary biomarkers can reliably reflect bone health changes across menopausal stages and serve as an early, non-invasive tool for identifying women at risk of bone loss and related oral health consequences. This study also assessed the correlation between menopausal stage and oral health status and bone turnover markers to understand the relationship between alveolar bone loss and systemic bone loss.

Salivary biomarkers such as ALP, osteocalcin, and vitamin D3 reflect bone turnover and mineral metabolism [9],

Both osteocalcin and ALP are bone formation markers [18]. However, in the context of periodontal disease, particularly among postmenopausal women, elevated salivary or crevicular ALP levels have been associated with bone resorption and periodontal tissue breakdown [19]. This dual role highlights the importance of interpreting ALP levels in relation to both systemic and local conditions.

Vitamin D3 is a calciotropic hormone that plays a significant role in calcium regulating network [20]. Combined evaluation of such biomarkers non-invasively, helps in dynamic measurement of bone remodelling especially in pre, peri and postmenopausal women much well before the changes are evident in screening tests such as Dual-Energy X-ray Absorptiometry (DEXA) scan. Also, with saliva there is better chance of repeatability at short intervals of time enabling quantification [21].

Taken together, the present study hypothesizes that the Salivary bone turnover markers (ALP, osteocalcin, vitamin D3) differ significantly across menopausal stages and are associated with oral health status, thereby serving as potential non-invasive indicators of bone health.

Materials and methods

Study population and study design

The present study was a hospital-based cross-sectional study. Female subjects attending the outpatient department of Oral Pathology, Sree Balaji Dental College and Hospital, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India, were recruited after obtaining approval from the Institutional Ethical Committee (SBDCH/IEC/12A/2021/6). Written informed consent was obtained from all participants prior to enrolment. Participants completed a questionnaire (Supplementary File 1) covering age, marital status, height, weight, parity, age at menarche, and menstrual and menopausal history. The study was conducted in accordance with the Declaration of Helsinki (2013 revision), and the STROBE checklist was followed for reporting.

Sample size calculation and grouping

A priori sample size estimation was performed for a one-way Analysis of Variance(ANOVA) with three equal groups ($\alpha = 0.05$). With 50 participants per group (total $N = 150$), the study had approximately 78% power to detect a medium effect size (cohen's $f = 0.25$; equivalent pairwise $d \approx 0.50$). Since the expected between-group differences in biochemical markers were anticipated to exceed this threshold, the chosen sample size was considered adequate. A convenience sampling strategy was employed, recruiting women who met the inclusion criteria. The study was conducted over a period of three months (May–July 2022), and participants were classified into three groups based on menopausal status:

- Group I: Premenopausal women ($n = 50$).
- Group II: Perimenopausal women ($n = 50$).
- Group III: Postmenopausal women ($n = 50$).

Inclusion and exclusion criteria

The inclusion and exclusion criteria are detailed in Table 1. Menopausal status was classified based on the Study of Women's Health Across the Nation(SWAN) criteria, a longitudinal, multi-ethnic study of midlife women examining biological, psychological, and social changes during the menopausal transition [22]. SWAN categorizes menopausal stages based on menstrual cycle patterns, ranging from premenopause, early and late perimenopause, to postmenopause.

Table 1 Inclusion and exclusion criteria

Inclusion	Exclusion
Age & Sex: Biological females, 35–60 years (For peri and postmenopausal women)and < 25 years for pre-menopausal women.	Menopausal Status: Late perimenopause, postmenopause, surgical menopause, or hysterectomy
Menopausal Status: Premenopause (no change in bleeding patterns), perimenopause (change in length or interbleed interval), natural postmenopause (no bleeding for 12 months)	Systemic conditions: Osteoporosis, hyper/hypoparathyroidism, uncontrolled thyroid disease, chronic renal/liver disease, diabetes
Dentition/Oral Status: ≥20 natural teeth; willing to undergo periodontal exam and saliva collection	Medications: Recent use of corticosteroids, antiresorptive, hormone replacement therapy, selective estrogen receptor modulators, oral contraceptives, anticonvulsants, heparin, proton pump inhibitors (≤ 3 months), long-term NSAIDs, or immunosuppressants
General Health: Healthy, with no conditions affecting bone/mineral metabolism.Random blood sugar < 140 mg/dL	Oral/Periodontal Confounders: Acute oral infections, recent periodontal therapy (< 6 months), antibiotics/antiseptic mouthwash (< 4 weeks), xerostomia
Medication/Supplement: No vitamin D or calcium supplements for ≥ 8 weeks	Lifestyle: smokers, tobacco chewers, alcohol use
Consent: Written informed consent and compliance with study procedures	Other: Pregnancy/lactation, recent major illness/surgery (≤ 3 months), inability to provide saliva or any condition affecting safety/interpretation

Screening for oral health

Oral examinations were conducted by two trained oral pathologists. Hard tissue changes were recorded, including decayed teeth, missing teeth, dental calculus, and tooth mobility (Miller Mobility Index). Supragingival calculus was recorded as absent or present. Periodontal assessments included probing pocket depth (PPD), bleeding on probing (BOP), and clinical attachment level (CAL) at six sites per tooth. Interdental CAL ≥ 5 mm or buccal/oral CAL ≥ 3 mm at ≥ 2 non-adjacent teeth was used to assess severity. BOP was reported as localized when ≤ 30% of sites were affected and generalized when > 30% of sites were affected. A 1 mm Williams probe (Jaypee Dental Instruments, Chennai, India) was used. Examiners were calibrated prior to data collection, with inter-examiner reliability assessed using cohen's Kappa (> 0.85, indicating excellent agreement).

Saliva collection

Unstimulated whole saliva (3 mL) was collected into sterile containers between 10:00 and 12:00 to minimize diurnal variation [23]. Participants were instructed to avoid eating, drinking, or using mouthwash for at least 1 h prior to collection. Samples were centrifuged at 2,000 rpm for 10 min, and the supernatant was stored at − 80 °C until analysis.

Assessment of salivary calcium and phosphorus

Salivary calcium levels were determined by the Arsenazo III colorimetric method (Biosystems, Barcelona, Spain), where calcium forms a stable complex measurable at 650 nm. A calcium standard of 10 mg/dL (2.5 mmol/L) was used for calibration. The minimum detection limit was 0.2 mg/dL (0.05 mmol/L), and the method was linear up to 18 mg/dL (4.5 mmol/L). The assay showed good precision, with intra-assay CV 1.2–1.7% and inter-assay CV 2.2–2.8%.

Inorganic phosphate was estimated by the acid molybdate method, (Biosystems, Barcelona, Spain), where phosphate forms a measurable complex at 340 nm. A standard of 5 mg/dL (1.61 mmol/L) was used for calibration. The minimum detection limit was 0.13 mg/dL (0.042 mmol/L), with linearity up to 20 mg/dL (6.46 mmol/L). The assay showed good precision, with intra-assay CV 0.7–1.3% and inter-assay CV 2.5–2.9% [24, 25].

Assessment of bone markers by ELISA

Osteocalcin assay Salivary osteocalcin levels were measured using an ELISA kit (Abbkine, Georgia, USA) according to the manufacturer's instructions. The lower limit of detection (LLOD) was 5 ng/mL. Intra-assay and inter-assay coefficients of variation (CV%) were < 8% and < 10%, respectively. All samples were analyzed in duplicate. Briefly, 50 µL of standard (75–1,200 ng/mL) or salivary sample was added to wells of a 96-well microplate, followed by 50 µL of streptavidin-HRP. Plates were incubated at 37 °C for 60 min, washed, and incubated with 50 µL each of chromogen A and B in the dark for 10 min. Reactions were stopped with 50 µL stop solution, and absorbance was read at 450 nm.

Vitamin D_3 assay Salivary vitamin D_3 levels were measured using an ELISA kit (Abbkine, Georgia, USA). The LLOD was 1 ng/mL. Intra-assay and inter-assay CVs were < 8% and < 10%, respectively. Assay procedures were identical to those described for osteocalcin, using standards of 10–160 ng/mL.

ALP assay Salivary ALP levels were measured using an ELISA kit (Abbkine, Georgia, USA) with an LLOD < 10 IU/L. Intra-assay and inter-assay CVs were < 8% and < 10%, respectively. All samples were processed in duplicate following procedures identical to osteocalcin measurement.

Statistical analysis

Data were analyzed using GraphPad Prism version 10 (GraphPad Software, Boston, MA, USA). Normality was assessed using the Shapiro–Wilk test. Descriptive statistics are presented as mean ± SD. Comparisons among the three groups were performed using one-way ANOVA, followed by Tukey's post-hoc test for pairwise comparisons. Associations between salivary biomarkers and clinical parameters, including oral health indices and duration of menopause, were assessed using Pearson correlation coefficients. A p-value < 0.05 was considered statistically significant.

Table 2 Baseline demographics of the study participants

	Pre menopause	Perimenopause	Post menopause	*p* Value
Age(Years)	21.06 ± 1.99	39.30 ± 5.17	51.72 ± 6.49	< 0.001***
Height (cm)	151.56 ± 25.43	157.17 ± 3.57	158.72 ± 0.95	< 0.05*
Weight (Kg)	57.25 ± 8.80	61.91 ± 11.15	63.11 ± 10.52	< 0.05*
Number of Children	Nil	1.73 ± 0.86	2.22 ± 0.80	

Values are represented as mean ± SD

*** represents $p < 0.001$

* $p < 0.05$

Results

This study included 150 women participants of pre, peri and postmenopausal status.

Baseline demographics

Baseline demographic information on age, height, weight, parity, age of menarche, and duration of menopause are presented in Table 2. The mean age of postmenopausal women was 51.72 ± 6.49 years, while that of perimenopausal women was 39.30 ± 5.17 years. The mean parity in premenopausal women was 1.73 ± 0.86 and in postmenopausal women was 2.22 ± 0.80. Height, weight, and parity did not show any statistically significant differences among the study groups. The average age of menopause was 47.2 years.

Salivary ALP, osteocalcin, vitamin D3, calcium, and phosphorus

We evaluated key biochemical markers—ALP, osteocalcin, Vitamin D3, calcium, and phosphorus—across three menopausal stages: premenopause, perimenopause, and postmenopause ($n = 50$ each). A one-way ANOVA revealed no significant differences for ALP (IU/L), $F(2, 147) = 2.38$, $p = 0.09$, $\eta^2 = 0.03$. Significant differences were observed for osteocalcin (ng/mL), $F(2, 147) = 3.51$, $p = 0.035$, $\eta^2 = 0.045$, with post-hoc Tukey comparisons showing higher levels in perimenopausal women compared to premenopausal ($p = 0.035$, $d = 0.85$) and postmenopausal women ($p = 0.035$, $d = 0.56$). Vitamin D3 (ng/mL) differed significantly, $F(2, 147) = 5.65$, $p = 0.0058$, $\eta^2 = 0.072$, with premenopausal women having higher levels than perimenopausal ($p = 0.0058$, $d = 0.52$) and postmenopausal women ($p = 0.0058$, $d = 0.87$). Highly significant differences were observed for calcium (mg/dL), $F(2, 147) = 42.8$, $p < 0.001$, $\eta^2 = 0.368$, and phosphorus (mg/dL), $F(2, 147) = 53.6$, $p < 0.001$, $\eta^2 = 0.422$, with post-hoc comparisons indicating significant differences between all groups. All post-hoc p-values were adjusted using Bonferroni correction. Detailed pairwise comparisons, including mean differences, cohen's d, and significance, are presented in Table 3.

Oral health and menopausal status

A comparison of oral and periodontal health across menopausal stages is presented in Table 4. The mean number of decayed teeth showed a progressive increase from the premenopausal (2.10 ± 1.49) to the postmenopausal group (3.16 ± 1.33), demonstrating a statistically significant difference ($p = 0.041$). Likewise, the number of missing teeth was significantly higher among postmenopausal women (4.28 ± 1.87) compared with

Table 3 Changes in salivary levels of bone markers, calcium and phosphorus

Marker	Comparison	Mean ± SD Difference	*p*-value	cohen's d	Significance
ALP (IU/L)	Pre vs. Peri	9.60 ± 4.2 vs. 10.54 ± 2.58	0.09	0.27	NS
	Pre vs. Post	9.60 ± 4.2 vs. 8.95 ± 3.94	0.16	0.16	NS
	Peri vs. Post	10.54 ± 2.58 vs. 8.95 ± 3.94	0.48	0.48	NS
Osteocalcin (ng/mL)	Pre vs. Peri	612 ± 202.39 vs. 855.87 ± 351.39	0.035*	0.85	Significant
	Pre vs. Post	612 ± 202.39 vs. 685.16 ± 248.91	NS	0.32	NS
	Peri vs. Post	855.87 ± 351.39 vs. 685.16 ± 248.91	0.035*	0.56	Significant
Vitamin D3 (ng/mL)	Pre vs. Peri	188.7 ± 59.71 vs. 137.35 ± 58.34	0.0058**	0.52	Significant
	Pre vs. Post	188.7 ± 59.71 vs. 110 ± 45.6	0.0058**	0.87	Significant
	Peri vs. Post	137.35 ± 58.34 vs. 110 ± 45.6	NS	1.48	NS
Calcium (mg/dL)	Pre vs. Peri	10.15 ± 1.13 vs. 9.75 ± 2.73	< 0.001***	0.19	Significant
	Pre vs. Post	10.15 ± 1.13 vs. 5 ± 2.5	< 0.001***	2.65	Significant
	Peri vs. Post	9.75 ± 2.73 vs. 5 ± 2.5	< 0.001***	1.81	Significant
Phosphorus (mg/dL)	Pre vs. Peri	4.25 ± 1.25 vs. 2.15 ± 1.3	< 0.001*	1.64	Significant
	Pre vs. Post	4.25 ± 1.25 vs. 1.3 ± 0.7	< 0.001*	2.92	Significant
	Peri vs. Post	2.15 ± 1.3 vs. 1.3 ± 0.7	< 0.001*	0.81	Significant

Values are represented as mean ± SD

NS Not significant

Effect size for pairwise group comparisons was calculated using cohen's d, which measures the standardized difference between two means

Values of d ≈ 0.2, 0.5, and 0.8 are interpreted as small, medium, and large effects, respectively

*** represents $p < 0.001$

** $p < 0.01$

* $p < 0.05$

perimenopausal (2.28 ± 1.69) and premenopausal participants (0) ($p = 0.028$).Postmenopausal participants also showed greater tooth mobility compared with perimenopausal women ($p = 0.029$).

Similarly, the number of teeth affected by generalized and localized periodontitis was higher in postmenopausal women and showed significant differences across the groups. Generalized periodontitis was particularly more frequent among postmenopausal women compared with peri- and premenopausal women.

Table 4 :Comparison of hard and soft tissue changes across the study group. *LE-1 comparison of oral signs among*

	Premenopause	Perimenopause	Postmenopause	*p* value
Hard Tissue				
Decayed Teeth	2.10 ± 1.49	2.34 ± 1.89	3.16 ± 1.33	0.041*
Missing Teeth	0	2.28 ± 1.69	4.28 ± 1.87	0.028*
Calculus	0.32 ± 0.14	0.68 ± 0.58	0.68 ± 0.25	0.21NS
Tooth Mobility	0	0.38 ± 0.17	1.82 ± 1.16	0.029*
Soft Tissue				
Loss of Attachment	0	0.87 ± 0.54	1.57 ± 1.26	0.037*
PPD	2.72 ± 0.78	4.08 ± 0.97	4.72 ± 0.89	< 0.001***
Localized Periodontitis	1.35 ± 0.07	2.26 ± 0.36	4.38 ± 1.02	0.016*
Generalised Periodontitis	0	1.28 ± 2.38	3.82 ± 1.24	0.035*

Values are represented as mean ± SD

NS Not significant, *PPD* Probing pocket depth

*** represents $p < 0.001$

* $p < 0.05$

Relationship between oral health, vitamin D3,ALP and menopause

Correlation analysis revealed that duration of menopause was strongly positively associated with poor oral signs ($r = 0.70$, 95% CI: 0.52–0.82, $p = 0.0038$) indicating deterioration of oral health with longer duration of menopause. and strongly negatively associated with salivary Vitamin D3 levels (r = -0.60, 95% CI: -0.77–-0.39, $p = 0.0036$). indicating decreasing vitamin D3 with longer duration of menopause.

Puberty age showed no significant correlation with menopause age (r = -0.061, 95% CI: -0.33–0.11, $p = 0.809$). Across the study population, oral signs correlated moderately with Vitamin D3 ($r = 0.60$, 95% CI: 0.39–0.75, $p = 0.038$), calcium ($r = 0.60$, 95% CI: 0.39–0.75, $p = 0.049$), and ALP ($r = 0.60$, 95% CI: 0.39–0.75, $p = 0.046$), while correlation with phosphorus was weaker and not statistically significant ($r = 0.40$, 95% CI: 0.14–0.61, $p = 0.073$). These results are summarized in Tables 5 and 6.

Table 5 Correlation analysis between oral health, salivary vitamin D3 levels, and duration of menopause. All correlation coefficients (r) are reported with p-values and 95% confidence intervals (CI) to indicate the precision of estimates

Correlation	*r*	*p* value	95% CI	Interpretation
Duration of menopause and poor oral signs	0.7	0.0038*	[0.52, 0.82]	Strong positive
Duration of menopause and VitaminD3 level	-0.6	0.0036*	[-0.77, -0.39]	Strong negative
Puberty and menopause attaining age	-0.061	0.809	[-0.33, 0.11]	No correlation

Table 6 Correlation between oral health and salivary levels of vitamin D3, calcium, phosphorus, and alkaline phosphatase across the study population. Correlation coefficients (r) are presented with p-values and 95% confidence intervals (CI)

Correlation	*r*	*p* value	95% CI	Interpretation
Between the oral signs and Vitamin D3	-0.6	0.038*	[0.39, 0.75]	Strong negative
Between the oral signs and calcium	0.6	0.049*	[0.39, 0.75]	Moderate positive
Between the oral signs and Phosphorus	0.4	0.073	[0.14, 0.61]	Weak/ moderate
Between the oral signs and Alkaline phosphatase	0.6	0.046*	[0.39, 0.75]	Moderate positive

Discussion

Natural menopause is defined as the spontaneous and permanent cessation of menstruation for at least 12 consecutive months between the ages of 45 and 55 years (mean 50–52) [26]. The average age of menopause in India is reported as 46.2 ± 4.9 years [27], while in the present study population it was 47.2 years. Early menopause in Indian women increases the risk of osteoporosis, bone loss, and fractures for approximately a decade earlier than in Caucasians [28]. The present evaluation of salivary bone turnover markers, in conjunction with periodontal status highlights their potential utility as early indicators of menopause-related bone loss.

Alkaline phosphatase (ALP) is a key enzyme involved in osteoid development and bone mineralization [29]. ALP is synthesized by immature osteoblasts, whereas osteocalcin is produced by mature osteoblasts. Both serum osteocalcin and ALP levels are influenced by age, gender, ethnicity, and menopausal age [30]. Previous studies have reported higher salivary ALP levels in postmenopausal women compared to premenopausal women [31, 32]. In contrast, our study found that perimenopausal women exhibited higher ALP levels than postmenopausal women. This may reflect the marked estrogen fluctuations and accelerated bone resorption characteristic of the perimenopausal transition, compared with the more stable low-estrogen environment of postmenopause [33]. Furthermore, salivary ALP is also recognized as a marker for periodontitis [34], and studies have reported increased salivary ALP levels in postmenopausal women with periodontitis [35].

The present study also assessed salivary osteocalcin, a non-collagenous protein hormone that influences bone mineral density during menopause. Studies have reported osteocalcin gene polymorphisms as potential predictive markers for osteoporosis [36]. In our study, perimenopausal women exhibited higher salivary osteocalcin levels than postmenopausal women, which may be explained by increased bone loss during the perimenopausal transition, a period associated with the greatest predicted bone loss [37, 38].

Vitamin D_3 plays a critical role in maintaining bone health, particularly in the elderly, and nearly one billion people worldwide suffer from vitamin D insufficiency or deficiency [39]. Estrogen significantly contributes to vitamin D_3 activation and increases the expression of vitamin D receptors. Consequently, estrogen deficiency in menopause increases the risk of vitamin D deficiency [40]. Consistent with existing literature, salivary vitamin D_3 levels were significantly lower in postmenopausal women in this study, and levels declined further with advancing menopausal age.

Although prior studies have reported increased salivary calcium and phosphorus levels in menopause and periodontitis [41], our findings contrast with these reports, as postmenopausal women exhibited lower salivary calcium and phosphorus levels compared with healthy controls [42–44]. This discrepancy may reflect differences in sample size, methodology, or dietary and systemic factors. The decline in calcium levels in postmenopausal women likely reflects estrogen deficiency–related disturbances in calcium absorption and excretion, consistent with previous findings by Qureshi et al. (2010) [45].

Menopause-associated estrogen deficiency also increases vulnerability to plaque accumulation, gingivitis, and severe periodontitis [46, 47]. Kemar et al. highlighted the association between menopause and clinical attachment loss [48]. Accordingly, in addition to the biochemical markers, this study assessed oral hard and soft tissue changes. Compared with premenopausal women, postmenopausal women demonstrated increased tooth mobility, tooth loss, and dental decay.

The higher prevalence of decayed and missing teeth in postmenopausal women indicates a detrimental impact of menopause on oral health, possibly mediated by estrogen deficiency–induced alterations in salivary physiology. Further research is warranted to clarify the underlying mechanisms and assess the potential role of hormone replacement therapy in mitigating these effects [49].

Soft tissue assessment revealed a higher prevalence and severity of localized and generalized periodontitis in postmenopausal women. Salivary ALP and vitamin

D_3 levels showed moderate positive correlations with oral health, while salivary calcium and phosphorus levels correlated positively with oral hard and soft tissue parameters. Salivary osteocalcin, however, did not show a correlation with periodontal health in this study.

Overall, our findings regarding altered salivary levels of vitamin D_3, osteocalcin, calcium, phosphorus, and ALP across menopausal stages align with growing evidence that salivary biomarkers reflect periodontal and bone metabolic changes in postmenopausal women. For instance, John and Shenoy (2022) reported a strong inverse correlation between salivary vitamin D levels and clinical periodontal parameters such as CAL, PD, PI, and BOP in postmenopausal women [50]. Similarly, elevated salivary osteocalcin levels have been positively correlated with probing depth and alveolar bone loss, supporting its use as a marker for periodontal bone degradation [51]. ALP has also been proposed as an early salivary indicator of periodontal disease linked to altered bone metabolism in menopausal women [52]. Finally, a cross-sectional study comparing reproductive-age and postmenopausal women reported both increased alveolar bone loss and elevated remodelling markers (β-CTx, osteocalcin) in the postmenopausal group [53]. These studies validate and complement our results, highlighting the potential of salivary markers to reflect both periodontal health and menopause-related bone changes.

To our knowledge, this study adds to the understanding of the relationship between salivary vitamin D_3 and bone markers (ALP and osteocalcin) in relation to oral health status, including periodontitis, across peri- and postmenopausal women. The inclusion of perimenopausal women is particularly notable, given that the greatest bone loss is predicted during the perimenopausal transition.

The study has several limitations. Age-related changes in bone turnover and periodontal status may confound associations with menopausal stage, as both systemic bone metabolism and cumulative periodontal disease naturally vary with age. Classification into pre-, peri-, and postmenopausal groups was based primarily on menstrual history, which may not fully capture the heterogeneity of menopausal transition. The cross-sectional design limits causal inference, and longitudinal studies with larger, age-matched cohorts are needed to validate the predictive utility of salivary ALP, osteocalcin, and vitamin D_3 as markers of bone and periodontal health. Although participants with normal random blood sugar levels were included to exclude diabetes, undiagnosed or early-stage metabolic alterations cannot be entirely ruled out. Since diabetes has a direct impact on periodontal health and bone metabolism. Additional confounders such as diet, lifestyle, sun exposure, salivary flow rate, and pH were not assessed. Finally, the lack of bone mineral density (BMD) measurements precludes assessment of systemic bone loss.

Further longitudinal studies are warranted to validate the relationship between these salivary markers and periodontal health, which may ultimately enable non-invasive, chairside assessment of bone loss in peri- and postmenopausal women at an early stage.

Conclusion

Salivary bone turnover markers, including ALP, osteocalcin, calcium, phosphorus, and vitamin D_3, reflect menopause-related changes in bone metabolism and correlate with periodontal health. Perimenopausal women exhibited higher ALP and osteocalcin levels, suggesting accelerated bone remodelling during the menopausal transition, while postmenopausal women showed reduced vitamin D_3, calcium, and phosphorus levels. These findings highlight the potential of salivary biomarkers as non-invasive tools for early detection of bone loss and periodontal deterioration in peri- and postmenopausal women. Further longitudinal studies are warranted to validate their predictive utility in clinical practice.

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Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12905-025-04238-5.

Supplementary Material 1

Patient declaration of consent

Written informed consent was obtained from all individual participants included in the study. The confidentiality and anonymity of all participants were maintained throughout the study

Authors' contributions

Mathangi. R: Conceptualization, design, Methodology, Supervision, Writing, Investigation, review and editing; Aravindha Babu- Design, Investigation, data collection Dr.Aafiya, Kowsalya Shankar and KMK Masthan– writing, data collection.

Data availability

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the from the Institutional Ethical Committee(SBDCH/IEC/12A/2021/6)of Sree Balaji Dental College and Hospital, BIHER. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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