

Limited utility of salivary mineral content in prediction of fragility fractures among postmenopausal women

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Conflict of interest

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Abstract

Background. Osteoporosis is a metabolic disease characterized by increased bone fragility. As it is characterized as a general skeletal disease, changes can also be seen in the stomatognathic system (edentulism, wrong fitting of dentures, etc.). The question is whether early changes in the salivary mineral content and acid-base balance may reflect skeletal status and risk of bone fracture.

Objectives. The objective of the study was to evaluate whether minerals in the saliva were associated with skeletal fractures in a population of postmenopausal women.

Materials and methods. In this observational study, dental examinations along with the collection of saliva were conducted in 117 randomly recruited women (mean age 64.6 ± 5.9 years). The study group included 23 study participants with fractures, of which 10 had a history of osteoporotic fractures. Saliva samples for mineral content including copper (Cu), zinc (Zn), calcium (Ca), and phosphorus (P), as well as salivary pH were collected and analyzed to determine associations between salivary mineral content and fracture risk.

Results. As a result, the median pH value was 6.8, and the median levels for Cu ($0.35 \mu\text{mol/L}$), Zn ($0.61 \mu\text{mol/L}$), Ca (0.7 mmol/L), and P (6.64 mmol/L) were observed. No differences were noted in salivary mineral content and acid-basic balance between the fractured and non-fractured participants.

Conclusions. The results of our study suggest that salivary mineral content has limited usability in predicting skeletal fragility in postmenopausal women when used alone.

Key words: minerals, osteoporosis, saliva, dental status, fracture

Background

Osteoporosis is a metabolic disease characterized by low bone mass and deterioration of the bone microarchitecture and quality, usually leading to increased bone fragility, which in turn results in severe negative health outcomes.^{1,2} There are numerous risk factors that can contribute to age-related decreases in bone mass in women. Hormonal disturbances, such as hypogonadism and estrogen deficiency, long-term glucocorticoid therapy, thyroid diseases, and a number of chronic endocrine diseases are essentially associated with bone loss and may be, indirectly, responsible for clinically significant fractures.^{3–5} Women after menopause, due to a rapidly progressing estrogen deficiency, are exposed to the detrimental effects on bone and, therefore, are at a higher risk of osteoporosis. Along with hormonal insufficiency, they also experience various co-morbidities influencing calcium-phosphate and mineral metabolism that can increase bone resorption and negatively affect bone quality and strength. All those factors, along with a history of any previous fracture (including all sustained significant fractures beyond those related to osteoporosis), make postmenopausal women more susceptible to increased skeletal fragility.⁶

A generalized compromised skeletal status resulting from osteoporosis may be associated with alterations in other mineralized tissues, including the teeth, and the alteration of microelement levels, although the causal pathway remains unclear.^{7–11} It has been reported that clinical manifestations of osteoporosis in the stomatognathic system may result in periodontal disease and a loss of teeth with all the consequences, like wrong fitting of dentures and several dental and oromandibular dysfunctions.^{12–18} In the adult population, the number of natural teeth can be recorded in the assessment of osteoporosis, particularly to estimate the risk of hip fracture.¹⁹ The connections through dental status, oral diseases, bone mass, bone loss, and risk of osteoporosis have been largely studied in adult and older populations. However, available data are inconsistent to some extent and cannot be generalized, mainly due to the variety of different research methods used.^{20–25}

A question arises whether the aforementioned changes in the masticatory system may reflect any changes in salivary content and the acid-base balance. Another issue is whether salivary characteristics and other markers measured in the saliva may accurately indicate, or correspond to, general metabolic processes or measurable dysfunctions of the skeleton.

Objectives

This study aimed to evaluate the relationship between salivary mineral content, saliva characteristics, and the prevalence of fragility fractures in women after menopause.

Materials and methods

Patients

The study group was randomly selected from the population of Zabrze in Silesia, Poland. The recruitment part consisted of acquiring a list of 3,000 women aged over 55 years (which was 10% of the gender and age-specific population) and sending them invitations to participate. Of these women, 365 responded to the invitation. Out of them, only 117 women agreed to visit the dental office and adhered to the entire study protocol.

Specimen characteristics, assay methods

At the following stage, saliva was collected after a precise preparation of the standard to test salivary pH, calcium (Ca), copper (Cu), phosphorus (P), and zinc (Zn) content.^{26–28} Prior to collection, all the participants were told to refrain from eating, drinking, gum chewing, tobacco smoking, and oral hygiene procedures (mouth rinses, flossing, brushing) for a minimum of 1 h before the collection, and to avoid any dental work within 24 h prior to sample collection. Visits occurred between 8:00 AM and 11:00 AM. At the start of the appointment, study participants were asked to rinse their mouth with deionized water for 1 min. Afterwards, they spit the water out. Five minutes after the oral rinse, unstimulated saliva was gathered into a sterile graduated test tube. The spitting method was conducted in accordance with standardized guidelines. The collection lasted 5 min. The samples were stored on ice and then refrigerated at -80°C immediately after the saliva collection.

A questionnaire-based direct interview, medical records and a clinical examination ascertained by the physician were used to obtain data on past history of fractures, nutritional habits, smoking, and long-term medical therapies. At least 1 non-accidental fracture event after the age of 40 years was included in the analysis, and all fractures were confirmed using routine radiographs. Standard anthropometric methods were used to evaluate weight and height and to calculate body mass index (BMI) based on a standard formula.

Study design

The research was a part of the Silesia Osteo Active Study, which was conducted in 2015.^{26,29,30} A detailed description of the Ca, Zn, Cu, P, and pH assessment methods was published in our previous work.²⁶ The study obtained the approval from the Ethics Committee at the Medical University of Silesia (approval No. KNW/0022/KB1/22/III/14/15). Informed consent was collected from each participant prior to the examinations.

Statistical analyses

All statistical analyses were performed using Statistica v. 13.3 program (StatSoft, Tulsa, USA). The assumption of normality was tested with density functions and Shapiro–Wilk tests. Normally distributed descriptive data were presented as mean values \pm standard deviations ($M \pm SD$), whereas data with a non-normal distribution were presented using the median and quartiles (1st quartile (Q1) and 3rd quartile (Q3)). Data with non-normal distribution were analyzed using Mann–Whitney U and Spearman rank tests. Data with normal distribution were analyzed with the use of t-tests. Homogeneity tests were performed using the Levene and Brown–Forsythe tests. A p-value of less than 0.05 was considered statistically significant.

Results

The study group included 117 Caucasian women aged 64.6 ± 5.9 years, all of whom were at least 1 year post-menopause, as self-reported. The mean BMI was 29.5 ± 4.7 kg/m². Full demographic data with main fracture-related factors are presented in Table 1. The average number of teeth presented was 13.2 ± 9.1 (min 0, max 30). Twenty-three participants (19.7%) experienced previous fractures that had occurred after the age of 40 years. In 10 women (8.5% of the whole study group), it was a clinically significant result of low-energy trauma (low-energy fracture (LEF)). In 16 participants (13.7%), the fracture location was the wrist (in 7 cases, the wrist, radius, and/or ulna fractures were classified as LEF), in 6 cases (5.1%), the ankle (including 3 cases of LEF), and in 1 woman, a vertebral fracture in the lumbar spine was found and assigned to a LEF.

The median value of salivary pH was 6.8 (Q1–Q3: 6.5–7.1), while the median Ca concentration was 0.7 (Q1–Q3: 0.53–1.19) mmol/L, Cu 0.35 (Q1–Q3: 0.19–0.57) μ mol/L, Zn 0.61 (Q1–Q3: 0.15–1.22) μ mol/L, and P 6.64 (Q1–Q3: 5.73–8.07) mmol/L.

Mean salivary pH did not correlate with salivary mineral content (Spearman's rank correlation test $p > 0.05$). A significant positive correlation was found between Cu and Zn ($r = 0.48$) and Cu with P ($r = 0.38$) content (Spearman's rank test, $p < 0.05$). Concise results of the Spearman's rank test analysis are presented in Table 2. Next, participants were stratified with regard to the history of any fractures

Table 1. Basic characteristics of the study group including demographic data and morbidity

Variable	Value	
Mean age [years]	64.6 ± 5.9	
Mean age of menopause [years]	49.4 ± 5.2	
variable	number of participants	percentage of group
BMI [kg/m ²]	29.5 ± 4.7	
Underweight	0	0
Normal weight	21	17.9
Overweight	45	38.5
Obese	51	43.6
Past and current smoking	56	47.8
History of osteoporosis (based on a previous single DXA scan and interpretation)	27	23.1
Chronic kidney disease	7	6
Hypothyroidism	45	38.5
History of long-term glucocorticoid therapy (>3 months)	8	6.8
Hormone replacement therapy (>3 years)	43	36.8

BMI – body mass index; DXA – dual energy X-ray absorptiometry.

Table 3. Homogeneity test results (Levene's test)

Analyzed parameter	F	df	p-value
Age	2.6	115	0.111
Body mass index	0.18	115	0.669
Salivary pH	2.3	115	0.128
Salivary Ca concentration	1.2	115	0.277
Salivary Cu concentration	0.4	115	0.531
Salivary Zn concentration	18.2	115	<0.001
Salivary P concentration	0.6	115	0.432

Ca – calcium; Cu – copper; Zn – zinc; P – phosphorus; df – degrees of freedom.

that had occurred after the age of 40 years. To test for homogeneity of variance, a Levene's test was performed (Table 3). It confirmed the homogeneity assumption of the variance ($p > 0.05$) with respect to age, BMI, salivary pH, Cu, P, and Ca concentrations. As it comes to Zn, Levene's test indicated heterogeneity between the groups. Therefore, the comparison of the groups with respect to Zn

Table 2. Correlation between salivary mineral content (Spearman's test), $n = 117$

Analyzed parameter	Salivary Ca concentration	Salivary Cu concentration	Salivary Zn concentration	Salivary P concentration
Salivary pH	$r = -0.17, p = 0.106$	$r = -0.03, p = 0.768$	$r = -0.06, p = 0.627$	$r = -0.16, p = 0.202$
Salivary Ca concentration	$r = 1$	$r = 0.14, p = 0.360$	$r = 0.2, p = 0.171$	$r = 0.12, p = 0.319$
Salivary Cu concentration	$r = 0.14, p = 0.360$	$r = 1$	$r = 0.48, p < 0.001$	$r = 0.38, p = 0.034$
Salivary Zn concentration	$r = 0.2, p = 0.171$	$r = 0.48, p < 0.001$	$r = 1$	$r = 0.12, p = 0.501$
Salivary P concentration	$r = 0.12, p = 0.319$	$r = 0.38, p = 0.034$	$r = 0.12, p = 0.501$	$r = 1$

Table 4. Comparison of the salivary mineral content between the subgroups according to fracture prevalence

Compared parameter	Without fracture	With fracture	Test used	Test value	df	p-value
Number and % of participants	94 (80.3% of the study group)	23 (19.7% of the study group)	–	–	–	–
Age (M \pm SD (95% CI)) [years]	63.9 \pm 6.6 (62.6, 65.2)	66.3 \pm 7.7 (62.7, 69.4)	t-test	t = –1.39	115	0.256
BMI (M \pm SD (95% CI)) [kg/m ²]	29.5 \pm 4.8 (28.5, 30.4)	29.9 \pm 4.3 (28.03, 31.8)	t-test	t = –0.43	115	0.648
pH (median; Q1–Q3)	6.85; 6.5–7.3	6.8; 6.5–7	U Mann–Whitney	U = 959.5	–	0.405
Cu (median; Q1–Q3) [μ mol/L]	0.36; 0.2–0.62	0.33; 0.15–0.54	U Mann–Whitney	U = 326.5	–	0.417
Zn (median; Q1–Q3) [μ mol/L]	0.61; 0.15–1.07	0.61; 0.1–1.38	U Mann–Whitney	U = 358	–	0.716
Zn (median; Q1–Q3) [mmol/L]	0.67; 0.53–1.13	0.81; 0.68–1.22	U Mann–Whitney	U = 561	–	0.533
P (median; Q1–Q3) [mmol/L]	6.7; 5.8–7.8	6.46; 5.6–8.06	U Mann–Whitney	U = 337	–	0.83

Cu – copper; Zn – zinc; P – phosphorus; BMI – body mass index; M – mean; SD – standard deviation; 95% CI – 95% confidence interval; Q1 – 1st quartile; Q3 – 3rd quartile; df – degrees of freedom.

concentration was performed with 2 independent sample comparisons of the means test with unequal variance (Welch's test), but the p-value remained greater than 0.5 ($w = -0.95$, $df = 11.07$, $p = 0.363$), as well. In respect to age and BMI, t-tests for independent groups were used, but no significant differences were found ($p > 0.05$). For group comparisons with respect to salivary pH, Cu, Ca, and P, the Mann–Whitney U test was performed ($p > 0.05$). No significant differences between fractured and non-fractured participants were found (Table 4).

Discussion

This study attempted to evaluate the relationship between salivary mineral characteristics and clinically significant fractures in the female population. Despite negative results, our report provides information on poorly studied (so far) associations indicating that further research, preferably prospective studies, is needed. The relationship between all fractures (osteoporotic and non-osteoporotic) was analyzed, assuming that the prognostic power of prior fractures as a risk of subsequent fractures is not affected by the nature of the fracture. This was based on a recent observation by Leslie et al.⁶ There is still very little information available on mineral content concentrations in saliva, as well as the importance of selected individual minerals in postmenopausal osteoporosis. Furthermore, it is believed that the composition of saliva changes with age.³¹

The importance of Ca and P in bone turnover and the role of minerals such as Zn and Cu in bone metabolism are well known, although not all of their mechanisms of action in postmenopausal osteoporosis are fully understood. Zinc and Cu are important for bone integrity and elasticity, whereas Cu induces low bone turnover by suppressing both osteoblastic and osteoclastic functions. As a cofactor for lysyl oxidase, Cu is required in the cross-linking of collagen and elastin. Zinc is a constituent of about 300 enzymes. Moreover, it regulates the secretion of calcitonin from the thyroid gland and impacts bone turnover. Zinc

deficiency causes a reduction in osteoblastic activity, collagen and chondroitin sulfate synthesis, and alkaline phosphatase activity.^{32–34}

There is a growing interest in diagnostic procedures based on saliva testing. Saliva is increasingly used for the early diagnosis of osteoporosis and fracture risk assessment. The methods for collecting this biological material are simple, cost-effective, painless, and, most importantly, non-invasive and safe for both the patient and the operator.^{27,28} Saliva is regarded as a “mirror of the body” and has a number of important roles, mainly in maintaining the health of the oral cavity, while it may also indirectly affect the entire human metabolism. One of the essential functions of saliva is to maintain and protect the hard tissues of the tooth by reassuring physicochemical balance and providing a unique source of Ca and phosphate ions.²⁸

Saliva contains ingredients that can act as buffers when the pH is below or above their isoelectric point.³⁵ The buffers maintain the resting saliva pH between 5.7 and 6.2. However, to ensure the integrity of the tooth structure, the oral pH should be around 6.3 or higher. It has been well documented that the dissolution of enamel occurs when the pH falls below a critical pH, i.e., 5.5, and that the acidic environment promotes demineralization of the inorganic substance of the tooth. Median values obtained in the present study proved to be quite similar in both subgroups (6.8–6.85) and matched normal values.^{36,37} Slightly lower pH values were found in study participants with fractures, but the difference was not significant. Our results were similar to those observed by Pereira et al., who compared postmenopausal women with low bone mineral density (BMD) to controls without bone loss and found a pH proportion of 7.1–7.2. However, a weak correlation has been shown between BMD and pH in the latter study.¹¹ An impact of hormone replacement therapy on an increase of salivary pH and, at the same time, the reduction of Ca levels in saliva in postmenopausal patients has been reported elsewhere.^{38,39} Additionally, Tabor et al. conducted an epidemiological study of randomly recruited postmenopausal women. The study

demonstrated that a high calcium content in saliva, co-existing with a low pH, was associated with lower femoral and spinal bone density in postmenopausal women.²⁶ Contrary to our results, Wasti et al. demonstrated that Ca content in saliva may have been strongly indicative of the presence or absence of osteoporosis in postmenopausal women.⁴⁰ Saha et al. also found a significant increase in salivary Ca and alkaline phosphatase in study participants with osteoporosis and osteopenia compared to healthy controls.⁹ Noticeably, the methodological approach and the design of a given study may, to a great extent, affect the interpretation of the results. Osteoporosis is commonly understood as bone loss and decreased BMD, according to the current practice guidelines and definition. However, the core element of the disease is low energy fractures, implying major clinical issues around menopause. Thus, the connection between BMD and other mineralized tissues, such as teeth, and the relationship between salivary minerals and bone minerals may be obscured or misleading. Our study was, therefore, focused strictly on the prevalence of fractures.

Limitations

The study has some limitations. Firstly, prospective observation was not performed, and the study population was limited only to women. Further, the number of study participants with fractures was relatively small, yet reflected a general rate of fragility in the population due to the randomized approach. It is also possible that a few silent fractures may have been omitted due to a lack of radiographic evidence. Nevertheless, this appears to be the first report evaluating salivary characteristics and postmenopausal fractures. Data from larger populations, using a prospective design, would be necessary to support or contradict our observations.

Conclusions

There was no association between salivary mineral content and the prevalence of fracture. The results of our study suggest that salivary mineral content has limited usability in predicting skeletal fragility in postmenopausal women, and should therefore be regarded with caution in routine practice.

Supplementary data

The Supplementary materials are available at <https://doi.org/10.5281/zenodo.10429621>. The package contains the following files:

Supplementary Fig. 1. Scatterplots illustrating the relationships between the data analyzed by the Spearman correlation (salivary Ca, Cu, P, Zn, pH).

Supplementary Fig. 2. The violin plots illustrating data distribution in two compared groups (fractured compared to non-fractured).

Data availability

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

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References

1. Kanis JA, Melton LJ, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. *J Bone Miner Res*. 1994;9(8):1137–1141. doi:10.1002/jbmr.5650090802
2. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy. Osteoporosis prevention, diagnosis, and therapy. *JAMA*. 2001;285(6):785–795. doi:10.1001/jama.285.6.785
3. Khosla S, Pacifici R, Marcus R, Dempster DW, Cauley JA. Estrogen deficiency, postmenopausal osteoporosis, and age-related bone loss. In: Marcus R, Dempster DW, Cauley JA, Feldman D, eds. *Osteoporosis*. 4th ed. Amsterdam, the Netherlands-New York, USA: Elsevier; 2013:1113–1136. doi:10.1016/B978-0-12-415853-5.00046-7
4. Kanis J, Stevenson M, McCloskey E, Davis S, Lloyd-Jones M. Glucocorticoid-induced osteoporosis: A systematic review and cost-utility analysis. *Health Technol Assess*. 2007;11(7):iii–iv, ix–xi, 1–231. doi:10.3310/hta11070
5. Tuchendler D, Bolanowski M. The influence of thyroid dysfunction on bone metabolism. *Thyroid Res*. 2014;7(1):12. doi:10.1186/s13044-014-0012-0
6. Leslie WD, Schousboe JT, Morin SN, et al. Fracture risk following high-trauma versus low-trauma fracture: A registry-based cohort study. *Osteoporos Int*. 2020;31(6):1059–1067. doi:10.1007/s00198-019-05274-2
7. Rabiei M, Masooleh IS, Leyli EK, Nikoukar LR. Salivary calcium concentration as a screening tool for postmenopausal osteoporosis. *Int J Rheum Dis*. 2013;16(2):198–202. doi:10.1111/1756-185X.12003
8. Passos-Soares JDS, Vianna MIP, Gomes-Filho IS, et al. Association between osteoporosis treatment and severe periodontitis in postmenopausal women. *Menopause*. 2017;24(7):789–795. doi:10.1097/GME.0000000000000830
9. Saha MK. Evaluation of correlation between salivary calcium, alkaline phosphatase and osteoporosis: A prospective, comparative and observational study. *J Clin Diagn Res*. 2017;11(3):ZC63–ZC66. doi:10.7860/JCDR/2017/24960.9583
10. Kumbhojkar S, Kale A, Kumbhojkar V, Desai K. Salivary calcium as a diagnostic tool for screening of osteoporosis in postmenopausal women. *J Oral Maxillofac Pathol*. 2019;23(2):192. doi:10.4103/jomfp.jomfp_133_19
11. Pereira IF, Brasileiro CB, Kleperon NP, et al. Comparative study of oral and salivary parameters in patients with and without loss of bone mass. *Braz Oral Res*. 2018;32:e54. doi:10.1590/1807-3107bor-2018.vol32.0054
12. Darcey J, Horner K, Walsh T, Southern H, Marjanovic EJ, Devlin H. Tooth loss and osteoporosis: to assess the association between osteoporosis status and tooth number. *Br Dent J*. 2013;214(4):E10. doi:10.1038/sj.bdj.2013.165
13. Nicopoulou-Karayianni K, Tzoutzoukos P, Mitsea A, et al. Tooth loss and osteoporosis: The osteodent study. *J Clin Periodontol*. 2009;36(3):190–197. doi:10.1111/j.1600-051X.2008.01365.x
14. Sachelarie L, Farcas DM, Dartu L, et al. Comparative study of diseases of the stomatognathic system and specific parameters of osteoporosis. *Osteoporos Int*. 2016;27(2):845–848. doi:10.1007/s00198-015-3251-6
15. Koth VS, Salum FG, De Figueiredo MAZ, Cherubini K. Repercussions of osteoporosis on the maxillofacial complex: A critical overview. *J Bone Miner Metab*. 2021;39(2):117–125. doi:10.1007/s00774-020-01156-4

16. Bandela V. Osteoporosis: Its prosthodontic considerations. A review. *J Clin Diagn Res*. 2015;9(12):ZE01–ZE04. doi:10.7860/JCDR/2015/14275.6874
17. Wang CW, McCauley LK. Osteoporosis and periodontitis. *Curr Osteoporos Rep*. 2016;14(6):284–291. doi:10.1007/s11914-016-0330-3
18. Savić Pavičin I, Dumančić J, Jukić T, Badel T. The relationship between periodontal disease, tooth loss and decreased skeletal bone mineral density in ageing women. *Gerodontology*. 2017;34(4):441–445. doi:10.1111/ger.12290
19. Yu YH, Cheung WS, Miller DR, Steffensen B. Number of teeth is associated with hip fracture and femoral neck bone mineral density in the NHANES. *Arch Osteoporos*. 2021;16(1):105. doi:10.1007/s11657-021-00970-1
20. Moghaddam SA, Zakeri Z, Fakour SR, Moghaddam AA. Does salivary calcium and phosphate concentrations adequately reflect bone mineral density in patients with chronic periodontitis? *Glob J Health Sci*. 2016;8(10):282. doi:10.5539/gjhs.v8n10p282
21. Ozola B, Slaidina A, Laurina L, Soboleva U, Lejnicks A. The influence of bone mineral density and body mass index on resorption of edentulous jaws. *Stomatologija*. 2011;13(1):19–24.
22. Singh A, Sharma RK, Siwach RC, Tewari S, Narula SC. Association of bone mineral density with periodontal status in postmenopausal women. *J Invest Clin Dent*. 2014;5(4):275–282. doi:10.1111/jicd.12047
23. Qi J, Liu E, Guo YF, et al. Association between periodontal disease and osteoporosis in postmenopausal women: A protocol for systematic review and meta-analysis. *BMJ Open*. 2021;11(9):e049277. doi:10.1136/bmjopen-2021-049277
24. Sewón L, Laine M, Karjalainen S, Doroginskaia A, Lehtonen-Veromaa M. Salivary calcium reflects skeletal bone density of heavy smokers. *Arch Oral Biol*. 2004;49(5):355–358. doi:10.1016/j.archoralbio.2003.11.004
25. Pepelassi E, Nicopoulou-Karayianni K, Archontopoulou A, et al. The relationship between osteoporosis and periodontitis in women aged 45–70 years. *Oral Dis*. 2012;18(4):353–359. doi:10.1111/j.1601-0825.2011.01881.x
26. Tabor E, Hüpsch H, Rokicka J, et al. Salivary content might be associated with skeletal status in postmenopausal women: SilesiaOsteoActive study results. *J Clin Densitom*. 2021;24(1):14–21. doi:10.1016/j.jocd.2020.02.001
27. Chiappin S, Antonelli G, Gatti R, De Palo EF. Saliva specimen: A new laboratory tool for diagnostic and basic investigation. *Clin Chim Acta*. 2007;383(1–2):30–40. doi:10.1016/j.cca.2007.04.011
28. Bhattarai KR, Kim HR, Chae HJ. Compliance with saliva collection protocol in healthy volunteers: Strategies for managing risk and errors. *Int J Med Sci*. 2018;15(8):823–831. doi:10.7150/ijms.25146
29. Tabor E, Kuźniewicz R, Zagórski P, Martela K, Pluskiewicz W. The relationship of knowledge of osteoporosis and bone health in postmenopausal women in Silesia OsteoActive Study. *J Clin Densitom*. 2018;21(1):98–104. doi:10.1016/j.jocd.2016.08.005
30. Tabor E, Zagórski P, Martela K, Glinkowski W, Kuźniewicz R, Pluskiewicz W. The role of physical activity in early adulthood and middle age on bone health after menopause in epidemiological population from Silesia OsteoActive Study. *Int J Clin Pract*. 2016;70(10):835–842. doi:10.1111/ijcp.12874
31. Xu F, Laguna L, Sarkar A. Aging-related changes in quantity and quality of saliva: Where do we stand in our understanding? *J Texture Stud*. 2019;50(1):27–35. doi:10.1111/jtxs.12356
32. Gür A, Çolpan L, Nas K, et al. The role of trace minerals in the pathogenesis of postmenopausal osteoporosis and a new effect of calcitonin. *J Bone Miner Metab*. 2002;20(1):39–43. doi:10.1007/s774-002-8445-y
33. Lin S, Yang F, Ling M, Fan Y. Association between bone trace elements and osteoporosis in older adults: A cross-sectional study. *Ther Adv Musculoskelet Dis*. 2022;14:1759720X2211259. doi:10.1177/1759720X221125984
34. Ceylan MN, Akdas S, Yazihan N. Is zinc an important trace element on bone-related diseases and complications? A meta-analysis and systematic review from serum level, dietary intake, and supplementation aspects. *Biol Trace Elem Res*. 2021;199(2):535–549. doi:10.1007/s12011-020-02193-w
35. Carpenter GH. The secretion, components, and properties of saliva. *Annu Rev Food Sci Technol*. 2013;4(1):267–276. doi:10.1146/annurev-food-030212-182700
36. Dawes C. What is the critical pH and why does a tooth dissolve in acid? *J Can Dent Assoc*. 2003;69(11):722–724.
37. Deng F, Sakai H, Kitagawa H, et al. Fabrication of pH-responsive Zn²⁺-releasing glass particles for smart antibacterial restoratives. *Molecules*. 2022;27(21):7202. doi:10.3390/molecules27217202
38. Sewón L, Laine M, Karjalainen S, Leimola-Virtanen R, Hiidenkari T, Helenius H. The effect of hormone replacement therapy on salivary calcium concentrations in menopausal women. *Arch Oral Biol*. 2000;45(3):201–206. doi:10.1016/S0003-9969(99)00137-5
39. Wang L, Zhu L, Yao Y, Ren Y, Zhang H. Role of hormone replacement therapy in relieving oral dryness symptoms in postmenopausal women: A case control study. *BMC Oral Health*. 2021;21(1):615. doi:10.1186/s12903-021-01966-6
40. Wasti A, Wasti J, Singh R. Estimation of salivary calcium level as a screening tool for the osteoporosis in the post-menopausal women: A prospective study. *Indian J Dent Res*. 2020;31(2):252. doi:10.4103/ijdr.IJDR_879_19