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Salivary calcium concentration as a screening tool for postmenopausal osteoporosis

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Abstract

Aim: Measurements of salivary calcium level may be a useful screening tool for osteoporosis in postmenopausal women. The purpose of this study was to clarify whether this measure is valid compared with dual-energy X-ray (Bone Mineral Density) screening tools in osteoporosis.

Methods: A case-control study was carried out in 40 postmenopausal women with osteoporosis (*T*-score ≤ -2.5) and 40 women without osteoporosis (*T*-score > -1 bone mineral density). Salivary samples were collected and calcium concentrations were measured and expressed as mg/dL. Receiver operating characteristic curve analyses was used to determine the optimal cut-off thresholds for salivary calcium in healthy postmenopausal women.

Results: The cut-off point for salivary calcium was 6.1 mg/dL. The sensitivity and specificity, respectively, for identifying women with osteoporosis, were 67.5 (95%CI 52.33–82.67) and 60% (95%CI 44.62–75.38). The area under curve (AUC) was 0.678 (95%CI 0.56–0.79), the positive predictive value (PPV) was 62.79 (95%CI 47.74–77.84) and negative predictive value (NPV) was 64.86% (95%CI 49.27–80.46). The positive likelihood ratio was 1.688 and the negative likelihood ratio was 0.542.

Conclusions: Salivary calcium concentration discriminates between women with and without osteoporosis and constitutes a useful tool for screening for osteoporosis.

Key words: calcium, osteoporosis, saliva, screening.

INTRODUCTION

Identifying women with osteoporosis remains a clinical challenge and diagnosing osteoporosis before a fracture occurs is far preferable to waiting for a fracture to occur.[1,2] All women aged $\geq$ 50 years should be evaluated for risk factors for osteoporosis.[3]

There are different strategies for screening for osteoporosis. The gold standard is bone mineral densitometry test so-called central dual-energy X-ray (DXA). Further tests may be quantitative ultrasound (QUS), radiography and clinical prediction rules (CPRs).[1] It is of the utmost importance to find new low-cost and available tools for screening for osteoporosis, which may decrease fracture risks.

In recent years, saliva-based diagnostic tests have increased in popularity because of their non-invasive nature. Salivary biomarkers have been used to assess the risk of: developing oral, ovarian and breast cancers; HIV infection; Sjögren's syndrome; and dental caries and periodontal diseases. Using saliva rather than serum has benefits: it is non-invasive, easy to obtain, painless and there is no need to employ specially trained personal for sample collection.[4,5]

Calcium is an important skeletal mineral. Several studies indicate that salivary calcium and phosphate

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concentrations show a clear increase with increasing age. Calcium is the only electrolyte which does not show correlation with salivary flow rate.[6–8] Heavy smokers seem to have lower bone mineral density and higher salivary calcium than their non-smoking counterparts. The high salivary calcium concentration in smokers is related to skeletal calcium disturbances.[9] It has been reported that, in response to hormone replacement therapy, calcium concentration decreased ($P = 0.037$), sodium increased ($P = 0.019$), while no change was observed in the potassium concentrations in stimulated whole saliva during the follow-up period.[10]

The optimal strategies for screening postmenopausal women for osteoporosis are not clear.[11] The objective of this study was to measure salivary calcium concentration as a screening strategy and calculate the sensitivity, specificity and discriminant values of salivary calcium in postmenopausal osteoporosis.

MATERIALS AND METHODS

Study population

Eighty postmenopausal women participated in a case-control study. They had been consecutively referred to the Guilan University Centre for Metabolic Bone Diseases, Iran, for bone densitometry. The study group had to be functionally independent.

None of the women included in the study were receiving hormone therapy, selective estrogen receptor modulators (SERMs) or diuretics. The participants were aged between 41 and 75 years (mean 57.12 ± 8.04 years) and had not had a menstruation cycle for at least 12 months. None of the participants were smokers or obese (body mass index $\geq$ 24), had a systemic disease (including Sjögren's syndrome) and all were free of oral candidiasis. Forty patients with osteoporosis, *T*-score < -2.5 at the spine or hip DXA, formed the case group and 40 whose *T*-score was > -1 formed the control group. After obtaining all participants' informed consents, all tests were performed in a set sequence. The study protocol was approved by the Guilan University of Medical Ethics Committee. Participants signed informed consent forms before being recruited into this study.

Measurement of saliva secretion rates

The women were asked to answer a questionnaire describing a list of symptoms associated with xerostomia to confirm that there was no subjective dry mouth.

- Does the amount of saliva in your mouth seem too little?
- Does your mouth feel dry when eating a meal?
- Do you have difficulty swallowing any food?
- Do you sip liquids to aid in swallowing dry food?[5]

Participants answered no to these four questions. None complained of a burning sensation in the mouth.

All saliva secretion rates were measured between 8 am and 1 pm; they had to brush and floss in advance for the study. The participating individuals were instructed not to eat or drink or perform any oral hygiene procedures for at least 2 h prior to the measurements. Before saliva collection, they rinsed their mouths thoroughly with deionized water. A standardized protocol for collecting whole saliva was used.[4,12] The flow rate was calculated in g/min, which is almost equivalent to mL/min.

Calcium analyses

Saliva was then centrifuged (3600 *g*) and the supernatants of saliva were separated. After collection, the saliva calcium concentrations were immediately measured.

Whole saliva Ca^{+2} concentrations were assessed colorimetrically by atomic absorption spectrometry (Model 35, Perkin-Elmer, Norwalk, CT, USA) and using Arsenazona reaction with affiliated kits (Pars Azmon Diagnostics, Tehran, Iran). Findings are expressed in mg/dL.

Statistical analyses

For statistical analysis, the data are presented as means ± SD. Differences in characteristics between the case and control groups were compared using independent unpaired *t*-tests. Receiver operator characteristic (ROC) curve analyses were used to determine the optimal cut-off thresholds of salivary calcium. Chi-squared tests were used to compare calcium concentrations in the case and control groups. Sensitivity, specificity and their corresponding 95% confidence intervals (CI) were calculated at the recommended cut-off points. The positive predictive value (PPV) and negative predictive value (NPV) were estimated by considering 95% CIs. Predictive values were calculated in dichotomous 2 × 2 tables in case and control groups. The likelihood ratios (LR) with 95% CIs were calculated.

By means of multiple logistic regressions with considering cut-off point in unadjusted and adjusted model by age and time of menopause, the risks of osteoporosis were estimated. We calculated 95% CIs.

Statistical significance was set at $P < 0.05$. Hypothesis tests were two-tailed. All data were analysed by SPSS

version 16 (SPSS Inc., Chicago, IL, USA) and SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS

The study population consisted of 80 postmenopausal women, of whom 40 had osteoporosis (defined as a DXA-determined *T*-score below –2.5 at the hip or spine). The case and control group characteristics are shown in Table 1.

By independent *t*-tests, the mean salivary calcium concentrations in the case group were significantly higher than in the non-osteoporotic women ($P < 0.001$). The cut-off point for calcium in this study with Pearson chi-squared test was 6.084 ($P < 0.014$) (Table 2), with sensitivity 67.50 (95%CI 52.33–82.67) and specificity 60.00 (95%CI 44.62–75.38).

The ROC curve for identifying women with osteoporosis is plotted in Figure 1. The area under the curve (AUC) was 0.678 (95%CI 0.560–0.797) (Fig. 1). The PPV was 62.79 (95%CI 47.74–77.84) and the negative predictive value (the proportion of patients with a negative test who had no osteoporosis) was 64.86 (95%CI 49.27–80.46). Of the women with salivary calcium lower than the cut-off point, about 64.86% had no osteoporosis.

The positive LR and negative LR were 1.688 (95%CI 1.091–2.61) and 0.542 (95% CI 0.324–0.905), respectively. The odds ratio (OR) for women with osteoporosis and calcium over the cut-off point (6.1) was 3.8 (95%CI 1.29–7.78). The Pearson correlation revealed that there is a significant correlation between time of menopause with level of salivary calcium ($\chi^2 = 0.402$, $P < 0.014$). The risk of osteoporosis in postmenopausal women with salivary calcium over the cut-off would rise 3.12 times more than non-osteoporoses in an unadjusted model (OR: 3.12; 95%CI 1.29–7.76) while in an adjusted model the risk may increase 3.8 times.(OR: 3.8; 95%CI 7.78–1.29). By increasing per unit in salivary calcium the risk of osteoporosis may rise 1.6 times (OR: 1.68; 95%CI 0.6–4.7). The mean salivary calcium in women more than 10 years past menopause had higher risks (41.5 mg/dL *vs*. 18.7 mg/dL) rather than women under 10 years past menopause.

Table 1 Characteristics of case and control group participants

	Case group	Control group
Age, years (SD)	58.47 (7.24)	55.77 (8.65)
Time since menopause, Years (SD)	15.8 (6.64)	9.48 (6.14)
Salivary flow rate, mL/min (SD)	0.355 (0.236)	0.318 (0.242)
Ca^{++} mg/dL (95%CI upper–lower)	6.92 (95% CI 6.187–7.667)	5.63 (95%CI 6.273–5.001)

Table 2 Salivary calcium concentration in case and control groups

Cut-off Ca (mg/dL)	Non-osteoporosis *n* (%)	Osteoporosis *n* (%)	Total *n* (%)	Pearson
< 6.1	24 (60)	13 (32.5)	37 (46.25)	$\chi^2 = 6.084$
> 6.1	16 (40)	27 (67.5)	43 (53.75)	$P < 0.014$
Total	40	40	80	

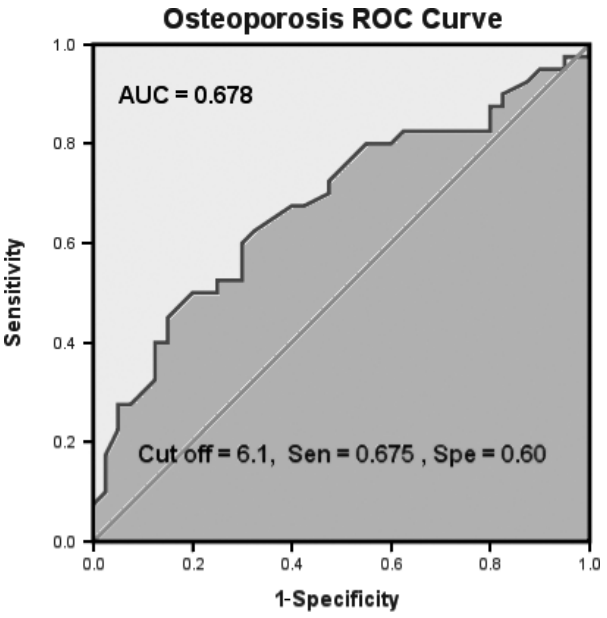

Figure 1 ROC curve for osteoporosis.

DISCUSSION

It is important to identify women at risk of osteoporotic fractures. Salivary calcium levels may vary in osteoporotic and osteopenic women compared with the controls.[14]

Our results reveal that calcium concentration significantly above the cut-off (6.1) occurred frequently in postmenopausal osteoporosis. This finding is in agreement with the findings of other investigators.[6–10,14] Salivary calcium concentration identified about 67.5% of patients with osteoporosis, while 60% of women with salivary calcium under the cut-off point were without osteoporosis. The AUC was 0.678, indicating

the probability of the correct risk rating of a randomly selected diseased/healthy pair of subjects. Osteoporosis was present in 62.7% of patients with a positive calcium concentration test above the cut-off; of the women with lower salivary calcium concentration than the cut-off, about 64.86% had no osteoporosis (NPV). A good screening test must have a high sensitivity, so as not to miss the few cases of disease that are present, and a high specificity, to reduce the number of false-positive results which entail further diagnostic testing.[13]

The sensitivity, specificity and AUC of salivary calcium in this study were similar to those of the QUS test (sensitivity 68, specificity 70 and AUC 0.72%). Nevertheless, sensitivity, specificity and AUC of digital X-ray radiogrammetry (DXR) and radiographic absorptiometry (RA) were higher than the corresponding values for salivary calcium.[1,14,15]

Sensitivity of 67.50 means that 67.5% of patients with salivary calcium concentration above the cut-off point (6.1 mg/dL) had osteoporosis, and specificity of 0.60 means that 60% of women with salivary calcium below the cut-off point did not have osteoporosis. The AUC (0.678) indicates the probability of correctly rating the risk of randomly selected diseased or healthy subjects (Fig. 1). This cut-off value would obviate the need to perform DXA in 62.79% of the population. An NPV of 64.8 indicates that 62.7% of patients with a salivary calcium concentration above the cut-off had osteoporosis, given that many postmenopausal women have one or more risk factors for osteoporosis.

Clinical prediction rules (CPRs) are designed to assist medical decision-making. Simple Calculated Osteoporosis Risk Assessment Estimation (SCORE) and the Osteoporosis Risk Assessment Instrument (ORAI) are two simple tests that have been applied in different populations. The CPRs were quite sensitive in population-based samples (98–100%), but none were very specific (10–40%). SCORE and ORAI were much more specific in postmenopausal women younger than 65 years compared with those 65 years or older.[1,13,16] A clinical tool such as Osteorisk is a valid tool for screening for women at low risk of osteoporosis, so they may avoid densitometry. It has sensitivity near that of salivary calcium (64%) but has lower specificity (6.7%, PPV 30.6 and NPV 89%).[17]

In recent decades, dentists have proposed different screening tools for identifying women at high risk of fracture. These include panoramic radiographic and computed tomography indices (CTI) on cone beam computed tomography (CBCT) images for an assessment of bone mineral density in postmenopausal osteoporotic women.[18–20] The CBCT images were obtained from the unilateral mental foramen region using a PSR-9000N^{TM} Dental CT system.[20] Measurements of mandibular inferior cortical shape and width detected on dental panoramic radiographs may be a useful screening tool for spinal osteoporosis in postmenopausal women. The sensitivity was 86–92% for cortical width and cortical shape, and specificity was 35.0–63.6% in postmenopausal women with or without histories of oophorectomy or estrogen use. Nevertheless, in comparison, salivary calcium had higher specificity, cost less and offered easier access.[21] Tooth counts are also another easy screening tool; fewer than 20 teeth can reasonably lead the clinician to screen further for osteoporosis but it requires further investigation.[22]

Screening is of interest when the specificity and sensitivity are good, and when screening has lower costs (monetary or clinical) than the diagnostic test that would otherwise be undertaken. Screening by salivary analysis is less disruptive for the person than a bone mineral density test, which requires radiography equipment. Using approximately current rates, salivary Ca^{++} concentrations costs can be estimated at about 1 euro, while bone mineral density costs about 30 Euro in Iran today.

Screening all women with DXA would be more effective than no screening or screening only women with at least one risk factor.[2] Many strategies for postmenopausal osteoporosis screening are effective and cost-effective, including strategies involving screening initiation at age 55 years.[11] Cost-effectiveness studies may be useful tools for helping decision-makers, and further models based on different assumptions should be performed to improve the level of evidence on cost-effectiveness ratios of usual screening strategies for osteoporosis.[2]

Using saliva rather than serum and radiographic findings has benefits: it is non-invasive, easy to obtain, painless and there is no need to employ specially trained personnel for sample collection. Salivary calcium concentration is an easy-access and low-cost alternative method that could help physicians reduce the need for DXA, the expensive gold standard examination for diagnosing osteoporosis.

Overall, our data suggest that salivary calcium may be at least as effective as pre-screening methods for targeting cases where DXA testing in high-risk postmenopausal women could be useful.

CONCLUSION

Our findings suggest that salivary calcium concentration and appropriate cut-off values may offer suitable sensitivity and specificity for screening postmenopausal women for osteoporosis.

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CONFLICT OF INTEREST

The authors report no conflicts of interests. The authors alone are responsible for the content and writing the paper.

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