Original Article

Salivary calcium as a diagnostic tool for screening of osteoporosis in postmenopausal women

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Abstract

Background: Women's health undergoes physiological, pathological and psychological changes after menopause. Reduced estrogen levels have been implicated in the pathogenesis of osteoporosis in postmenopausal women. Estrogen is also known to affect the salivary gland functions. To understand the association between serum estrogen, osteoporosis and salivary calcium, the present cross-sectional study was undertaken.

Aim: The aim of this study is to determine salivary calcium levels and its use for the diagnosis of osteoporosis in postmenopausal women.

Methodology: Ninety individuals divided into three groups of healthy controls, pregnant women and postmenopausal women were selected. Serum estrogen, salivary calcium and bone mineral density (BMD) at the heel region were estimated. Statistical analysis using the Mann–Whitney U-test was done to compare the results within the groups.

Results: Mean estrogen levels were 115.8 ± 80.18 pg/mmol in control group, 7729.4 ± 907.6 pg/mmol in pregnant group and 51.2 ± 74.51 pg/mmol in postmenopausal group, respectively. The mean salivary calcium in control, pregnant and postmenopausal groups was 3.12 ± 0.63, 3.19 ± 0.62 and 7.12 ± 0.79 µg/dl, respectively. Paired comparison within the groups showed high statistical significance ($P = 0.0000$) in the salivary calcium levels. The mean BMD of −2.3 (standard deviation [SD] ± 0.83) in the postmenopausal group was significantly lower than −0.6 (SD ± 0.99) and −0.2 (SD ± 1.42) of pregnant and control groups, respectively.

Conclusion: A negative correlation was found between estrogen and bone density. A significant difference in salivary calcium was noted in the study groups, highlighting the role of salivary calcium in the detection of early bone changes in postmenopausal women.

Keywords: Calcium, estrogen, osteoporosis, postmenopause, saliva

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INTRODUCTION

Osteoporosis is defined as a progressive systemic skeletal disorder characterized by low bone mineral density (BMD), deterioration of the microarchitecture of bone and an increased susceptibility to fractures.[1] The World Health Organization (WHO) (1994) has proposed a clinical definition of osteoporosis as –" A patient is osteoporotic

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if the BMD measurement is below 2.5 standard deviations of typical bone mass of young healthy white women."[2] Postmenopausal osteoporosis affects women who are postmenopausal but younger than 70 years of age. These women are said to have postmenopausal osteoporosis when the WHO BMD criteria are observed within 15–20 years after the onset of menopause.[1]

Several theories have been put forth for the underlying induction of high bone turnover after menopause. Direct action of estradiol on osteoclasts is the highly accepted one. At present, the diagnostic modalities for determination of osteoporosis include BMD estimation as done by dual-energy X-ray absorptiometry (DEXA), conventional radiography, quantitative computerized tomography and quantitative ultrasound. DEXA is considered to be the gold standard among these and its measurements given as a T-score is used as a reference to arrive at a diagnosis of osteoporosis (T-score ≤2.5). However, all these methods involve a high cost-to-benefit ratio coupled with exposure to radiation. Hence, a diagnostic tool that eliminates both these factors or at least considerably reduces them is desirable.[3,4]

Saliva has been used as a diagnostic fluid in medicine, and it meets the demand for inexpensive, noninvasive and easy to use diagnostic aids for oral and systemic diseases.[5] Calcium and phosphorous are present as the main inorganic components of saliva, which quantitatively accounts for the main mineral component of the human skeletal system. We, therefore, planned to explore the use of salivary calcium levels as a diagnostic biochemical marker for osteoporosis.

METHODOLOGY

After obtaining institutional ethical clearance, 90 individuals divided into three groups of 30 each of healthy controls, pregnant women and postmenopausal women were included in the study. Pregnant and postmenopausal women visiting the Outpatient Department of Gynecology and Obstetrics, Dr. Prabhakar Kore Hospital and MRC, Belgaum, were selected for the study.

Sample criteria

Group A – Healthy controls

Inclusion criteria – All women between the age groups of 20 and 35 years with regular menstrual cycle and body mass index (BMI) <24 were included in the study.

Exclusion criteria – Women with a history of any systemic illness, drug intake (e.g., hormonal replacement therapy [HRT] and calcium supplements) and tobacco use were excluded from the study.

Group B – Pregnant females

Inclusion criteria – Women in the age range of 20–35 years and with first pregnancy (across all the three trimesters) were included in the study. BMI criteria were not considered for pregnant individuals.

Exclusion criteria – Women with a history of oral contraceptive use prior to conception, drug intake (e.g., HRT and calcium supplements) and tobacco use were excluded from the study.

Group C – Postmenopausal women

Inclusion criteria – All women in the age group of 45–55 years and above, with the onset of menopause at least 1 year prior and BMI <30 and with a history of 2–3 parturitions only were included in the study.

Exclusion criteria – Women with a history of systemic illness, drug intake, calcium supplements and tobacco use were excluded from the study.

After obtaining written informed consent, each participant of the study was subjected to assessment of serum estrogen, salivary calcium and BMD.

Body mass index

BMI was calculated using the equation as below:[6]

$$BMI = (kg)/m^2$$

Where kg is weight in kilograms and m is height in meters.

Assessment of serum estrogen

Under aseptic measures, 5 ml of venous blood was drawn from the cubital vein and was transferred to gel-coated tubes for transport to the laboratory. Each sample was then centrifuged for 10 min at 3000 rpm. The supernatant serum obtained was then processed for quantitative estimation of estradiol using the Abbott's AxSYM automated system.

Assessment of salivary calcium

Collection of salivary sample

Two milliliters of unstimulated whole saliva was collected based on the standard procedure. The participants were asked to refrain from eating, drinking or any other oral hygiene procedures, for 1 h prior to the sample collection. Drinking water was then given to the participants to rinse their mouth. Five minutes after the oral rinse, 2 ml of unstimulated was collected in 50 ml sterile plastic sample containers. The samples were then subjected to biochemical estimation of calcium.

Estimation of salivary calcium levels

It was done using a commercially available kit Erba Mannheim, Trans Asia Biomedicals Ltd. The principle is based on O-cresolphthalein complexone (OCPC) method. Calcium, in an alkaline medium, combines with OCPC to form a purple-colored complex. The intensity of the color formed is directly proportional to the amount of calcium present in the salivary sample. After incubation of samples for 5 min, the absorbance values of standard and test samples were measured against the blank within 60 min by colorimeter under a wavelength of 570 nm.

Calculation – Calcium in mg/dl = Abs. T/Abs. S × 10

Assessment of bone mineral density

BMD at the heel region was measured by ultrasound (Paltech System CM200). Participants were asked to sit in the chair and place their foot on the machine. Subsequently, readings were recorded.

BMD is categorized on the basis of T-scores as follows

- Normal: T-score of −1
- Osteopenic: T-score of −1 to −2.5
- Osteoporotic: T-score is below −2.5.

T-score is the bone density compared with that expected in a normal healthy adult of matched age and sex. The T-score is the number of units – standard deviations (SDs) that the bone density is above or below the standard.

RESULTS

The mean age of women was 21, 23 and 57 years for the healthy control, pregnant and postmenopausal groups, respectively [Table 1]. The mean serum estrogen levels were 115.87 ± 80.19, 7729.37 ± 907.68 and 51.23 ± 74.52 pg/ml in the healthy control, pregnant and postmenopausal groups, respectively. Intergroup comparison among all the three groups revealed a highly significant ($P = 0.0000$) difference in the mean serum estrogen levels [Tables 2 and 3].

The mean salivary calcium levels were 3.12 ± 0.63, 3.19 ± 0.62 and 7.12 ± 0.79 μg/ml in the healthy control, pregnant and postmenopausal groups, respectively. Intergroup comparison among the three groups showed a highly significant ($P = 0.0000$) difference in the salivary calcium levels between the postmenopausal groups when compared with healthy control/nonpregnant and pregnant groups. The comparison between the healthy controls and the pregnant group with respect to salivary calcium levels was not significant ($P = 0.7901$) [Tables 4 and 5].

Table 1: Mean and standard deviation age of study participants according to groups

Group	Mean	Median	SD
Healthy control	21.90	21.00	2.59
Pregnant	22.80	22.00	2.43
Postmenopausal	57.13	56.00	7.52

SD: Standard deviation

Table 2: Comparison of three groups with respect to estrogen level (pg/ml) by Kruskal–Wallis one-way ANOVA by ranks

Group	Mean	Median	SD	Sum of ranks	*H*	*P*
Healthy control	115.87	92.00	80.19	1217.00	68.2624	0.0000*
Pregnant	7729.37	7834.00	907.68	2265.00		
Postmenopausal	51.23	22.50	74.52	613.00		

*Significant at 5% level of significance ($P<0.05$). SD: Standard deviation

The mean BMD expressed as T-score was 0.20 ± 1.42, −0.6633 ± 0.99 and −2.38 ± 0.83 for the healthy control/nonpregnant, pregnant and postmenopausal groups, respectively. Intergroup comparison showed a very highly significant ($P = 0.0000$) difference in the BMD in the postmenopausal group when compared with the healthy control/nonpregnant and pregnant groups. There was a significant difference ($P = 0.0333$) in the BMD score when the comparison between healthy controls and the pregnant group was made [Tables 6 and 7]. Comparison for all the groups showed a significant correlation between BMD and salivary calcium levels ($P = 0.0000$) and between salivary calcium and serum estrogen levels ($P = 0.0000$) [Table 8].

DISCUSSION

Menopause is defined as a permanent cessation of menstruation resulting from loss of ovarian follicular activity.[7,8] It is associated with wide-ranging implications on both systemic and oral health. Major oral symptoms of menopause are xerostomia, burning mouth and disturbances in taste perception and increased susceptibility to osteoporosis.[7,8] Osteoporosis, a chronic skeletal disorder, is characterized by a low bone mass and microarchitectural deterioration with a consequent increase in bone fragility and increased susceptibility to pathologic fractures. Osteoporosis has a strong negative influence on oral health as a whole and dental health in particular.[7,8]

The past five decades have focused on the assessment of markers such as alkaline phosphatase and osteocalcin in urine. Urinary calcium levels along with collagen degradation products served as indicators of bone resorption.[9,10] Increased understanding of the influence of estrogen on salivary calcium levels over the years[10] prompted us to investigate this potential importance of salivary calcium as a biomarker for osteoporosis in our study.

Table 3: Pairwise comparison of three groups with respect to estrogen level (pg/ml) by Mann–Whitney U-test

Group	Mean	Median	SD	Sum of ranks	*U*	*Z*	*P*
Healthy control	115.87	92.00	80.19	465.00	0.00	−6.6530	0.0000*
Pregnant	7729.37	7834.00	907.68	1365.00			
Healthy control	115.87	92.00	80.19	1217.00	148.00	−4.4649	0.0000*
Postmenopausal	51.23	22.50	74.52	613.00			
Pregnant	7729.37	7834.00	907.68	1365.00	0.00	−6.6530	0.0000*
Postmenopausal	51.23	22.50	74.52	465.00			

*Significant at 5% level of significance ($P<0.05$). SD: Standard deviation

Table 4: Comparison of three groups with respect to salivary calcium level (μg/ml) by Kruskal–Wallis one-way ANOVA by ranks

Group	Mean	Median	SD	Sum of ranks	*H*	*P*
Healthy control	3.12	2.85	0.63	897.00	59.6259	0.0000*
Pregnant	3.19	3.10	0.62	934.50		
Postmenopausal	7.12	7.30	0.79	2263.50		

*Significant at 5% level of significance ($P<0.05$). SD: Standard deviation

The sample comprised 90 females subdivided into three groups of 30 each. The study participants in the pregnant group comprised only those in their first pregnancy (across all three trimesters) and those in the postmenopausal group comprised participants who had attained menopause within a minimum of 1 year prior to the conduct of the study. This was done so as to minimize the effect of calcium deficiency which was time dependent and strongly correlated to the functional levels of estrogen in both these groups when compared to controls.

The BMI was standardized for all the three groups as per the Indian population standards (≤24 for the control group and ≤30 for the postmenopausal group; BMI was not applicable to the pregnant individuals).

Mean estrogen levels were observed to be 115.8 ± 80.18 pg/mmol in healthy control/nonpregnant group, 7729.4 ± 907.6 pg/mmol in pregnant group and 51.2 ± 74.51 pg/mmol in postmenopausal group, respectively [Tables 2 and 3]. This can be attributed to the physiological changes in the various stages of the reproductive life in women in the control and pregnant groups. During pregnancy, the placenta secretes both estrogen and progesterone with the levels of the former increasing by about 30 times the normal toward parturition.[7] Menopause is a burn out of the ovaries resulting in a cessation of estrogen synthesis resulting in a decrease below the critical levels and nearing almost to zero. Estrogen is produced in subcritical levels but only for a short time.

In our study, we observed that the mean salivary calcium levels in healthy control/nonpregnant, pregnant and postmenopausal groups were 3.12 ± 0.63, 3.19 ± 0.62 and 7.12 ± 0.79 μg/dl, respectively. Paired comparison within the three groups by the Mann–Whitney U-test revealed a high statistical significance ($P = 0.0000$) in the mean salivary calcium levels among healthy control/nonpregnant versus postmenopausal group and pregnant versus postmenopausal group. This can be explained on the basis of the fact that estrogen is believed to directly affect the physiologic absorption of calcium from the gut. A deficiency or a drastic fall in estrogen levels as seen in postmenopausal phase triggers a release of parathormone which in turn actively induces calcium resorption from the skeletal system increasing the serum levels of calcium[11] which in turn reflects in the raised levels of calcium in the saliva.

Leimola-Virtanen *et al*.[12] made a similar observation in their study that salivary calcium was estrogen dependent. Ben Aryeh *et al*.[13] and Nagler and Hershkovich[14] have also observed that salivary calcium levels are significantly higher in the elderly age group compared to the young.

Agha-Hosseini *et al*.[15] also demonstrated that the mean salivary calcium concentration in whole stimulated saliva was significantly higher in individuals with a feeling of oral dryness compared to controls. Hence, it can be conclusively presumed that there is a definite inverse correlation between salivary calcium levels and the quantum of salivary secretion which in turn is age and estrogen dependent.

Puskulian *et al*.[16] observed that calcium concentration in submandibular saliva was low during ovulation. They further observed that salivary calcium levels were lower during pregnancy than labor which directly correlated with the high estrogen levels. However, they could not appreciate any difference in the levels of salivary calcium between control and pregnant groups.

Sewón *et al*.[17] reported that salivary calcium concentration showed a significant reduction with HRT in healthy postmenopausal women. Sewón *et al*.[18] further observed that salivary calcium levels were significantly increased in women exhibiting a low BMD compared to controls.

Bone loss associated with estrogen deficiency in postmenopausal women is accompanied by increased

Table 5: Pairwise comparison of three groups with respect to salivary calcium level (μg/ml) by Mann–Whitney U-test

Group	Mean	Median	SD	Sum of ranks	*U*	*Z*	*P*
Healthy control	3.12	2.85	0.63	897.00	432.00	−0.2661	0.7901
Pregnant	3.19	3.10	0.62	933.00			
Healthy control	3.12	2.85	0.63	465.00	0.00	−6.6530	0.0000*
Postmenopausal	7.12	7.30	0.79	1365.00			
Pregnant	3.19	3.10	0.62	466.50	1.50	−6.6308	0.0000*
Postmenopausal	7.12	7.30	0.79	1363.50			

*Significant at 5% level of significance ($P<0.05$). SD: Standard deviation

Table 6: Comparison of three groups with respect to bone mineral density by Kruskal–Wallis one-way ANOVA by ranks

Group	Mean	Median	SD	Sum of ranks	*H*	*P*
Healthy control	0.2067	0.6500	1.4290	1904.00	44.938	0.0000*
Pregnant	−0.6633	−0.7500	0.9942	1586.00		
Postmenopausal	−2.3800	−2.6250	0.8367	605.00		

*Significant at 5% level of significance ($P<0.05$). SD: Standard deviation

bone resorption. This may partly be due to a reduction in the influence which estrogen exerts on osteoclasts and their precursors and also on the levels of chemical mediators such as interleukin (IL)-1. IL-6 and tumor necrosis factor-α all of which enhance osteoclastic activity which can be suppressed by administration of physiologic doses of estrogen.

Numerous studies have previously reported that increased urinary calcium levels are markers for bone resorptive activity and are a result of the increase in serum levels of calcium that in turn gets filtered and excreted in the urine.[7,8] A similar explanation can be given for increased salivary calcium levels as saliva can be considered an ultrafiltrate of plasma.

To endorse this alteration in the skeletal system under the influence of estrogen, we carried out an assessment of BMD in all the three groups. It was done using an ultrasound device at the heel region. We observed in our study that the mean BMD of −2.3 (SD ± 0.83) in the postmenopausal group was significantly lower than the −0.6 (SD ± 0.99) and −0.2 (SD ± 1.42) of pregnant and control groups, respectively. Further, there was a highly significant correlation ($P = 0.0000$) between the BMD score and salivary calcium levels indicative of the fact that a reduction in the mean BMD scores is associated with a concurrent increase in the salivary calcium levels. It was also found that there was also a highly significant correlation ($P = 0.0000$) between serum estrogen levels and salivary calcium. This establishes the scientific link between the three entities, that is, serum estrogen, BMD and salivary calcium.

Thus, we understand that there exists a definite interrelationship between serum estrogen, BMD and salivary calcium and that salivary calcium levels can be used as a risk marker for osteoporosis in postmenopausal women. It is a comparatively easy, safe, reliable, inexpensive and noninvasive method when compared to its current investigative peers such as serum or urinary calcium estimation and radiographic aids used in the diagnosis of osteoporosis.

CONCLUSION

Conclusions that can be inferred from the observations made in our study are that in the postmenopausal group demonstrated a significantly higher mean salivary calcium levels compared to the pregnant group and healthy controls indicative that it could be a potential indicator of osteoporosis. This was further endorsed with a concurrent reduction in the BMD also coinciding with the reduced serum estrogen levels in this group. The two parameters of salivary calcium and BMD, however, did not differ in the healthy controls when compared to the pregnant group. The salivary calcium levels exhibited a correlation with BMD among all the three groups; however, a negative correlation was seen between serum estrogen and salivary calcium only in the postmenopausal group. This substantiates the point that salivary calcium levels can definitely indicate the possibility of the presence or absence of osteoporosis in postmenopausal women. Its advantages outlined in this manuscript earlier, therefore, warrant its application as a diagnostic marker of osteoporosis on a routine basis in the future.

The observations made in our study are based on investigations conducted on a small sample size. However, they can be extrapolated to arrive at a scientific understanding of the interrelationship that exists between the triad of serum estrogen, salivary calcium and BMD during the pre- and postmenopausal life of the women population. The same needs to be re-examined and substantiated in a larger sample of population.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Table 7: Pairwise comparison of three groups with respect to bone mineral density by Mann–Whitney U-test

Group	Mean	Median	SD	Sum of ranks	*U*	*Z*	*P*
Healthy control	0.2067	0.6500	1.4290	1059.00	306.00	−2.1290	0.0333*
Pregnant	−0.6633	−0.7500	0.9942	771.00			
Healthy control	0.2067	0.6500	1.4290	1310.00	55.00	−5.8398	0.0000*
Postmenopausal	−2.3800	−2.6250	0.8367	520.00			
Pregnant	−0.6633	−0.7500	0.9942	1280.00	85.00	−5.3963	0.0000*
Postmenopausal	−2.3800	−2.6250	0.8367	550.00			

*Significant at 5% level of significance ($P<0.05$). SD: Standard deviation

Table 8: Correlation between bone mineral density, salivary calcium level (μg/ml) and estrogen level (pg/ml) by Karl Pearson's correlation coefficient in total samples (normal + pregnant + postmenopausal group)

Correlation between	Correlation coefficient	*t*	*P*
BMD and salivary calcium levels	−0.6357	−7.7252	0.0000*
BMD and estrogen levels	0.1401	1.3276	0.1877
Salivary calcium levels and estrogen levels	−0.4541	−4.7811	0.0000*

*Significant at 5% level of significance ($P<0.05$). BMD: Bone mineral density

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