Original Research

Salivary Calcium Concentration, A Cost Effective Diagnostic Resource For Predicting Post Menopausal Osteoporosis, A Hospital Based Study

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A R T I C L E   I N F O

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A B S T R A C T

Aim: To detect the levels of salivary calcium concentration in post menopausal osteoporotic female subjects.

Materials and methods: The study included 90 diagnosed osteoporotic female subjects with no previous history of mensturation for the past 12 months reporting to the OPD of Medical Hospital and Research Centre. As per the WHO guidelines, T score values were used to distinguish between normal, osteopenic and osteoporotic female subjects. Each group included 30 subjects. The subjects were asked to rinse there mouth several times with water and relax for five minutes. 5 ml of saliva was collected in 30 ml air tight plastic containers using spitting method. Commercially available kit   Cresolpthalein complexone (CPC, Crest Bio systems, India) was used to estimate the salivary calcium levels. Statistical analysis was done using two way ANOVA test. P value of < 0.05 was considered to be statistically significant.

Results: The mean salivary calcium levels for osteoporotic, osteopenic and control groups were 7.35 mg/dl, 6.80 mg/dl and 4.59 mg/dl respectively. There was significant increase in salivary calcium levels among the osteoporotic and osteopenic female subjects when compared to the normal female subjects.

Conclusion: The present study revealed correlation of salivary calcium levels among osteoporotic, osteopenic and normal female subjects. Highest salivary calcium levels were detected in osteoporotic female subjects compared to the osteopenic and normal female subjects. Salivary calcium levels can be used as a screening tool to distinguish the women with or without osteoporosis.

INTRODUCTION

Identifying women with osteoporosis remains a clinical challenge. Osteoporosis is a risk factor for all women aged more than 50 years. It is a very common skeletal disease that progressively reduces bone mass density and changes its micro-architectural structure. This loss of bone mass increases the risk for future fractures[1]. World Health Organization (WHO) defines osteoporosis, osteopenic and normal female subjects based on the T scores. Normal females have T score greater than -1.0,

Osteopenic females have between -1.0 and -2.5 and Osteoporotic females have T score -2.5 or below.[2] Many histologic and radiographic researches, on jaw bones have demonstrated positive correlation between jaw bone loss and osteoporosis. Other methods to detect the loss of bone mass density are Dual-photon absorptiometry (DPA), Single-photon absorptiometry (SPA), Quantitative CT (QCT) and more recently is the Dual-energy X-ray absorptiometry (DEXA). However, these are expensive methods which increase the treatment

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cost.[3]. The gold standard test for osteoporosis is Dual energy X ray Absorptiometry (DEXA) due its high accuracy and precision. In recent years, saliva based diagnostic tests have gained popularity due to the painless procedure, non- invasive method, low cost screening test and does not require trained personels for the sample collection.[4] Recently salivary biomarkers have been used to assess the risk for jaw fractures, infections like measles, mumps, oral cancer, leukoplakia, oral candidiasis, herpes infection caused by HIV 1 virus, dental caries, periodontal diseases, breast cancers, drug monitoring, hormonal levels, diabetes and Sjogren's syndrome [5]. Salivary collection requires no special skills nor any special equipments for its storage. These type of methods have high patient acceptance as compared to the collection of other fluids such as blood. Stimulated saliva is collected by asking the patient to chew paraffin wax or by application of citric acid on the patient's tongue [5,6]. Saliva collected by the stimulatory method not only affects the concentration and constituents of saliva but also the pH of the fluid. Unstimulated saliva is collected without any exogenous masticatory and gustatory stimulation. Various methods to collect saliva are draining, spitting, suction, and swabbing method. The best two ways to collect whole saliva are the spitting method, in which the subject expectorates the saliva in a test tube and the draining method, in which saliva is allowed to drip saliva off the lower lip.[5,6,7] In this study unstimulated saliva was collected using spitting method.

MATERIALS AND METHODS

SOURCE OF DATA: The unstimulated saliva of the subjects was collected from the OPD of Medical Hospital and Research Centre. The ethical clearance was obtained from the Institutional Ethical Committee.

INCLUSION CRITERIA

Diagnosed osteoporotic, osteopenic and normal female subjects with no history of mensturation for past 12 months were selected from the Hospital and Medical Research Centre. Informed consent was obtained from the subjects.

EXCLUSION CRITERIA

The following exclusion criteria were considered for the selection of the subjects.

Patients with systemic diseases such as hyperpara thyroidism, salivary gland disorders such as sialadenitis, hormone replacement therapy, oral candidiasis were excluded from the study. Patients on drugs that alter salivary flow such as diuretics and anti cholinergics were also excluded from the study.

METHOD OF COLLECTION OF THE SALIVA

A total of 90 female subjects were included in the study. All the subjects underwent DEXA at the Radiology Department of the Hospital. The subjects were divided into three equal groups, according to the T score given by WHO for Normal, Osteopenic and Osteoporotic female subjects. Each group with 30 female subjects were divided into normal(group I), osteopenic (group II) and osteoporotic(group III). The subjects were given instructions not to eat or drink for one hour before the collection of saliva. Before the collection of saliva, they were asked to rinse their mouth several times with water to remove any remaining food particles followed by relaxation for five minutes. Unstimulated saliva was collected by spitting method. In spitting method, the subjects had to expectorate the saliva after every five minutes for another fifteen minutes. Approximately, 5 ml of saliva was collected in an air tight 10 ml plastic beaker. The plastic beakers were labeled with the

subject's name and dispensed immediately to the Basic research laboratory (BSR) at the Institution. Saliva was then transferred from the plastic beaker using micropippete into another fresh beaker. Later it was centrifuged at the rate of 200 rotations per second to separate the food debris and other large particles from the clear saliva fluid. Calcium was estimated using a commercially available kit Cresolpthalein complexone (CPC, Crest Bio systems, India) using a semi automatic analyzer.

STATISTICAL ANALYSIS

The statistical analysis was carried out using Tukeys multiple prosthoc procedures and one way ANOVA among the three groups.

RESULTS

Comparison of salivary calcium values among group I, group II and group III are shown in Table 1 and 2. The normal standard salivary calcium value as per the literature is 5.2 mg/dl (1.35mMol/L).The mean salivary calcium levels for osteoporotic group, osteopenic group and normal groups were 7.35 mg/dl, 6.80 mg/dl and 4.59 mg/dl respectively. There was significant increase in salivary calcium levels between the osteoporotic and osteopenic female subjects when compared to the normal female subjects. The above findings revealed negative correlation of the salivary calcium with the bone mass density. Highest mean salivary calcium level was observed in the osteoporotic female subjects suggesting that the rate of salivary calcium levels increases among the postmenopausal women.

Pair wise comparisons of the three groups with calcium values in mg/dl by Tukeys multiple posthoc procedures is shown in Table 1. There is statistically significant difference between group I and II, group I and III, group II and III ($p<0.05$).

Pair wise comparisons of the three groups by one way ANOVA is shown in Table 2, revealing significant difference among the three groups. ($p<0.05$)

Figure1 shows standard deviation and comparison of the mean salivary calcium values among the three groups. Highest mean salivary calcium level was present in the osteoporotic group.

Table 1 shows pair wise comparisons of the three groups with calcium values in mg/dl by Tukeys multiple posthoc procedures
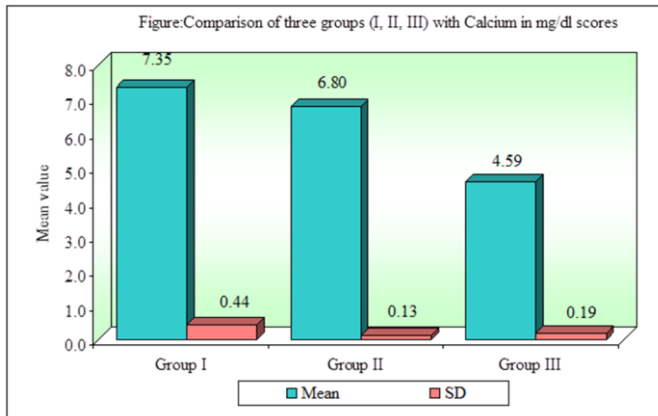
	Group I	Group II	Group III
Mean	7.35	6.80	4.59
SD	0.44	0.13	0.19
Group I	-		
Group II	P = 0.0008*	-	
Group III	P = 0.0001*	P = 0.0001*	-

*$p<0.05$

Table 2 shows pair wise comparisons of the three groups by one way ANOVA

Source of the variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	P-value
Between groups	2	42.74	21.3680	254.8246	0.0001*
Within groups	27	2.26	0.0839		
Total	29	45.00			

*$p<0.05$

Fig 1: shows standard deviation and comparison of the mean salivary calcium values among the three groups

DISCUSSION

Calcium is one of the main constituents of the bones. It plays a major role in the bone remodeling.[8] Among postmenopausal women, osteoporosis is a common skeletal disease. Osteopenia and osteoporosis are characterized by increased bone fragility and decreased bone mass density. The World Health Organization (WHO) defines osteoporotic, osteopenic and normal female subjects on the basis of T scores. The T scores indirectly measures the Bone Mineral Density(BMD)[2,9]. In the anterior mandible, jaw bones have higher densities as compared to the density of the jaw bones in the posterior mandible. Hardness and density of the jaw bones are of great importance as it affects the outcome of the implant procedures. Osteoporosis if not diagnosed at an early stage may lead to accidental jaw fractures during implant procedures and failure of the implants.[10] Resorption of the bone may also affect the periodontal health status of an individual causing mobility and early tooth loss.[11] Osteoporosis not only determines the success of the implant procedures, bone grafting but also causes difficulties during the extractions of the teeth and incidence of pathologic fractures.[12]

Literature reveals various biochemical markers being discovered for the resorption of the bones. Phosphorus, calcium, osteocalcin and alkaline phosphatase are the most commonly used biochemical markers for the diagnosis of osteoporosis.[13] However, the procedures to detect the biochemical markers are expensive, invasive and painful. Estimating calcium in the saliva is gaining popularity as it is an easy, non-invasive, inexpensive, less painful and no trained or skilled people are required for the saliva collection.[14, 15]

In the present study, the mean salivary calcium levels for osteoporotic, osteopenic and control groups were 7.35 mg/dl, 6.80 mg/dl and 4.59 mg/dl respectively. There was significant increase in the salivary calcium levels among the osteoporotic and osteopenic female subjects compared to the normal female subjects. A similar study was done by Reddy et al who found the salivary calcium levels in osteoporotic and control groups were 7.36 mg/dl and 4.57 mg/dl respectively.[5] These values were almost similar to the values of present study which strongly suggested that the calcium bone loss has a positive correlation with the increase in the salivary calcium levels. However, the mean salivary calcium levels in the osteopenic females was 7.80 mg/dl which was not in accordance with results of the present study. This difference could be attributed to smaller sample size, racial differences and lack of technique sensitive calcium estimation method.

Another study was conducted by Rabiei et al, who also found increased salivary calcium levels in the osteoporotic women compared to the women without osteoporosis. The above study strongly suggests the positive correlation of the salivary calcium levels in the osteoporotic females which was similar to the results of present study.[16]

Wowern *et al* conducted a study to find relationship between the osteoporotic women and periodontal status. The study reported a greater loss of attachment in the osteoporotic women than the normal women[17]. Periodontal health status of the subjects was not included in the present study and hence its relationship among the osteoporotic women was not assessed. Since the present study revealed that the salivary calcium levels among osteoporotic women were highest, thus indirectly suggesting the loss of calcium from the jaw bones and the compromised periodontal health status of affected women.

The present study demonstrated that the salivary calcium level had increased among the post menopausal osteoporotic women when compared to the normal women. Hence, salivary calcium levels can be used as a screening tool to distinguish the women with or without osteoporosis.

The limitations of the study were smaller sample size, lack of the precise method to estimate the salivary calcium levels and standardization. Future studies should be encouraged with larger sample size and more accurate methods to authenticate the correlation of osteoporosis with salivary calcium levels.

ACKNOWLEDGMENTS

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