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Original Article

Dentistry Section

Evaluation of Correlation between Salivary Calcium, Alkaline Phosphatase and Osteoporosis- A Prospective, Comparative and Observational Study

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ABSTRACT

Introduction: Fixed and/or removable prosthodontics caters to the restorative needs of the largest count of geriatric patients whose rehabilitative procedures depend on the quality and quantity of available bone. The common diagnostic parameters for quantifying bone may involve an invasive blood examination, an expensive bone densitometry procedure or a urine analysis. Sialometry involving the basic biochemistry of saliva analysis may be proposed as an alternative to the conventional investigative protocol and its usefulness may be attributed owing to its non invasive and simpler procedure.

Aim: The aim of this study was to compare the salivary calcium and alkaline phosphatase among osteoporotic, osteopenic and normal edentulous subjects.

Materials and Methods: A prospective, comparative and observational study was carried out on 80 selected edentulous subjects (40 males and 40 females) aged 55-75 years (30 osteoporotic, 30 osteopenic and 20 control). A sample of saliva was taken for the study. Salivary calcium was measured by Arsenazo reaction; and alkaline phosphatase by the International Federation of Clinical Chemistry (IFCC) method. Statistical analysis was done by unpaired t-test. A comparison was made in the levels of salivary calcium and alkaline phosphatase with that of osteoporosis and osteopenia.

Results: The mean salivary calcium and alkaline phosphatase levels were found to be significantly higher in the osteoporotic and osteopenic edentulous subjects as compared to the control group.

Conclusion: Salivary calcium and alkaline phosphatase are increased significantly in case of osteoporosis and osteopenia. Hence, screening of salivary samples of patients may be an effective indicator for the detection of underlying disorders of bone metabolism.

Keywords: Edentulous patient, Osteoporotic patients, Salivary analysis, Screening

INTRODUCTION

Fixed and/or removable prosthodontics caters to the restorative needs of the largest count of geriatric patients whose rehabilitative procedures depend on the quality and quantity of available bone. The common diagnostic parameters for quantifying bone may involve an invasive blood examination, an expensive bone densitometry procedure or a urine analysis [1,2].

The systemic health is reflected by the oral cavity more than any other part of the body. The assessment of salivary components both qualitative and quantitative may serve as important diagnostic tools for underlying systemic diseases and also to monitor the normal body functioning [3].

The chief mineral components of the skeletal system i.e., calcium and phosphorus are also found as components of saliva. Along with these alkaline phosphatase is also secreted in saliva. All these can serve as markers for turnover of bone [4,5].

The techniques currently available for measurement of bone mass in vivo are expensive as well as invasive. They may also be not very effective for screening of general population. Therefore, saliva, the ultrafiltrate of plasma can serve as an efficient, simple, economic and noninvasive resource [4].

Hence, an early diagnosis and intervention strategy will definitely contribute to better and more predictable treatment outcomes.

The present study was aimed to estimate the salivary calcium and alkaline phosphatase levels and correlate the findings in normal and osteoporotic subjects.

The null hypothesis for the present study was that a decrease in skeletal bone density may increase the amount of calcium and alkaline phosphatase in saliva.

MATERIALS AND METHODS

A prospective, comparative and observational study was carried out which consisted of 80 completely edentulous patients. Forty males and 40 females in the age range of 55-75 years who reported to the Department of Prosthodontics, College of Dental Science and Hospital, Indore, Madhya Pradesh, India, over a period of five months (from December 2015 to April 2015) for complete denture treatment participated in the study.

As per the previous studies on osteoporotic females [4], the maximum sample size was 45. A sample size larger than this was to be chosen. The number of patients reported to the hospital within our research period was 95 of which 15 were excluded. Thus, a sample size of 80 was selected.

Inclusion criteria: The patient should be a non smoker without a history of oral candidiasis and without any other systemic diseases like osteomalacia, arthritis, multiple myeloma and primary or secondary hyperparathyroidism. The subject must not be receiving any hormone therapy, diuretics or Selective Estrogen Receptor Modulators (SERMs).

Ethical clearance was obtained from Institutional Ethical Committee which was in accordance with Declaration of Helsinki. The procedure was explained to the subject and voluntary written informed consent was obtained. The subjects were then assessed for the Bone Mineral Density (BMD) by a Dual X-ray Absorptiometry (DEXA) scan.

Grouping: As per the World Health Organization (WHO), BMD of spine is categorized as follows: Normal if T score is above -1.0; Osteopenic if T score is between -1 and -2.5 and Osteoporotic if T score is below -2.5 [6]. Based on this criteria and according to the BMD results the subjects were assigned to three groups:

Group I (the osteoporotic group) comprised of 30 established osteoporotic edentulous subjects (12 males and 18 females) with T score of < - 2.5 on DEXA scan.

Group II (the osteopenic group) comprised of 30 established osteopenic edentulous subjects (12 males and 18 females) with T score of -1 and -2.5 on DEXA scan.

Group III (control group) which comprised of 20 subjects (16 males and four females) without osteoporosis (T score > -1.0 on DEXA scan) or without any of the aforementioned disease.

METHODOLOGY

Salivary calcium and alkaline phosphatase levels were estimated for all the three groups. Prior to salivary sample collection, patients were instructed not to eat or drink anything for at least two hours. Saliva was collected by stimulating salivary flow by making the patient chew on a standard cube of paraffin wax (10x10x2 mm) [2,7]. Approximately 5 ml of saliva was collected in a test tube and tested for calcium and alkaline phosphatase levels. Calcium was estimated by a commercially available kit (Accucare Calcium) containing arsenazo III system which functions on the following mechanism: Calcium with arsenazo III at neutral pH yields a blue colored complex. This was done with the help of semi autoanalyser which digitally displays the values. (All tests performed at Joshi Pathology lab. Mhow, Indore, India).

Alkaline phosphatase was estimated by using a commercially available alkaline phosphatase kit (ACCUCARE ALKALINE PHOSPHATASE-SLR, manufactured by LAB-CARE DIAGNOSTICS (INDIA) PVT. LTD.), which functions as per the standards of IFCC method. The analyses of-values were done by a semi auto analyser [Table/Fig-1] (ErbaChem 5 Plus).

STATISTICAL ANALYSIS

The data were recorded on a Microsoft Excel sheet (Microsoft Office Excel; Microsoft Corp, Redmond, WA, USA) for statistical evaluation using a commercial program (Mini Tab version 7.0). Patient's salivary calcium levels and alkaline phosphatase levels were tabulated and the values were compared using unpaired t-test and ANOVA. A prospective, comparative and observational study was carried out. A p-value of <0.05 was taken as statistically significant. The software used for statistical analysis was IBM SPSS version 20.0.

RESULTS

[Table/Fig-2] shows the comparison of salivary calcium and alkaline phosphatase levels in control, osteopenic and osteoporotic groups. There is a statistically significant difference among these values showing that the levels of salivary calcium and alkaline phosphatase in control group is less than that of osteoporotic and osteopenic groups.

[Table/Fig-3] shows the comparison of mean values of salivary calcium and alkaline phosphatase between control-osteopenic groups, control-osteoporotic groups and osteoporotic-osteopenic groups. The differences are statistically significant indicating that values of both salivary calcium and alkaline phosphatase are higher in osteoporotic and osteopenic groups as compared to control groups.

[Table/Fig-4] shows the mean values of salivary calcium and alkaline phosphatase in males and females of control, osteoporotic and osteopenic groups along with their standard deviations.

[Table/Fig-1]: Semi autoanalyser.

Character	Control		Osteoporotic		Osteopenic		F-value	p-value
	Mean	±SD	Mean	±SD	Mean	±SD		
Salivary calcium	4.25	0.45	8.42	1.33	7.67	1.06	120.86	≤0.001 HS
Alkaline phos-phatase	57.84	5.90	168.94	8.11	163.44	7.79	1535	≤0.001 HS

[Table/Fig-2]: Mean values and respective comparisons between control, osteoporotic and osteopenic groups. (Test applied: ANOVA).
*HS – Highly Significant (p<0.05= statistically significant; p<0.001= highly significant);
SD – Standard Deviation

Group	Mean	Mean	t-value	p-value
Salivary calcium				
Control and Osteoporotic	4.25 (control)	8.42 (osteoporotic)	17.28	0.001 HS
Control and Osteopenic	4.25 (control)	7.67 (osteopenic)	17.10	0.001 HS
Osteoporotic and Osteopenic	8.42 (osteoporotic	7.67 (osteopenic)	2.67	0.009 Sig
Alkaline phosphatase				
Control and Osteoporotic	57.84 (control)	168.94 (osteoporotic)	53.40	0.001 HS
Control and Osteopenic	57.84 (control)	163.44 (osteopenic)	49.74	0.001 HS
Osteoporotic and Osteopenic	168.94 (osteoporotic)	163.44 (osteopenic)	2.64	0.010 Sig

[Table/Fig-3]: Descriptive table representing the mean values of salivary calcium and alkaline phosphatase amongst males and females in control group, osteoporotic group and osteopenic group.
(test applied: unpaired t-test) HS – Highly significant Sig – Significant; p<0.001= highly significant

A comparison of salivary calcium and alkaline phosphatase levels of osteoporotic males and females, osteopenic males and females and males and females of the control group shows no significant difference indicating that there is no effect of gender on the salivary calcium and alkaline phosphatase values of the same group [Table/Fig-5].

[Table/Fig-6,7] show mean values and intergroup comparisons of salivary calcium and alkaline phosphatase among males and females. There is a statistically significant difference among these values showing that the levels of salivary calcium and alkaline phosphatase in males and females of control group is less than that of osteoporotic and osteopenic groups.

DISCUSSION

The prosthodontic rehabilitation of the currently increasing geriatric population is largely governed by the quality and quantity of the

Character	Control				Osteoporotic				Osteopenic			
	Male		Female		Male		Female		Male		Female	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Salivary calcium	4.19	0.45	4.42	0.47	8.66	1.22	8.26	1.19	7.94	0.92	7.47	0.94
Alkaline phosphatase	56.40	5.79	62.14	4.18	170.83	8.53	167.68	7.51	163.03	8.12	163.75	8.44

[Table/Fig-4]: Mean values of salivary calcium and alkaline phosphatase in males and females of all the groups.

Group	Mean	Mean	t-value	p-value
	Control and male	Control and female		
Salivary calcium	4.19	4.42	0.96	0.345 NS
Alkaline phosphatase	56.40	62.14	2.03	0.058 NS
Osteoporotic male and female				
Salivary calcium	8.66	8.26	0.88	0.383 NS
Alkaline phosphatase	170.83	167.68	1.06	0.294 NS
Osteopenic male and female				
Salivary calcium	7.94	7.47	1.36	0.183 NS
Alkaline phosphatase	163.03	163.75	0.23	0.815 NS

[Table/Fig-5]: Comparison of means of salivary calcium and alkaline phosphatase among males and females of various groups. (test applied: unpaired t-test).
NS – Non Significant

Group	Mean	Mean	t-value	p-value
Salivary calcium				
Control Vs Osteoporotic	4.19 (control)	8.66 (osteoporotic)	12.04	<0.001 HS
Control Vs Osteopenic	4.19 (control)	7.94 (osteopenic)	13.41	<0.001 HS
Osteoporotic Vs Osteopenic	8.66 (osteoporotic)	7.94 (osteopenic)	1.67	0.106 NS
Alkaline phosphatase				
Control Vs Osteoporotic	56.40 (control)	170.83 (osteoporotic)	41.47	<0.001
Control Vs Osteopenic	56.40 (control)	163.03 (osteopenic)	40.41	<0.001
Osteoporotic Vs Osteopenic	170.83 (osteoporotic)	163.03 (osteopenic)	2.34	0.02 sig

[Table/Fig-6]: Mean values and intergroup comparison of salivary calcium and alkaline phosphatase among males.
(Test Applied: unpaired t-test). HS – Highly Significant Sig – Significant NS – Non Significant
p<0.001= highly significant

Group	Mean	Mean	t-value	p-value
Salivary calcium				
Control Vs Osteoporotic	4.42 (control)	8.26 (osteoporotic)	6.95	<0.001 HS
Control Vs Osteopenic	4.42 (control)	7.47 osteopenic)	6.89	<0.001 HS
Osteoporotic Vs Osteopenic	8.26 (osteoporotic)	7.47 osteopenic)	2.16	0.037 Sig
Alkaline phosphatase				
Control Vs Osteoporotic	62.14 (control)	167.68 (osteoporotic)	29.82	<0.001 HS
Control Vs Osteopenic	62.14 (control)	163.75 osteopenic)	25. 69	<0.001 HS
Osteoporotic Vs Osteopenic	167.68 (osteoporotic)	163.75 osteopenic)	1.45	0.154 NS

[Table/Fig-7]: Mean values and intergroup comparison of Salivary Calcium and alkaline phosphatase among females.
(Test Applied: unpaired t-test). HS – Highly Significant Sig – Significant; p<0.001= highly significant

available bone. Osteoporosis, a chronic bone disease, commonly affecting the elderly, may often remain unreported. Osteoporosis often results in low bone mass with micro-architectural deterioration, resulting in increased fragility of the bones thus increasing the susceptibility to fracture [6-8]. Evidence suggests that osteoporosis and osteopenia have a direct effect on jaw bones, resulting in resorption and reduction in bone density [4]. Generalized osteoporosis also has a significant deleterious effect on the periodontal health status, thus compromising tooth support. The osseointegration of implants may also be compromised in osteoporotic individuals. It has been variously postulated that, apart from the various other local and systemic factors, osteoporosis remains a primary reason causing residual ridge resorption; dietary calcium insufficiency and excess phosphorus consumption further contributes to the disease progression [9]. The proper management of osteoporosis involves early diagnosis of the disease through complementary tests, assessment of serum and urinary markers for bone turnover, analysis of the risk of bone fracture and monitoring of response to therapy. It thus becomes imperative that edentulous elderly patients be routinely screened for osteoporosis as a prognostic indicator for successful rehabilitation. Conventional diagnostic parameters include urine analysis or radiographic bone densitometry. The capability to detect and assess the initiation and progression of any underlying illness through a non-invasive approach remains one of the primary aims of the health-care researcher and provider. Hence, evaluation of saliva, an ultra-filtrate of plasma, could be used as a simple and effective diagnostic and screening procedure, the basis being that saliva is an interstitial fluid from blood capillaries which enters via the salivary gland ducts wherein it is modified from isotonic into hypotonic fluid [1]. Screening by salivary analysis offers the advantages of being less invasive, easily obtainable and comparatively inexpensive. Also, it can be carried out as a chair-side procedure alongside routine dental treatment or at the time of case history recording. Further, it eliminates the need to employ specially trained personnel for sample collection [5]. Hence, salivary screening was used as a simple diagnostic tool for evaluating systemic health status in this study.

The resorption of bone leads to release of calcium in serum which is filtered into urine and excreted [4]. Likewise the present study also shows an increased level of salivary calcium and alkaline phosphatase in saliva of osteoporotic and osteopenic edentulous subjects which is an ultrafiltrate of plasma. Hence, salivary parameters should be used as predictors for these diseases and further investigation should be done to support any definite conclusions [4].

Many studies have been performed to assess bone turnover markers for the prediction of bone loss and to evaluate the correlation of markers with bone mineral density [4,9,10]. The common markers for osteoporosis and osteopetrosis include calcium, phosphorus, type I collagen related peptides, osteocalcin and alkaline phosphatase which are assessed in the blood. Biochemical markers of bone turnover are said to be related to the current bone mass and help in predicting future bone loss. Calcium and phosphorus which quantitatively account as the main mineral component of the human skeletal system are present as inorganic components in the saliva [10]. Several previous studies however have reported that there is no significant difference in salivary phosphorus levels between osteoporotic and non-osteoporotic individuals [10]. Hence, in the present study salivary calcium and alkaline phosphatase levels alone were compared between normal, osteopenic and osteoporotic individuals in order to determine a significant correlation if present.

The results of the present study revealed that there was a significant increase in the salivary calcium and alkaline phosphatase levels in the osteoporotic and osteopenic individuals when compared to healthy controls. This is in accordance with the previous studies. Reddy et al., reported that the normal/standard value mentioned as per literature was 5.2 mg/dl (1.35 mMol/L) for salivary calcium. According to their study there was a significant increase in calcium levels of the osteoporotic and osteopenic patients when compared to the healthy controls [4].

A study was conducted by Sewon L et al., to analyse the effect of smoking on salivary calcium and bone density. It was concluded from the study that high salivary calcium concentration in smokers is in connection with skeletal calcium disturbances. Decrease of skeletal bone density may increase the amount of calcium in saliva [11].

Ross PD et al., and Taguchi A et al., reported that the levels of serum total alkaline phosphatase and bone-specific alkaline phosphatase are increased in subjects with low BMD [10,12]. In the same way the present study also showed significantly higher levels of salivary alkaline phosphatase in osteoporotic subjects. However, since there are no standard mean values published in literature for salivary alkaline phosphatase, the values obtained in the osteoporotic subjects were compared to those of the control group.

Overall, our data suggest that salivary calcium and alkaline phosphatase may be at least as effective as pre-screening methods for targeting cases where DEXA testing in osteoporotic edentulous individuals could be useful.

LIMITATION

Estimation of the sample size was done on the basis of previous studies. No statistical formula was used to estimate the sample size. Thus, further studies can be planned by determining the sample size statistically.

The present study did not include phosphorus as a diagnostic parameter. Phosphorus being an important skeletal component should be taken into consideration in further studies.

CONCLUSION

The findings of the present study suggest that assessment of salivary parameters such as calcium and alkaline phosphatase can serve as important indicators for detection of an underlying disease of bone metabolism and can serve as a pre screening method for targeting cases where DEXA scanning could be useful.

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