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Original Research Paper

# Evaluating the efficacy of salivary and serum calcium, phosphorus, vitamin D and alkaline phosphatase level in post-menopausal women for assessing the risk of osteoporosis

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**ABSTRACT**:
Osteoporosis is a condition that leads to weakened and brittle bones, postmenopausal women are at increased risk due to the decline in oestrogen, a hormone that helps protect bone density. This study was undertaken to investigate the changes in salivary and serum calcium, phosphorus, vitamin-D and alkaline phosphatase for assessing the risk of osteoporosis in post-menopausal women. The aim of this study was to compare serum and saliva calcium, phosphorus, vitamin-D and Alkaline phosphatase level and to compare the change in serum levels with those in saliva. The study was conducted in the department of Biochemistry, Rajas Dental college and hospital and was carried out on 15 selected post-menopausal women aged 45-60 years and 15 healthy women volunteers of age group 20-25 years. Quantitative analysis of Calcium, Phosphorus, Vitamin-D and Alkaline Phosphatase was calculated. Statistical analysis of independent t test was used. The mean serum Calcium, Phosphorus, ALP levels and mean salivary Calcium and Phosphorus were significantly higher in the post-menopausal women. Based on the P value, there was no significant difference between groups regarding all the parameters expect salivary ALP and Vitamin-D. Calcium, Phosphorus, Vitamin-D and Alkaline Phosphatase appear to be associated with risk of osteoporosis in Post-menopausal women.

***Keywords: Osteoporosis, Menopause, Calcium, phosphorus, alkaline phosphatase***.

## INTRODUCTION:

Osteoporosis is a progressive systemic skeletal disease which is associated with reduced bone mass/density and micro architectural deterioration of bone tissue in human body[1]. It is usually diagnosed by weakened bones and as cause of pain and debilitating fractures[2]. Osteoporosis is called a "silent disease" because it progresses without symptoms until a fracture occurs. The fractures caused by osteoporosis have a great impact on public health, as they are often associated to increased morbidity, mortality, reduced quality of life, long hospital stays and high economic cost[3]. Menopause is described as a permanent cessation of menstruation caused by a decrease in ovarian follicular activity. It has far-reaching ramifications for both systemic and dental health. Xerostomia, a burning tongue, changes in taste perception, and a higher risk of osteoporosis are some of the oral symptoms of menopause. Low bone mass and microstructural degradation are the hallmarks of osteoporosis, a chronic skeletal disorder that makes bones more brittle and susceptible to pathologic fractures. Dental health specifically and oral health in general are both significantly harmed by osteoporosis [4,5]. Saliva has long been utilized in medicine as a diagnostic fluid because it satisfies the need for low-cost, non-invasive, and simple diagnostic tools for both oral and systemic disorders[6].The main inorganic elements of saliva are calcium and phosphorus, which quantitatively make up the majority of the minerals that make up the human skeletal system. As a result, we intended to investigate the potential of using salivary calcium levels as a biochemical diagnostic marker for osteoporosis[7]

## MATERIALS AND METHODS:

After obtaining institutional ethical clearance, Group-I comprising of 15 post-menopausal women of age group 45 to 60 years and Group-II comprising of 15 young healthy individuals of age group 20-25 years were included in the study. The criteria for inclusion were postmenopausal women ranging in age from 45 to 60 years. Women below 45 years and above 60

years of age and those with a history of any systemic illness, drug intake (eg: hormonal replacement therapy, calcium supplements, Vitamin -D supplements) steroid therapy, bisphosphonates therapy and tobacco use were excluded from the study. Stimulated saliva is collected by rinsing 10 ml of warm water by passive drooling method in addition to that,5 ml of blood is collected from the anti- cubital vein and kept in EDTA coated vacutainers under aseptic measures. Quantitative analysis of salivary and serum calcium, phosphorus, vitamin-D and alkaline phosphatase level was estimated. Calorimetric method use chemical reagents that produce intense violet color changes in the presence of calcium ions. One commonly used reagent for calcium determination is O-cresolphthaleincomplexone (OCPC), which forms a colored complex with calcium ions. The intensity of the color change determines the amount of calcium present in the sample[8].The ammonium molybdate method is a commonly used technique for the estimation of phosphorus (phosphate) levels in various biological samples, including serum and saliva. This method is based on the formation of a blue colored complex between phosphate ions and ammonium molybdate under specific acidic conditions. The intensity of the color produced is proportional to the concentration of phosphate ions in the sample[9]. Immunoturbidimetric assay was used for the estimation of Vitamin-D. In an immunoturbidimetric assay for vitamin D, antibodies specific to vitamin D or its metabolites are coated onto latex particles. When these particles encounter vitamin D in a sample, they form aggregates or complexes, leading to an increase in turbidity. The intensity of the turbidityis proportional to the concentration of vitamin D in the sample[10]. The pNPP (p-nitrophenyl phosphate) method is a common enzymatic assay used to measure alkaline phosphatase (ALP) activity in biological samples. It involves the hydrolysis of p-nitrophenyl phosphate by ALP to form p-nitrophenol, which produces a yellow color that can be measured spectrophotometrically and is directly proportional to the amount of ALP activity in the sample.[11]. The statistical test used was Independent t test in the SPSS software version 21. Pearson correlation test was used for comparison of the serum and saliva. P value less than 0.05 is considered as statistically significant.

## RESULTS:

Comparative evaluation between two groups revealed a statistically significant result in mean serum Calcium, Phosphorus, Vitamin -D and Alkaline phosphatase level. In saliva, intergroup comparison reveals statistically significant result for calcium and phosphorus and shows insignificant result for ALP and Vitamin-D. The mean values of salivary and serum are listed in Table 1 and Table 2.

**Table 1: Intergroup Comparison (Serum)**

| | | N | Mean | Standard Deviation | p-value |
|---|---|---|---|---|---|
| Calcium (µg/ml) | Group 1 | 15 | 114.03 | 22.64 | 0.001* |
| | Group 2 | 15 | 97.15 | 8.79 | |
| ALP (U/L) | Group 1 | 15 | 95.84 | 25.85 | 0.007* |
| | Group 2 | 15 | 88.98 | 25.24 | |
| Vitamin - D (ng/ml) | Group 1 | 15 | 18.00 | 2.16 | 0.024* |
| | Group 2 | 15 | 21.19 | 6.52 | |
| phosphorus (µg/ml) | Group 1 | 15 | 38.80 | 8.83 | 0.017* |
| | Group 2 | 15 | 33.74 | 5.52 | |
| p-value based on Independent-*t*-test<br>* = Statistically Significant ($p < 0.05$) | | | | | |

Table 1: Mean values and respective comparison between postmenopausal women and young individuals in serum.

**Table 2: Intergroup Comparison (Saliva)**

| | | N | Mean | Standard Deviation | p-value |
|---|---|---|---|---|---|
| Calcium (µg/ml) | Group 1 | 15 | 8.93 | 1.52 | 0.045* |
| | Group 2 | 15 | 5.71 | 2.94 | |
| ALP (U/L) | Group 1 | 15 | 8.55 | 2.82 | 0.127 |
| | Group 2 | 15 | 9.33 | 2.35 | |
| Vitamin - D (ng/ml) | Group 1 | 15 | 18.66 | 2.27 | 0.291 |
| | Group 2 | 15 | 20.08 | 3.02 | |
| phosphorus (µg/ml) | Group 1 | 15 | 135.83 | 14.20 | 0.036* |
| | Group 2 | 15 | 125.65 | 6.63 | |
| p-value based on Independent-*t*-test<br>* = Statistically Significant ($p < 0.05$) | | | | | |

Table 2: Mean values and respective comparison between postmenopausal women and young individuals in saliva.

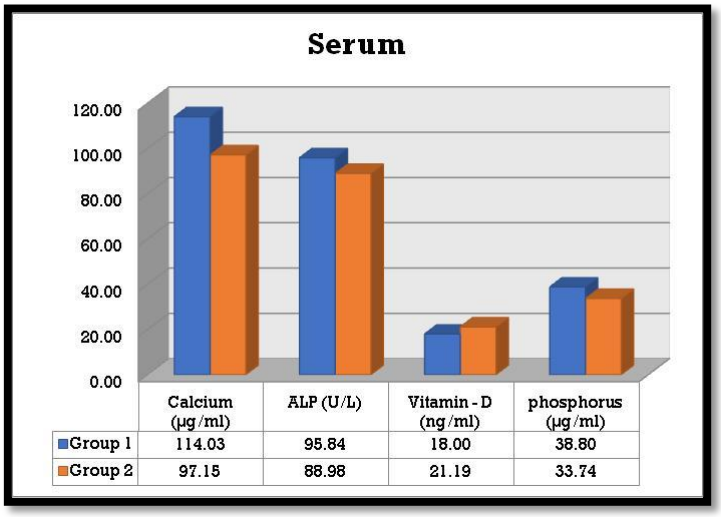


**Chart 1: The mean serum calcium, ALP, Vitamin-D and Phosphorus of Post menopausal women and young healthy individuals.**

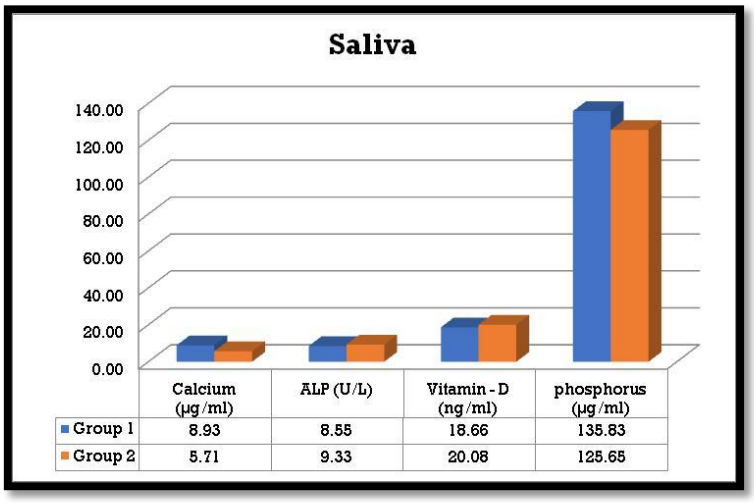


**Chart 2: The mean salivary calcium, ALP, Vitamin-D and Phosphorus of Post-menopausal women and young healthy individuals.**

## DISCUSSION:

Osteoporosis, a chronic bone disease that primarily affects the elderly, is frequently under reported. It causes low bone mass with micro-architectural degeneration, resulting in increased bone fragility and susceptibility to fracture[12,13,14].

The result of the present study revealed that there was a significant increase in serum and salivary calcium, phosphorus, alkaline phosphatase and Vitamin -D levels were significantly lower in post-menopausal women than the younger age group.

This is in accordance with the previous studies Sewón et al. [15] further observed that salivary calcium levels were significantly increased in women exhibiting a low BMD compared to controls.

Shweta Vinayak Kumbhojkar et al.[16] conducted a study by dividing the subjects into three groups of healthy controls, pregnant women and postmenopausal women to determine salivary calcium levels and its use for the diagnosis of osteoporosis in postmenopausal women and concluded that a negative correlation was found between estrogen and bone density. Asignificant 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.

Sewón et al. [17] conducted a study and reported that salivary calcium concentration was found to be reduced in healthy postmenopausal women.

Rabiei M et al [18] carried out a case control study in 40 postmenopausal women with osteoporosis and 40 women without osteoporosis and demonstrated the salivary calcium concentration discriminates between women with and without osteoporosis and constitutes a useful tool for screening for osteoporosis.

Ben Aryeh et al. [19] and Nagler and Hershkovich[20] have also observed that salivary calcium levels are significantly higher in the elderly age group compared to the young.

Heike A. Bischoff-Ferrari et al [21] and Lips P et al [22] reported positive association between 25-hy droxy vitamin D levels and bone mineral density. The consequences of Vitamin-D deficiency are secondary hyperparathyroidism and bone loss, leading to osteoporosis and fractures, mineralisation defects, which may lead to osteomalacia in the long term.

Agha-Hosseini F et al [23] conducted a case–control study was carried out on 60 selected menopausal women aged 45–79 years and found that the mean serum phosphorus and alkaline phosphatase, stimulated and unstimulated saliva calcium and alkaline phosphatase levels were significantly higher in the menopausal women and there were no significant differences between groups regarding saliva phosphorus and serum calcium concentration.

Bijender Kumar Bairwa et al [24] conducted a study to investigate the changes in salivary and serum calcium and alkaline phosphatase in osteoporosis patients and demonstrated a serum calcium has strong positive correlation with salivary calcium (r=0.726) while serum ALP and salivary ALP had weak positive correlation (r =0.453) and concluded that saliva can be used to measure calcium level instead of serum as it is non-invasive, quick and easy method.

Saha MK et al [25] and showed that the mean salivary calcium and alkaline phosphatase levels were found to be significantly higher 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.

Overall, our data suggest that salivary calcium, phosphorus, vitamin-D and alkaline phosphatase may be at least as effective as serum estimation for assessing the risk of osteoporosis in post-menopausal women.

## CONCLUSION:

Conclusions that can be inferred from the observations made in our study are that in the postmenopausal group demonstrated a significantly higher mean levels of calcium, phosphorus and ALP compared to young healthy individuals. Osteoporosis which is a silent disease needs more awareness and corrective measures. Diagnosis and monitoring of this disease often needs painful invasive procedures such as biopsies and repeated blood draws. Saliva based molecular biomarkers bypass these measures. Here we compared the serum and salivary biomarkers for the earlier detection of osteoporosis to explore the importance of saliva as a diagnostic tool.


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## CONFLICT OF INTEREST: Nil


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