

Original Research Article

A comparative study of salivary and serum calcium and alkaline phosphatase in patients with osteoporosis

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Received: 10 April 2019

Accepted: 04 May 2019

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ABSTRACT

Background: This study was undertaken to investigate the changes in salivary and serum calcium and alkaline phosphatase in osteoporosis patients. The objective was to compare the change in serum levels with those in saliva.

Methods: The study was conducted in the department of biochemistry, National Institute of Medical Sciences and Hospital, Shobha Nagar, Jaipur, Rajasthan, India. Subjects were selected from department of orthopedics, National Institute of Medical Sciences and Hospital, Shobha Nagar, Jaipur, Rajasthan, India. At the same time one hundred adult osteoporosis patients confirmed by DEXA were taken. Calcium and alkaline phosphatase were measured in serum and saliva of each patient. The data obtained was statistically analyzed.

Results: Serum calcium has strong positive correlation with salivary calcium ($r=0.726$) while serum ALP and salivary ALP had weak positive correlation ($r=0.453$).

Conclusions: Saliva can be used to measure calcium level instead of serum as it is non-invasive, quick and easy method.

Keywords: Calcium and alkaline phosphatase, Osteoporosis

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 In the developed world, depending on the method of diagnosis, 2% to 8% of males and 9% to 38% of females are affected.³ Rates of disease in the developing world are unclear.⁴ About 22 million women and 5.5 million men in the European Union had osteoporosis in 2015.⁵

Osteoporosis primarily affects older people, particularly women, and is associated with 80% of fractures in people older than age 60 years. 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.⁶

Menopause is a physiological process occurring due to decrease in levels of estrogen, in the fifth decade of life in women, involving permanent cessation of menstruation. Menopause is accompanied by physiological and sensorial oral changes in select individuals. The prevalence of oral symptoms was found to be significantly greater in menopausal women (43%) than in premenopausal women (6%).⁷ Osteoporosis have greater risk in women after menopause and certain oral changes like xerostomia and burning mouth syndrome, which are a caused of dry mouth, lead to decrease salivary flow.⁸ Osteoporosis is asymptomatic and the condition usually presents only after bone fracture and is usually associated with low trauma ‘fragility fractures. Osteoporotic (fragility) fractures are fractures that result from

mechanical forces that would not ordinarily result in fracture. Osteoporotic fractures are defined as fractures associated with low bone mineral density (BMD) and include spine, forearm, hip and shoulder fractures.⁹ BMD of the hip is a stronger predictor of future fracture risk than spine BMD. The risk of fracture increases 1.5-3 times each standard deviation of BMD (T score) below the reference population.¹⁰ Normal BMD is indicated by a T score of 1 to -1, while a T score ≥ -2.5 is diagnostic for osteoporosis. T score values between -1 and -2.5 identify a condition known as osteopenia which is associated with low to medium fracture risk, but frequent progression to osteoporosis. All cases of osteoporosis an imbalance exists between bone resorption and formation, the rate of bone formation is often normal, whereas resorption by osteoclasts is increased.¹¹

X-ray absorptiometry (DEXA) is presently considered the gold standard imaging technique which is used for the early detection of osteoporosis and risk of fracture, which is expensive and difficult in result interpretation.¹² Saliva is important for maintenance of the oral tissues health and can be used for assessing the hormones levels, drugs, and inflammatory factors. Saliva contains organic and inorganic components which may vary both qualitatively and quantitatively. So, saliva examination may be a new tool for the diagnosis of osteoporosis.¹³

In a longitudinal study, Sewon L et al, suggested that salivary calcium concentration decreases in stimulated saliva when hormone replacement therapy was initiated in menopausal women. They concluded that this may indicate that individual salivary calcium concentration is modified and/or regulated by factors other than salivary flow.¹⁴ Wardrop RW et al, also reported that menopausal women with oral discomfort were relieved of symptoms after systemic hormone replacement therapy, supporting the fact that there is a correlation between oral discomfort and level of hormones in menopausal women.¹⁵

Calcium is an important nutrient which is essential for bone health. Resorption of bone may lead to diffusion of calcium into blood and further into the saliva.¹⁶ Increased salivary Ca can be used as a potential screening tool for assessing the risk for osteoporosis. According to Maryam Rabiei M et al, the highest salivary calcium level is 6.1 mg/dl and above which (i.e. >6.1 mg/dl) can be used as a screening tool to identify osteoporosis risk in postmenopausal women.¹⁷

Alkaline phosphatase and Ca level in osteoporosis

Alkaline phosphatase is the enzymes mainly derived from the liver, bones and in lesser amounts from intestines, placenta, kidneys and leucocytes. Alkaline phosphatase enzyme plays an important role in bone metabolism and bone homeostasis by probably accumulating calcium ions and matrix vesicles during calcification process. Along with alkaline phosphatase, calcium also plays a major role in the bone homeostasis. Level of calcium depletes

with age thereby resulting in the reduction of bone strength. Thus, blood levels of alkaline phosphatase and calcium become inconsistent with age especially in females. Saliva considered as an ultra-filtrate of serum can overtake blood as a proxy due to its non-invasive nature and can be used for estimation of alkaline phosphatase and calcium levels.¹⁸⁻²⁰ Serum calcium and alkaline phosphatase (ALP) are the bone turnover markers which help in bone formation and mineralization.²¹ Menopause and ageing are known to associate with accelerated loss of cortical bone. Bone loss occurs when the balance between formation and resorption is upset and resorption is excessive resulting in a negative remodeling balance.²² Post-menopausal stage and ageing alter the serum calcium and ALP levels. Bhattra et al, reported the decreased level of serum calcium in postmenopausal women compared with premenopausal women and ALP level was found to be slightly higher among postmenopausal women, these two are the key marker of bone mass reduction.²³

METHODS

This study was approved by the ethical committee of the institution. This study was conducted from June 2018 to December 2018 in the period of 7 months. The study population comprised of multiethnic groups of patients from Jaipur district. The sample consisted of 100 patients in the age range of 35 to 70 years of both sexes. The patients were taken from the outpatient department of orthopedics, NIMS medical college and hospital, Shobha Nagar, Jaipur, Rajasthan, India.

Clinical assessment

Each patient in osteoporotic group was asked about the history of systemic diseases, history of intake of drugs (hormonal therapy) and DEXA scan analysis report and their detailed health questionnaire was completed in our pre decided format. In case of female patients, they were asked about duration and age of menopause. Samples of blood and saliva were collected from all the patients for the measurement of calcium and alkaline phosphatase. The level of calcium and alkaline phosphatase in serum was compared with that in saliva. Serum total calcium was measured calorimetrically using ready to use kit (Arsenazo method).²⁴ Serum alkaline phosphatase was measured by using ready to use para nitro phenol reagent kit of human (Kinetic method).

Inclusion criteria

- Adult patients which are clinically diagnosed cases of osteoporosis of both genders.

Exclusion criteria

- Patients older than 40 years, who had not diabetes or metabolic disorders, hypertension, thyroid disorders and oncological disorders, who were taking

continuous medication other than that for the treatment of osteoporosis, and who refused to sign the informed consent form were excluded from the study.

Statistical analysis

The paired student’s t test was used to compare serum and saliva levels and P<0.05 was considered significant. Pearson’s correlation coefficient was used to find correlation between serum analytes and salivary analytes.

RESULTS

Mean±SD serum calcium in osteoporosis patient was 9.7±0.4mg/dl. The calcium level in saliva of the patients was 7.8±0.5mg/dl. The level in saliva was lower but there was a strong positive correlation (r=0.7) between the serum level and the salivary level. The Mean±SD alkaline phosphatase in serum and saliva respectively was 250.24±57.04IU/L and 31.5±6.9IU/L. The level in saliva was much lower. The correlation between serum alkaline phosphatase and salivary alkaline phosphatase was positive but weak. Hence, saliva may not be a substitute to serum for measurement of alkaline phosphatase.

In Table 1, correlation between serum calcium and salivary calcium in osteoporosis patients is shown in which Pearson’s correlation analysis showed a significant (r=0.0726) highly positive correlation of calcium in serum and saliva in osteoporosis patients with a p-value of <0.001.

Table 1: Calcium and alkaline phosphatase (ALP) levels in serum and saliva of 100 osteoporosis patients (values are Mean±SD).

Parameters	Mean± SD
Serum Ca (mg/dl)	9.7±0.4
Salivary Ca (mg/dl)	7.8±0.55
Serum ALP (IU/dl)	250.24±57.04
Salivary ALP (IU/dl)	31.55±6.9

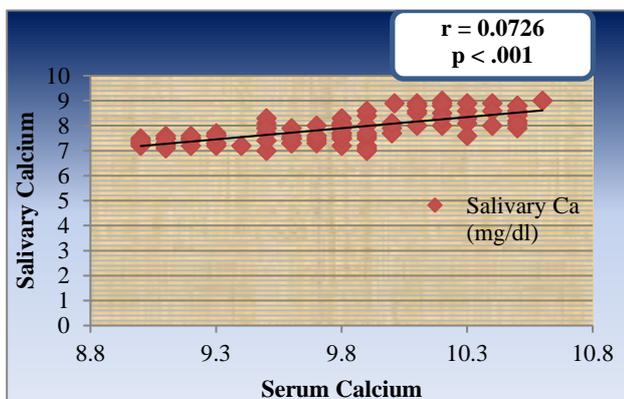


Figure: 1 Correlation of calcium (mg/dl) in serum and saliva in osteoporosis patients.

Figure 1 correlation between serum calcium and salivary calcium in osteoporosis patients is shown in which Pearson’s correlation analysis showed a significant (r = 0.0726) highly positive correlation of calcium in serum and saliva in osteoporosis patients with a p-value of <0.001.

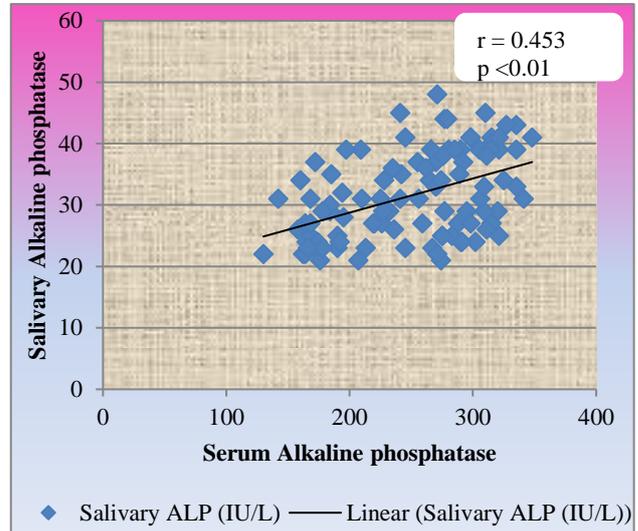


Figure: 2 Correlation of alkaline phosphatase (IU/L) in serum and saliva in osteoporosis patients.

In Figure 2, correlation between serum alkaline phosphatase and salivary alkaline phosphatase in osteoporosis patients is shown in which Pearson’s correlation analysis showed a significant (r = 0.453) but low positive correlation of calcium in serum and saliva in osteoporosis patients with a p-value of <0.01.

DISCUSSION

Calcium and phosphorus which quantitatively account as the main mineral component of the human skeletal system are present as inorganic components in the saliva. Calcium, phosphorus, type I collagen related peptides, osteocalcin and alkaline phosphatase are the common markers for osteoporosis 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. 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 bone mineral density.^{25,26}

The changes in serum calcium and alkaline phosphatase in osteoporosis are well documented but whether similar changes occur in salivary calcium and alkaline phosphatase is not known. This study was conducted to reveal whether changes in salivary calcium and alkaline phosphatase are similar to those in serum calcium and alkaline phosphatase and whether saliva can be used as a substitute to serum for measurement of calcium and alkaline phosphatase.

Calcium is the most important salivary electrolytes due to its effective role in bone structure and bone formation. It plays a significant role in bone regeneration, which is directly related to osteoporosis, a change that may occur as a consequence of reduced absorption of this electrolyte, which may occur in either the bone or saliva. Moghadam et al. did not find any correlation between salivary calcium and low bone mineral density. This difference in results may be due to the different age of patients in each sample and to the study designs as well.²⁷

Rabiei M et al, studied a group of similar patients and assessed salivary calcium, applying a cutoff point of 6.1 mg/dL. Salivary calcium concentration demonstrated that about 67.5% of the patients had osteoporosis, while 60% of women with salivary calcium levels below the cut-off point were free of osteoporosis. They came to the conclusion that salivary calcium can be used to diagnose bone mineral changes, thus obviating the need for bone densitometry.²⁸ The resorption of bone leads to release of calcium in serum which is filtered into urine and excreted. Reddy S et al, studied that an increased level of salivary calcium and alkaline phosphatase in saliva of osteoporotic 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.²⁹ Our results show that serum calcium had positive correlation with salivary calcium with an r value of 0.726 which showed strong correlation. Since salivary calcium levels paralleled serum calcium levels, authors can use salivary calcium as a diagnostic tool as it is non-invasive, quick and easy method of sample collection. Pearson analysis of serum alkaline phosphatase and salivary alkaline phosphatase revealed an r value of 0.453 which showed positive but weak correlation. Therefore, authors cannot use salivary alkaline phosphatase as a substitute for serum alkaline phosphatase.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Bairwa BK, Sagar M, Gupta RC, Gupta M. A comparative study of salivary and serum calcium and alkaline phosphatase in patients with osteoporosis. *Int J Res Med Sci* 2019;7:2412-6.