DOI:10.21276/sjodr.2016.1.3.2

Saudi Journal of Oral and Dental Research
Scholars Middle East Publishers
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

ISSN 2518-1300 (Print)
ISSN 2518-1297 (Online)

Original Research Article

Salivary Alkaline Phosphatase and Calcium – Diagnostic Marker for Bone Resorption in Post-Menopausal Women

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Abstract: Alkaline Phosphatase comprises a group of enzymes that catalyze the hydrolysis of phosphate esters in an alkaline environment. This enzyme plays an important role in the bone metabolism. Alkaline Phosphatase acts as a by-product and its serum level directly corresponds to the osteoblastic activity. The aim of the present study was to measure the serum and salivary alkaline phosphatase levels to detect the rate of bone resorption. In the present clinical study, serum and saliva samples were collected from the female patients between the age groups of 30-70 years and subjected for the biochemical analysis of alkaline phosphatase and calcium in semi-auto analyzer and were analyzed statistically. Serum and salivary alkaline phosphatase and calcium levels were decreased gradually with progressive age. By the present study, we concluded that levels of alkaline phosphatase and calcium will decrease with advancing age and saliva can be used as a diagnostic tool alternate to serum.
Keywords: salivary alkaline phosphatase, serum Alkaline Phosphatase, diagnostic tool

INTRODUCTION

The word ‘menopause’ is derived from two Greek words, ‘meno’ (month) and ‘paus’ (to stop) and is defined as the “permanent cessation of menses resulting from reduced ovarian hormone secretion that occurs naturally or induced by surgery, chemotherapy, or radiation”. This stage is generally considered as an oestrogen deficient stage [1]. Menopause and age are directly related to accelerated bone loss which results in the negative bone remodelling.

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. It is a well known fact that, levels 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 leverage and can be used in estimation of Alkaline Phosphatase and Calcium levels [2-4].

AIM AND OBJECTIVE

The aim of the present study was to estimate and correlate the Alkaline Phosphatase and Calcium levels in the serum and saliva of the women between 30-70 years of age.

METHODOLOGY

The study group included a total of 75 subjects which were divided into 4 groups based on their age. Group - I: 30-40 years, Group - II: 41-50 years, Group - III: 51-60 years and Group - IV: Above 60 years.

Table 1: Distribution of Sample

Groups	Age (years)	Number of patients
I	30-40	22
II	41-50	21
III	51-60	15
IV	Above 60	17

METHODOLOGY

5 ml of intravenous blood was collected in test tube and 5ml of unstimulated whole saliva was collected by spitting method from the study subjects in a sterile container. Both blood and saliva were centrifuged at 2500 rpm for 5 minutes to obtain serum and supernatant saliva. The supernatant was then separated into 1 ml aliquots and subjected for further biochemical analysis using the Alkaline Phosphatase kit (Liquixx-M, Erba Mannheim) and Calcium kit (Liquizyme, Beacon Diagnostics pvt. Ltd) in semi-autoanalyser (Prietest, Robonik). The level of Alkaline Phosphatase and Calcium in both the saliva and serum were tabulated and statistically analyzed.

RESULTS

The mean value of serum and salivary alkaline phosphatase was gradually decreased as age advances with the statistically significant P value (<0.0001) (Fig-1 & 2) (Table - 2). The mean value of serum and salivary calcium was also decreased as age advances with the statistically significant P value (<0.0001) (Fig - 3 & 4) (Table - 3).

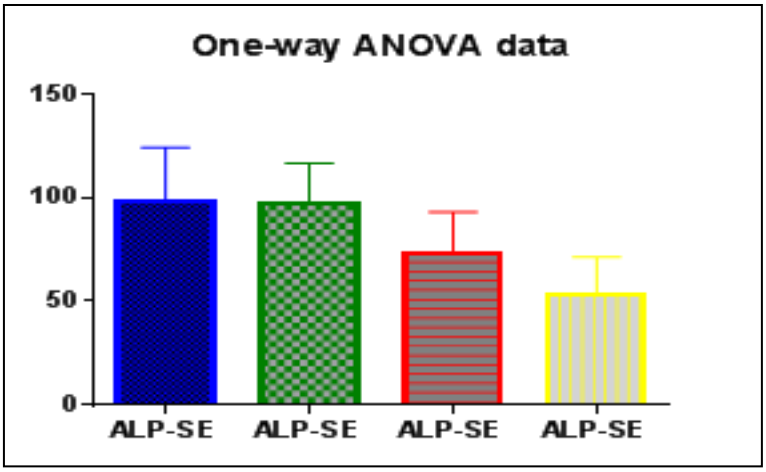

Fig-1: Showing the comparison of the Serum Alkaline Phosphatase levels within the study groups (ALP-SE-Serum Alkaline Phosphatase)

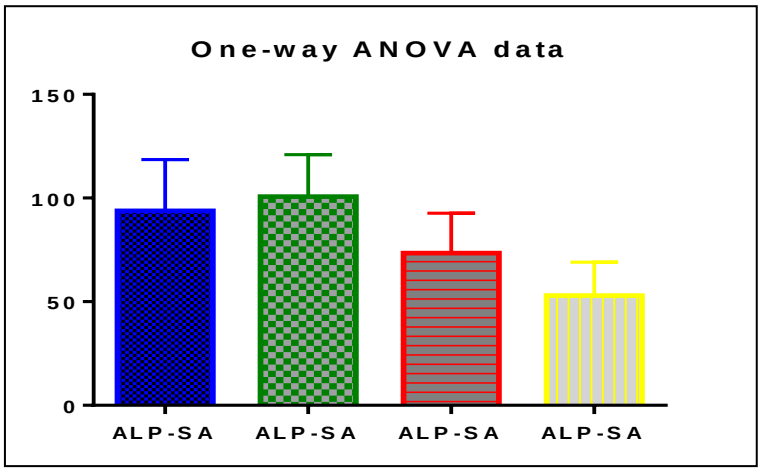

Fig-2: Showing the comparison of the Salivary Alkaline Phosphatase levels within the study groups (ALP-SA-Saliva Alkaline Phosphatase)

Table 2: Alkaline Phosphatase levels in serum and saliva

AGE GROUPS	SERUM	P VALUE	SALIVA	P VALUE
30-40	98±26.4	< 0.0001***	93.8±24.7	< 0.0001***
40-50	96.7±19.8		100±20.2	
50-60	73±20		73.3±19.2	
Above 60	52±18.3		52.9±16	
*** = SIGNIFICANT				

SERUM ALKALINE PHOSPHATASE			
DF	MS	F VALUE (DFn, DFd)	P VALUE
3 71 74	8581 472.7	F (3,71)=18.15	P < 0.0001

SALIVARY ALKALINE PHOSPHATASE			
DF	MS	F VALUE (DFn, DFd)	P VALUE
3 71 74	8616 427.8	F (3,71)=20.14	P < 0.0001

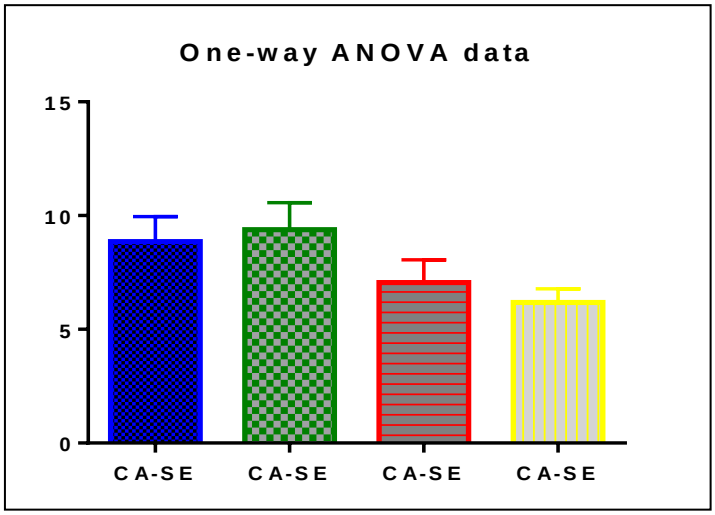

Fig-3: Showing the comparison of the Serum Calcium levels within the study groups (CA-SE- Calcium serum)

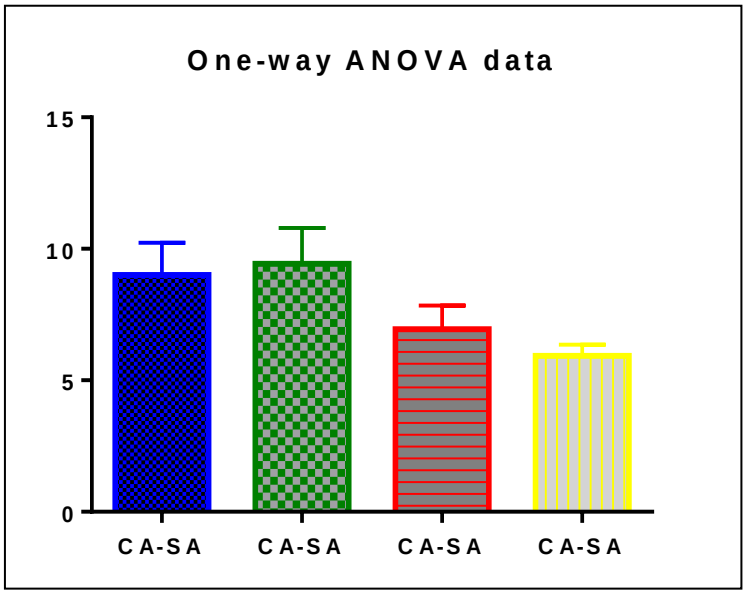

Fig-4: Showing the comparison of the Salivary Calcium levels within the study groups (CA- SA- Calcium saliva)

Table 3: Calcium levels in serum and saliva

AGE GROUPS	SERUM	P VALUE	SALIVA	P VALUE
30-40	8.8±1.1	<0.0001***	9±1.2	<0.0001***
40-50	9.3±1.1		9.4±1.3	
50-60	7±0.9		6.9±0.9	
Above 60	6±0.6		5.9±0.4	

*** = SIGNIFICANT

SERUM CALCIUM

DF	MS	F (DFn, DFd)	P VALUE
3 71 74	41.95 1.027	F(3,71)=40.85	P < 0.0001***

SALIVARY CALCIUM

DF	MS	F (DFn, DFd)	P VALUE
3 71 74	51.75 1.166	F(3,71)=44.39	P < 0.0001***

Positive correlation was seen while comparing the serum alkaline phosphatase and serum calcium with Pearson correlation coefficient in which P = < 0.0001 and r = 0.6076 (Fig 5). Positive correlation was also seen while comparing the salivary alkaline phosphatase and salivary calcium with Pearson correlation coefficient in which P = < `0.0001 and r = 0.5630 (Fig 6).

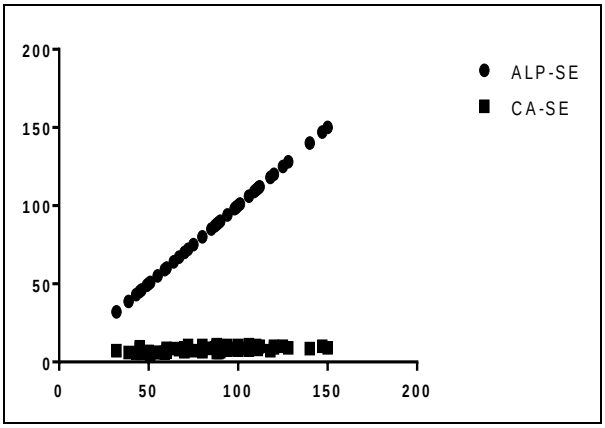

Fig-5: Showing the positive correlation between the serum alkaline phosphatase and serum calcium (ALP-SE: Serum Alkaline Phosphatase, CA-SE: Serum Calcium)

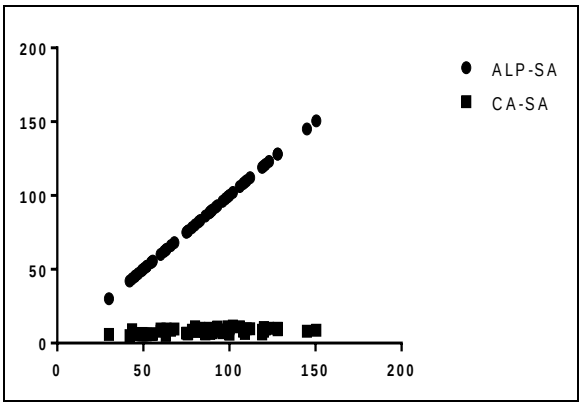

Fig-6: Showing the positive correlation between the salivary alkaline phosphatase and salivary calcium (ALP-SA: Salivary Alkaline Phosphatase, CA-SA: Salivary Calcium)

DISCUSSION

Alkaline Phosphatsase is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules including nucleotides, proteins and alkaloids in an alkaline environment [5]. Alkaline Phosphatase and calcium plays an important role in the

hard tissue formation and it increases the local concentration of inorganic phosphate and promotes mineralization thereby decreasing the concentration of extracellular pyrophosphate, an inhibitor of mineral formation [5]. Alkaline Phosphatase is active even in many other tissues, especially in the liver and kidneys, which helps in breakdown of the proteins.

ALPL (Alkaline Phosphatase, Liver/Bone/Kidney) is a Protein Coding gene. ALPL gene is present on 1p36.12 with base pairs of 21,508,981 to 21,578,411 and it provides the information for the Alkaline Phosphatase. Hypophosphatasia is a condition caused by the mutations in the ALPL gene. More than 190 mutations in the ALPL gene have been identified in people with hypophosphatasia. About 80 percent of these mutations change a single protein building block (amino acid) in the alkaline phosphatase enzyme. Other mutations insert or delete genetic material in the ALPL gene or change the way the gene's instructions are used to build the enzyme. Mutations in the ALPL gene lead to the production of an abnormal version of alkaline phosphatase that cannot participate effectively in the mineralization of developing bones and teeth. A shortage of alkaline phosphatase allows substances that are normally processed by the enzyme to build up abnormally in the body. Researchers believe that a build up of one of these compounds, inorganic pyrophosphate, underlies the defective mineralization of bones and teeth in people with hypophosphatasia [6].

ALPL mutations almost completely eliminate the activity of alkaline phosphatase resulting in more severe form of hypophosphatasia. Other mutations, which reduce but do not eliminate the activity of the enzyme, are often responsible for milder forms of the condition. Oestrogen, PTH and Cortisol are the most important hormones for bone metabolism. Oestrogen has direct effect on the osteoblast, osteoclast and also immune system. Apart from the Alkaline Phosphatase, calcium also an important mineral component of the skeletal system. It plays a crucial role in the mineralisation of skeleton and teeth and also control of excitability of nerves and transmission of impulses at synapses [6].

With age significant inverse relation was established between age and levels of Alkaline Phosphatase and Calcium indicating that as age increases both Alkaline Phosphatase and Calcium levels decreased contributed by a multitude of factors.

In the post menopausal women as there will be decrease in anabolic hormone that is oestrogen, the bone resorption occurs continuously, and there will be lag in its formation. After 60 years of age, there will be generalized atrophy of bone leading to senile osteoporosis. Alkaline Phosphatase is directly involved in the osteoid formation and helps in the mineralization of the bone. Whenever there is a deficiency in the oestrogen hormone such as in menopause this Alkaline Phosphatase will induce the synthesis of cytokines with the help of osteoblasts, monocytes and T cells, which causes some stimulation in the bone mineralization process which further results in the bone resorption by increasing the activity of the osteoclasts [7].

The gradual decrease in calcium levels with age can be attributed to lower intestinal absorption rate, lesser Vitamin D absorption and metabolism with age, decreased anabolic hormones, muscular proteins and flow of blood to bone [8, 9]. Since Calcium and Alkaline Phosphatase are interlinked with each other, the decreased availability of Calcium may weigh down the requirement of the Alkaline Phosphatase, thus decrease in its level with increasing age. This is evidenced by establishing a positive correlation between serum and salivary Alkaline Phosphatase and Calcium.

The corresponding serum and salivary levels of Alkaline Phosphatase and Calcium were almost analogous, thus releaving the fact that the saliva can acts as a mirror of blood, so saliva can also be used with equivalent efficacy as serum for the estimation of alkaline phosphatase and calcium levels [3, 4].

CONCLUSION

By the present study, we conclude that alkaline phosphatase and calcium decreases with age. So calcium supplements should be prescribed during the menopause to maintain the normal levels of alkaline phosphatase and calcium, thereby preventing bone resorption. Since salivary alkaline phosphatase and calcium levels proved to be a replica of serum levels, use of saliva as a diagnostic tool is accentuated as it is non-invasive quick and easy method of collection.

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