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Changes of serum acid phosphate and alkaline phosphatase in relation to age and the onset of osteoporosis among urban women

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Abstract
Analyzing biochemical turnover markers will be helpful to predict the bone health status of individuals. The present study aimed at studying the two essential enzymes involved in bone turnover process viz., serum acid phosphatase and serum alkaline phosphatase which are considered as bone resorption and bone formation marker respectively among urban women aged 35 to 74 years. The findings well demonstrated that resorption levels were increased markedly with the age as well as with the onset of osteoporosis by elevated levels both biochemical parameters studied. Serum acid phosphatase and serum alkaline phosphatase being considered as biochemical resorption marker and formation marker respectively found to be increased from younger to elderly and also as the bone density regressed from normal to osteopenia and further to osteoporosis. Adopting healthy dietary and life style practices may be helpful to reduce age-related bone loss and early onset of osteoporosis.

Keywords: Osteoporosis, Serum acid phosphatase, Serum alkaline phosphatase, Women

Introduction
Phosphatases are the enzymes which catalyze the splitting off of phosphoric acid from certain monophosphoric esters, a reaction of considerable importance in several body processes. Alkaline and acid phosphatases are the two types of phosphatases commonly estimated in serum along with serum calcium and phosphorus as concerned with calcification of bones and exhibit crucial role in bone turnover involving both bone formation and resorption of the bone. The rate of formation and degradation of the bone matrix can be assessed by measuring the enzymatic activity related to bone forming (osteoblasts) or resorbing cells (osteoclasts). Alkaline phosphatase is considered as bone formation markers and acid phosphatase as bone resorption markers due to their major role associated with osteoblastic and osteoclastic activity respectively.

Bone growth and remodeling are normal physiological events that occur at a high rate throughout childhood and adolescence and to a much lesser extent during adult years. It is the net result of the activity of two types of bone cells which have opposing actions: those that synthesize new bone material, with the involvement of bone formation markers like alkaline phosphatase mainly osteoblast and the other cells called osteoclasts, which are responsible for resorbing or breaking down of existing bone material due to the action of bone resorption markers like acid phosphatase. An exaggerate rate of bone resorption underlies in the patho-physiology of many human diseases – for example, postmenopausal osteoporosis, paget's disease, malignant hypercalcaemia, renal osteodystrophy, hyperthyroidism and hyperparathyrodism. The outcome is a progressive thinning of the bones and an increased risk of fractures [1].

Loss of bone tissue can be estimated by measuring bone mineral density (BMD), but BMD is unable to provide direct information on the micro architectural deterioration of bone. An increased rate of bone turnover is associated with more and deeper resorption sites, which weaken trabeculae due to the action of osteoclastic activity by the increasing activity levels of rersorption markers like serum acid phosphatase. To compensate the increased osteoclastic activity, the levels of bone formation markers like alkaline phosphatase simultaneously increased. Persistent high bone turnover likely leads to decreased trabecular thickness and ultimately trabecular perforation and progressively reducing bone strength [2].

Understanding the levels of both alkaline phosphatase and acid phosphatase may be helpful to predict the process of bone turnover with the ageing process as well as the onset of osteoporosis.

Materials and methods

Tirupati urban town of Chittoor district from Andhra Pradesh, South India was purposively selected as study area. The women who voluntarily participated in BMD campaign and willing to undergone serum biochemical analysis were included in the study. Age-related changes were evaluated by considering four different age groups viz., 35 to 44 years (young), 45 to 54 years (middle aged), 55 to 64 years (aged) and 65 to 74 years (elderly). The sample comprised of 60, 80, 80 and 40 subjects in the four respective age groups with a total sample size of 260 urban women.

Bone mineral density was analyzed using Quantitative Ultra Sound (QUS) bone densitometry measured at calcaneous bone. Based on WHO BMD T-score criteria women were divided into normal, osteopenic and osteoporotic women. Simultaneously with the BMD examination, biochemical evaluation was carried out for the estimation of serum acid phosphatase and serum alkaline phosphatase as per the standard procedures. The changes were evaluated in relation to age among four different age groups. The corresponding changes with the alteration in bone density were assessed by calculated t-values between normal and osteopenia; normal and osteoporosis and between osteopenia and osteoporosis women in each age group.

Results and discussion

Serum acid and alkaline phosphatases were the two major phosphatase enzymes selected to assess the bone turnover levels among urban women of different age groups and bone health conditions. The findings were evaluated for the age related changes and determined the changes associated with the onset of disease. The results were discussed further separately for the two different biochemical parameters individually under different subheads.

i) Serum acid phosphatase levels

Acid phosphatases are a family of enzymes that are widespread in nature and can be found in many animal and plant species. Acid phosphatase in the serum in raised amounts is used to detect the accompanying pathological bone resorption. Human acid phosphatases are normally found at low concentrations. However, pronounced changes in their synthesis occur in particular diseases, where unusually high or low enzyme expression is seen as part of the pathological process. This observation suggests that acid phosphatases could be diagnostically useful as serological and histological markers of disease, and could also be of use in the investigation of the pathophysiology of the associated disease [3].

The universal fact that bone deterioration would be accelerated with the advancing age and correspondingly the increase in osteoclastic activity and also the biochemical resorption markers associated with osteoclast cells. Hence, during the present study, the serum acid phosphatase being the bone resorption marker was analyzed. The changes were interpreted in relation to age and bone health status. The significant differences were assessed using t-values and the corresponding results were presented in table no-1.

Table 1: Mean serum acid phosphatase levels among urban women in relation to age and bone health - Calculated t-values for the differences and level of significance

Age (Years)	Mean serum acid phosphatase (U/L)			Calculated t-values		
	Normal Mean & ± S.D	Osteopenia Mean & ±S.D	Osteoporosis Mean & ± S.D	Normal Vs Osteopenia	Normal Vs Osteoporosis	Osteopenia Vs Osteoporosis
35-44(n=60)	2.22±(0.11)	2.55±(0.40)	2.87± (0.53)	5.02**	7.19**	1.18 ^{NS}
45-54(n = 80)	2.48±(0.37)	2.76±(0.58)	3.11± (0.55)	2.30*	4.61**	1.53 ^{NS}
55-64(n = 80)	2.87±(0.33)	2.99±(0.34)	3.37± (0.21)	1.43 ^{NS}	8.28**	6.48**
65-74(n = 40)	3.05±(0.33)	3.16±(0.30)	3.48± (0.46)	0.50 ^{NS}	1.28 ^{NS}	2.50*

Note: *= Significant at 5 percent level **= Significant at 1 percent level ^{NS} = Not Significant

The results from the table clearly denoted an increase in the serum acid phosphatase levels with the advancing age from young to elderly women. The important point need to be stressed that the resorption rate was to the maximum extent in elderly age. This was evidenced by the maximum levels of serum acid phosphatase irrespective of bone density condition including normal elderly women. Osteoclasts are well known for containing a large amount of acid phosphatase activity and this phenomenon has been used for many years to identify osteoclasts in tissue samples using histochemical techniques. Though the present study not conducted any osteoclastic histologic analysis, the changes in serum were adequately demonstrated the elevated bone resorption activity with the progressive age.

As the age advanced, there was a gradual reduction in bone mineral density which resulted in increased levels of bone resorption as evidenced by higher levels of bone resorption marker, serum acid phosphatase in the higher age groups. It was well known fact that an imbalance in bone turnover with increased bone resorption rather than bone formation lead to gradual shifting of normal bone status to ostepenia and further regressed towards osteoporosis. The risk status of osteoporosis with ageing process thus illustrated clearly in elderly groups of women. The findings indicate an immediate need to take appropriate measures to reduce the rate of bone resorption through suitable dietary and life style modifications in the women.

The serum acid phosphatase being the resorption marker lead to elevated levels in osteoporotic women significantly which was much evidenced by significant differences between the mean serum acid phosphatase levels of normal and osteoporotic women irrespective of age. It was also found that the trend of increasing levels of mean serum acid phosphatase was observed from normal to osteopenia and to osteoporosis. The changes were very clear though significant differences were not noticed in all cases between normal Vs osteopenia and osteopenia Vs osteoporosis.

Evidence from the existing literature showed that a pathological increase in bone resorption arised when osteoclasts were stimulated into resorption activity at an increased rate. This upset the normal balance between bone resorption and bone synthesis. The increase in osteoclast

activity was accompanied by an increase in the synthesis and secretion of acid phosphatase. Evidence indicated that acid phosphatase was involved in the bone resorption process. Furthermore, resorption events were marked by a corresponding rise in the total amount of acid phosphatase in the serum.

Bone kinetic assays and immunoassays showed that individuals with normally or pathologically high rates of bone turnover have increased levels of serum total acid phosphatase activity. The association between serum acid phosphatase and bone resorption was also confirmed by investigating bone density simultaneously with serum levels. A significant inverse correlation was observed between serum acid phosphatase and bone mineral density [4].

The available literature clearly explained the role of acid phosphatase as bone resorption marker. It was associated with osteoclasts, the bone break down cells and increase in the osteoclastic activity and hence reduced the bone mass through more of bone resorption. The current findings also supported the increasing activity of acid phosphatase with the advancing age and the resorption rates as the bone health shifted from normal to osteopenia and osteoporosis. Hence the serum acid phosphatase certainly become useful indicator in determining the extent of bone turnover as it was considered as bone resorption marker.

ii) Serum alkaline phosphatase levels

Investigation of the biochemical mechanism of calcification was initiated in 1923 by Robison's description of an enzyme which was capable of hydrolyzing phosphoric ester *in vitro*. In bone formation, two fundamental processes may be distinguished – the synthesis of a calcifiable protein matrix, and the deposition of mineral salts therein. The biochemical mechanism by which bone salts are formed and deposited has been studied extensively since Robinson's first description of a 'bone enzyme' with an optimum pH in the region of 9, which was referred to later as 'alkaline phosphatase' [5].

Bone resorption levels certainly increased with the progressive age which simultaneously would be accompanied by the bone formation to compensate the bone loss to certain extent. The role of alkaline phosphatase in osteogenesis has been investigated by histochemical techniques with particular attention to its relationship to phosphate metabolism and matrix elaboration. The enzyme appears to be intimately related to cellular metabolism and to the elaboration of a bone matrix that is chemically calcifiable. It remains possible however, that phosphatase may be in some way involved in making inorganic salts available to the calcifiable matrix [6]. Based on this background, the present research was also aimed at analyzing the serum alkaline phosphatase in relation to age and bone health. The results obtained were interpreted and presented in table no-2.

Table 1: Mean serum alkaline phosphatase levels among urban women in relation to age and bone health -Calculated t-values for the differences and level of significance

Age (Years)	Mean serum alkaline phosphatase (U/L)			Calculated t-values		
	Normal Mean & ± S.D	Osteopenia Mean & ± S.D	Osteoporosis Mean & ± S.D	Normal Vs Osteopenia	Normal Vs Osteoporosis	Osteopenia Vs Osteoporosis
35-44(n=60)	54.38±(12.92)	62.10± (10.63)	74.54± (15.66)	1.97 ^{NS}	2.59**	1.69 ^{NS}
45-54(n = 80)	60.58± (16.88)	69.27± (15.53)	81.16± (16.34)	1.80 ^{NS}	3.56**	1.83 ^{NS}
55-64(n = 80)	67.25± (18.62)	71.65± (18.11)	86.69± (14.17)	0.93 ^{NS}	3.43**	2.58*
65-74(n = 40)	68.55± (2.38)	77.03± (17.44)	93.16± (14.11)	0.83 ^{NS}	2.61*	3.15**

Note: WHO Classification of Osteoporosis by BMD T-Score

Normal: >-1.0 **Osteopenia:** -1.0 to -2.5 **Osteoporosis:** ≤ -2.5

*= Significant at 5 percent level **= Significant at 1 percent level ^{NS} = Not Significant

The important finding to be highlighted was the elevated serum alkaline phosphatase levels among osteopenic and osteoporotic women than normal women. When the bone density status shifted towards osteopenia and osteoporosis from normal bone density levels, then rate of bone resorption markedly increased with the lowering of bone densities. As mentioned earlier, the increase in the rate of bone turnover resulted in increasing levels of both formation and resorption markers. The serum alkaline phosphatase being the bone formation marker probably resulted in elevated levels in the conditions of osteopenia and osteoporosis due to increased rates of bone turnover process. This was much evidenced by the significant differences in the elevated levels of serum alkaline phosphatase in osteoporotic women against the normal women.

On the other hand, there was an increase in serum alkaline phosphtase levels with the advancing age. As the women aged around 45 years, usually the menstruation ceased due to attainment of menopausal condition. With the onset of menopause, estrogen deficiency status initiated and increased the rate of bone resorption by increasing osteoclastic activity. To overcome the increased levels of osteoclastic activity, there was an alternative compensatory mechanism in bone formation marker either by increasing the osteoblast number or activity. This compensatory phenomenon might be the possible reason for the age-related increase in serum alkaline phosphatase, which was considered as one of the bone formation marker.

The crucial observation to be noted down that serum alkaline phosphatase levels elevated significantly both in osteoporotic women as well as elderly against normal healthy individuals. Because osteoporotic bone was more porous due to increased number of osteoclast cells and the ageing process also increased osteoclastic activity, in both conditions, to fit the porous pits osteoblasts considerably increased and probably responsible for increased levels of serum alkaline phosphatase in osteoporotic elderly individuals.

One of the study results also noticed that higher serum concentrations of alkaline phosphatase were associated with increased risk of all-cause mortality and cardiovascular-related hospitalization in individuals. The greater risk of hospitalization was mainly due to any cause or related to infection or fracture. The results provided further support to the notion of a bone-vascular axis linking vascular calcification and bone health. The findings thus implicated the fracture risk with elevated levels of serum alkaline phosphatase [7].

The existing literature well explained the elevation of the serum alkaline phosphatase with the advancing age and with the decreased levels of bone mineral density. The other study

examined bone mineral density (BMD) in the radius region using single photon absorptiometry in 182 healthy Chinese women. The results indicated an increased levels of serum alkaline phosphatase with age especially post menopause condition and correlated negatively with BMD and suggested serum alkaline phosphatase can be used as an indicator to reflect bone remodeling and absorption. The present study results also indicated an inverse association of serum alkaline phosphatase with BMD as evidenced by significant elevated levels in osteoporotic women and also higher levels in higher age groups which constituted the representative sample of post-menopausal women [8].

The earlier studies suggested that estimation of total alkaline phosphatase levels in serum was a useful marker in assessing mineralization activity of osteoblast in postmenopausal women. The reseach conducted in postmenopausal women indicated the elevated bone resorption marker levels in parallel with elevated bone formation marker levels as supported by increased serum total alkaline phosphatase than premenopausal women [9]. These findings found to be in concurrent with the current research with elevated levels of total serum alkaline phosphatase from younger to higher age groups.

The biochemical bone turnover markers were analyzed in postmenopausal women in Calabar municipality and observed that both alkaline and acid phosphatases activities were increased in their serum levels. The elevation was mainly due to the inhibitory effects of estrogen on bone turnover rate which was dependent of age. The current results supported for the elevated levels of both serum alkaline and acid phosphatases with the advancing age and regression of bone density [10].

The overall results supported that serum alkaline phosphatase was being elevated with the natural ageing process as evidenced by number of studies quoted by increased levels of serum alkaline phosphatase among postmenopausal women. Gradually with the reduction in bone density levels towards osteoporotic condition, there was an increase in bone turnover during bone remodeling process and resulted in the elevation of both bone formation and resorption markers. The findings highlight the utmost need of planning appropriate measures to minimize the levels of bone turnover and to avoid the onset of osteoporosis or to reduce further consequences of osteoporosis and the fracture risk.

The findings well demonstrated that the mean serum acid phosphatase and alkaline phosphatase were raised with the advancing age as well as with the onset of osteoporosis. As the women reached on an average age of 45 years, many of them experienced the condition of menopause. The menopausal increase in bone turnover was reflected both by resorption and formation markers with elevated levels on par with the advancing age. This high turnover state sometimes referred to as “coupled”, though this term was somewhat inaccurate since bone was lost as a result of the net imbalance. The coupling process of bone remodeling might be related to the increase in the levels of both serum acid phosphatase and alkaline phosphatase as they found to be involved in bone resorption and formation and thus these markers said to be associated with bone remodeling process [11]. The results highlighted the need of protective measures to minimize the bone turnover, bone resorption and promotion of bone strength. The protective mechanism might be obtained through appropriate measures to reduce the rate of bone resorption by suitable dietary and life style modifications in the women.

Conclusion

Bone turnover process plays a crucial role in determining the bone density levels and strength in any individual. The rate of bone turnover is at accelerated rate in the higher age groups of women as they experienced menopausal condition which is accompanied by decreased levels of estrogen involved in bone formation. On the other hand, the enzymes also associated with the bone turnover process. The existing literature denoted that serum acid phosphatase and serum alkaline phosphatase are considered to be the bone resorption and bone formation markers respectively. The changes in these enzymes were studied among urban women in relation to age and bone health condition related to the onset of osteoporosis. The results indicated that both serum acid and alkaline phosphatases were being elevated with the advancing age as well as the bone heath regressed from normal to osteopenia and further to osteoporosis. The main reason was that due to resorption process, the serum acid phosphatase being resorption marker markedly increased in elderly and osteoporotic urban women. As compensatory mechanism to seal the osteoclastic activity, bone formation process was also initiated and resulted in increased levels of serum alkaline phosphatase being noted as bone formation marker.

Ageing is an unavoidable biological process affecting bone health in particular which need to be taken care of to reduce age-related bone loss and rate of bone turnover. The present findings clearly demonstrated elevated levels of bone turnover with the progressive age. The bone strength in the childhood is the major determinant of bone health in the late adulthood. However, suitable relevant measures in the dietary and life style habits such as balanced diet, calcium rich foods, exercise, avoiding smoking and drinking etc might be beneficial in minimizing age-related bone loss and early onset of osteoporosis and osteoporotic fractures.

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