Chemistry and Materials Research
ISSN 2224- 3224 (Print) ISSN 2225- 0956 (Online)
Vol.6 No.3, 2014

Validity of Osteocalcin and Alkaline Phosphatase Biomarkers in Postmenopausal Women With Low Bone Mineral Density

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Abstract

Background: Bone mineral density (BMD) can predict osteoporotic fractures which are major public health problem associated with increased morbidity, mortality, and health care cost amongst elderly**.**
Objective: To assess validity of osteocalcin and alkaline phosphatase (ALP) biomarkers in postmenopausal women with low bone mineral density (BMD)
Patients and Methods: This cross sectional study was conducted on postmenopausal women. BMD was measured by Dual energy x-ray absorptiometry (DEXA). Salivary and serum of both osteocalcin and alkaline phosphatase were measured from all postmenopausal women.
Results: Of a total 72 postmenopausal women, 48 had low BMD and 24 of normal BMD. Mean salivary and serum osteocalcin and mean serum alkaline phosphatase (ALP) levels were significantly higher in postmenopausal women with low BMD than those of normal BMD. Mean salivary ALP level was numerically more in women with low BMD than those with normal BMD but statistically not significant. Salivary and serum osteocalcin and ALP biomarkers score at cut off >1 had accuracy of 80.55 % to diagnose postmenopausal women with low BMD with positive predictive value of 88.64 %, sensitivity was 81.25 %, and specificity 79.17%.
Conclusions: Osteocalcin and ALP were valid biomarkers to diagnose postmenopausal women with low BMD. Biomarker score>1 had high accuracy and sensitivity to diagnose low BMD. This may suggest a new promising measure to early diagnose patients at high risk of low BMD and subsequently giving early appropriate treatment.
Key words: Osteocalcin, Alkaline phosphatase, bone mineral density, periodontal status

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1. Introduction

Bone Mineral Density (BMD) is a measure of the bone mass and a predictor of fracture. It has been well established that 90% of the variance in the bone strength is related to the BMD. The risk of fracture is known to be higher in women with low BMD, with the risk doubling for a reduction of one standard deviation in the BMD [Cummings et al, 1993]. Osteoporosis may be predicted from the bone turn over markers and BMD, because a low BMD is associated with a high turnover [Ravn et al, 1996]. It can also be predicted independently by BMD, since an increased bone turnover negatively affects the bone microarchitecture and the fragility [Seeman et al , 2006].

Osteoporotic fractures are a major cause of morbidity and mortality in the elderly population [Kumar et al, 2004]. Osteocalcin is an extracellular matrix protein produced mainly by osteoblast [Allison et al, 2000]. Alkaline phosphatase (ALP) is a ubiquitous enzyme that plays an important role in osteoid formation and bone mineralization. Both osteocalcin and ALP are markers of bone formation [Brown et al, 2009]. The biochemical markers of bone metabolism are tools of great importance in understanding the pathophysiologic basis for bone metabolic diseases. The determination of the protein fragments which are produced by osteoblasts like osteocalcin or enzymes which are secreted during osteogenesis, such as alkaline phosphatase, are commonly used to assess the osteoblastic activity. More recently, the bone turnover markers have been studied for their ability in predicting bone loss. This study was designed to assess validity of osteocalcin and alkaline phosphatase (ALP) biomarkers in postmenopausal women with low bone mineral density (BMD).

2. Patients and Methods

2.1 Study design and sample selection

This cross-sectional study was conducted at Mirjan Teaching Hospital, Babylon, Iraq from November 2012 till April 2013. A total of 72 consecutive postmenopausal women were included in the study. Informed consent was obtained from each participant included in this study according to the declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of Babylon University, College of Medicine, Medical Department.

Patients were excluded from the study if they had previous diseases like: diabetes, rheumatoid arthritis, systemic lupus erythematosus, thyroid, para-thyroid disease; neoplastic diseases, history of periodontal therapy within the last 3 months; current use of medications as corticosteroids or any immune suppressive agents within the previous 3 months, chemotherapy, or patients were smokers or alcohol users.

2.2 Data collection and measurements

Patients were asked for their ages. Body mass index (BMI) was measured according the equation BMI=weight / height ^{2}. BMD was measured by dual energy x-ray absorptiometry (central DEXA type DEXXUM 3, Korea). Low BMD was diagnosed when T-score < – 1SD (osteoporosis and osteopenia) according to World Health Organization [WHO, 1994].

About five ml of unstimulated whole saliva were collected under standardized conditions, venous blood samples of 5 milliliters (mLs) were drawn from all subjects by using disposable syringe (5 mLs). After centrifuging of saliva and blood. The supernatant of saliva and serum were separated and breezed until time of analysis. Osteocalcin was measured in serum and saliva using a chemiluminescence technique [Balwant et al, 2010] and Liaisoosteocalcine kit.

And ALP in saliva and serum were estimated by using alkaline phosphatase kit, which functions on the basis of Modified Kind & King's method [Reddy et al, 2008] using Alkaline phosphatase-Kit Colorimetric determination of alkaline phosphates activity.

2.3 Statistical analysis

Statistical software (SPSS version 22, IBM, USA) was used for data input and analysis. Kolmogorov-Smirnov test was used to assess the normal distribution of continuous variables. Statistical significance of differences between averages for normally distributed variables between 2 groups was assessed using the Student's t test. Continuous variables were presented as mean ± standard deviation (SD). A receiver operating characteristic (ROC) curve analysis was used to assess validity parameters and set optimum cut-off values for quantitative variables when used to predict a diagnosis of low BMD differentiating it from normal BMD. P value < 0.05 was considered statistically significant

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3. Results

Of a total 72 postmenopausal women, 48 had low BMD and 24 normal BMD. Mean age of postmenopausal women with low BMD was significantly more than those with normal BMD (61.63±6.86 vs 55.83 ± 4.66, p<0.001). Mean BMI was significantly lower in group of low BMD than in normal BMD group (30.59 ± 6.40 vs 36.54 ± 6.33, p<0.001). Additionally we found that mean salivary and serum osteocalcin was significantly higher in postmenopausal women with low BMD group compared to those with normal BMD group (1.27 ± 0.70 vs 0.76 ± 0.31, p <0.001; 20.54 ± 8.72 vs 14.13 ± 3.97, p<0.001 respectively). Mean serum ALP was significantly more in low BMD group than normal BMD group (111.11 ± 32.65 vs 81.98 ±31.51, p=0.001). Although mean salivary ALP was numerically more in low BMD group compared to normal BMD group but it was statistically not significant(p>0.05) as shown in table 1.

The validity of 4 biomarkers and the new biomarker score when used as test to diagnose low BMD cases differentiating them from normal BMD (controls) was tested by ROC method (Figure 1, Figure 2).
Salivary osteocalcin was associated with the highest validity (area under the curve = 0.752) followed by serum osteocalcine and serum ALP (AUC = 0.732 and 0.717 respectively). All the indices were associated with an ROC area which is significantly higher than the 0.5 area of an equivocal test as in table 2.

The optimal cut-off value for each of the 4 tested indices was ascertained by finding the shortest distance on ROC curve. Table 3 reports the optimal cut-off value for each of 4 biomarkers. A positive test result for low BMD was defined as a measured value for the index > the optimal cut-off value. Serum ALP at cut off value >63.28 was associated with the highest sensitivity while serum osteocalcin at cut-off>18.2 had the highest specificity. In overall the highest accuracy (80.56 %) was provided by serum ALP> 63.28 and would establish the diagnosis of low BMD with 78.33 % confidence. A negative serum ALP(when subject obtains value of ≤ 63.28) would exclude low BMD with 91.67 % confidence in a clinical context.

A summary score was calculated by summing the positive criteria out of 4 previously mentioned indices at their optimum cut off value. This new score is called biomarkers score of low BMD. The ROC analysis of this new overall score revealed that AUC was high (0.802%) and significantly different from the 0.5 area expected for an equivocal test (Table 2). The optimum cut-off value for this score was >1 positive criteria. This new test had the highest accuracy of 80.55 % and would establish the diagnosis of low BMD with 88.64% confidence (Table 3).

Table 1. Baseline characteristics of 72 postmenopausal women

Parameter	Low BMD=48	Normal BMD=24	P
Age (years), mean± SD	61.63±6.86	55.83 ± 4.66	<0.001
BMI (kg/m^2), mean± SD	30.59 ± 6.40	36.54 ± 6.33	<0.001
Salivary osteocalcin (ng/ml), mean± SD	1.27 ± 0.70	0.76 ± 0.31	<0.001
Serum osteocalcin (ng/ml) mean± SD	20.54 ± 8.72	14.13 ± 3.97	<0.001
Salivary ALP (IU), mean± SD	17.71± 6.46	14.52 ± 6.93	0.07
Serum ALP (IU), mean± SD	111.11 ± 32.65	81.98 ±31.51	0.001

BMD; bone mineral density; BMI, body mass index; ALP, alkaline phosphatase; SD, standard deviation

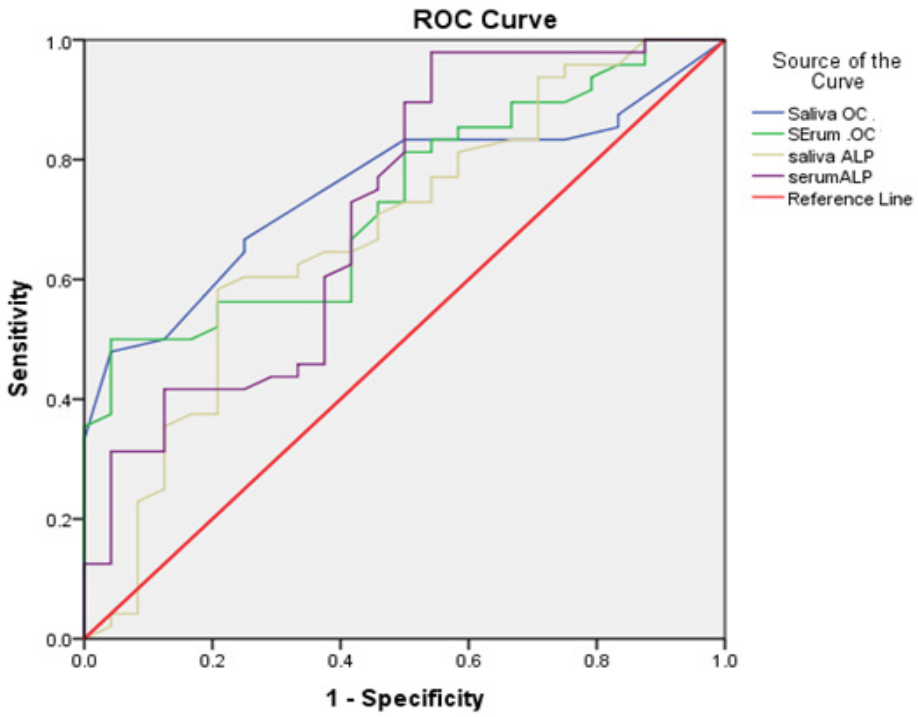

Figure 1: ROC curve showing the performance of selected biomarkers measurements in the diagnosis of low bone mineral density differentiating it from healthy controls

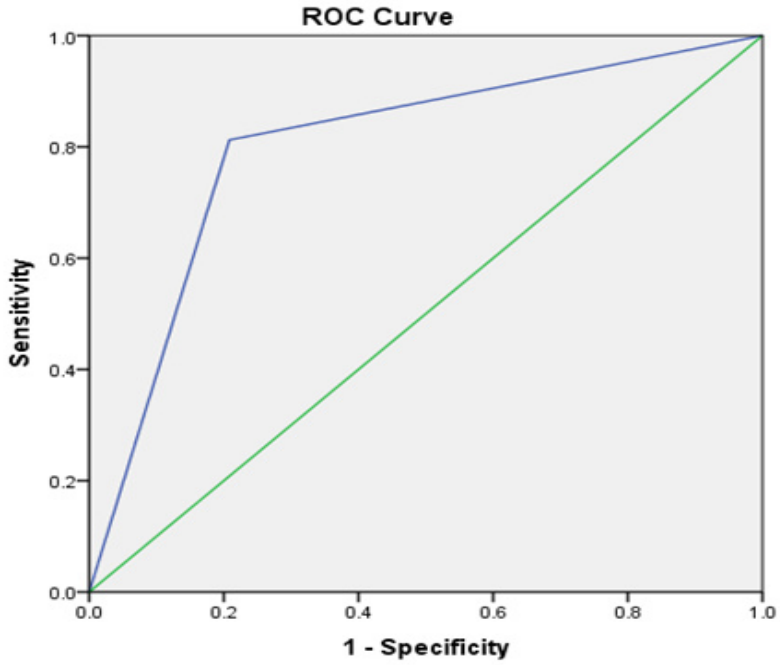

Figure **2**: ROC curve showing the performance of biomarker score (count of positive criteria) in the diagnosis of low BMD differentiating it from normal BMD (healthy controls).

Table 2. ROC area of selected biomarkers measurements when used as tests in the diagnosis of low BMD differentiating it from healthy controls.

Parameter	**AUC**	**P**
Biomarker score	0.802	<0.001
Salivary osteocalcin	0.752	0.001
Serum osteocalcin	0.732	0.001
Serum ALP	0.717	0.003
Salivary ALP	0.679	0.014

ALP, alkaline phosphatase; AUC, area under the curve

Table 3. Validity parameters of selected biomarkers when used as a test in diagnosis of low BMD differentiating it from healthy controls

Positive if > cut-off value	Sensitivity	Specificity	Accuracy	PPV	NPV
Biomarker score>1	81.25	79.17	80.55	88.64	67.86
Salivary osteocalcin >1.2 ng/ml	50	87.5	62.5	88.89	46.67
Serum osteocalcin >18.2 ng/ml	50	95.83	65.28	96	48.93
Salivary ALP>15.88 IU	58.33	79.17	65.28	84.85	48.72
Serum ALP > 63.28 IU	97.92	45.83	80.56	78.33	91.67

PPV, positive predictive value; NPV, negative predictive value; ALP, alkaline phosphatase

4. Discussion

Osteoporosis is a major growing public health problem with impact that crosses medical, social, and economic line [Gorial et al, 2013]. Since high bone turnover is associated with decreased bone mass, biochemical markers of bone remodeling may be used to assess osteoporosis and to predict fractures in elderly women, particularly those involving trabecular bone, and use of a combination of bone mineral density (BMD) and biochemical markers may improve fracture prediction [Merijanti Susanto et al, 2011].

The current study showed that mean salivary and serum osteocalcine were significantly higher in postmenopausal women with low BMD than those of normal BMD. Possible explanation of the high serum osteocalcin in postmenopausal women with low BMD is that serum osteocalcin is considered a specific marker of osteoblast function, as its levels have been shown to correlate with bone formation rates. However, since it is also released from bone matrix during bone resorption, it reflects the overall turnover of bone and is considered as a bone turnover marker. Osteocalcin has a high affinity for calcium and has a compact á helical conformation that is calcium dependent. The ã carboxyglutamic acid (Gla) residues of osteocalcin are capable of binding to bone matrix hydroxyapatite, thus leading to bone mineralization. Calcium- and phosphorus-deficient osteoporotic women may have a decreased rate of bone mineralization due to a reduction in hydroxyapatite crystal formation. In this condition, free osteocalcin may be present in the circulation [Filip et al, 2004]

Several studies have published data that confirmed our findings; however these studies either used serum or salivary test of osteocalcin as a biomarker of bone turnover that may detect low BMD. Rahanama et al [2010] reported that salivary test had higher concentration of osteocalcin in postmenopausal women. Jagtap et al [2011] measured osteocalcin and BMD in 60 clinically diagnosed postmenopausal osteoporosis women and 60 normal subjects (postmenopausal non-osteoporosis women).They stated that serum osteocalcin level was significantly increased as compared to control group. Atalay et al. [2012] observed that serum osteocalcin concentrations were significantly higher in women in the postmenopausal groups. Recently KalaiSelvi et al [2013] assessed association of serum osteocalcin with the bone mineral density in 60 post menopausal women and reported that mean serum values of osteocalcine was significantly more in osteoporotic than non osteoporotic subjects.

Another observation of note in the present study was that mean ALP level was significantly higher in postmenopausal women with low BMD than those of normal BMD. Mean salivary ALP was numerically more in women with low BMD than those with normal BMD but statistically not significant. The finding of high ALP in low BMD group may be related to increase of bone turn over in patients with low BMD rather than bone formation alone. Also periodontal disease may significantly increase the activity of salivary ALP [Dabra et al, 2012].

Various studies have assessed mean serum or salivary alkaline phosphatase as a biomarker of bone formation in postmenopausal women. Ross et al [2000] and Taguchi et al [2003] reported that the levels of saliva and serum total alkaline phosphatase were increased in subjects with low BMD. Reddy et al. [2008] evaluated salivary ALP in 45 postmenopausal women (15 osteoporotic, 15 osteopenic, and 15 normal BMD control group). They showed a significant increase in alkaline phosphatase level in the osteoporotic and osteopenia patients (low BMD group) when compared to the controls (normal BMD group). A more recent study done by Bhattarai et al [2014] measured serum ALP in 50 postmenopausal women (experimental group) and 50

premenopausal women (control group). They found that serum ALP level was significantly increased in postmenopausal women compared to controls.

In the current study, the non-significant numerical increase in mean salivary ALP in low BMD group compared to those with normal may be explained by small sample size of study.

The main limitations of the current study were the small size of the studied sample and short period of the study and these can be solved by larger and longer prospective study to support the reported data. Also the study performed only investigations on bone formation without investigating bone resorption. However, this study has points of strength like strict inclusion and exclusion criteria, and defined data measurement and collection. In addition we found a new biomarker score that detect low BMD in postmenopausal women. Salivary and serum osteocalcine and ALP biomarker score at cut off >1 had high accuracy to diagnose postmenopausal women with low BMD with high positive predictive value, sensitivity, and specificity.

In conclusion, osteocalcin and ALP were valid biomarkers to diagnose postmenopausal women with low BMD. Biomarker score>1 had high accuracy and sensitivity to diagnose low BMD. This may suggest a new promising measure to early diagnose patients at high risk of low BMD and subsequently giving early appropriate treatment.

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