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The importance of serum bone alkaline phosphatase in Metabolic Syndrome

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Abstract

Purpose: Serum alkaline phosphatase plays a role in vascular calcification. It is found in various tissues, whereas bone-spesific alkaline phosphatase (BAP) more specifically reflects mineral metabolism. The relationship of BAP with metabolic syndrome (MetS) is largely unknown. The aim of our study was to determine the optimal cut off level for BAP assess whether BAP could represent a novel, sensitive marker of bone mineral disease (BMD) in MetS patients.
Methods: 80 MetS patients (57 female and 23 male) and 50 healty individuals (33 female,17 male) were included in this study. BAP levels were measured using on Ostease Kit (Beckman Coulter , California, USA).
Results: Weight, BMI, waist circumference , hypertension, family history, blood pressure were significant higher in MetS group compared to the control group (p=0.0001 for all). HDL, fasting blood glucose ,TG, Insuline, HOMA-IR and BAP were significant higher in patients than control group (p=0.001, p=0.0001 respectively for all) The Receiver Operating Characteristics (ROC) analysis is used to measure the performance of BAP,Insuline and HOMA-IR in detecting bone mineral diseae in metabolıc syndrome .The cut off value off BAP was ≤ 15.1 µg/L. Area under the ROC curve was 0.839 (%95 CI ; 0.764-0.890, SE;0.038) (sensitivity; 83.75, spesifity; 76, PPV,84.4, NPV,74.5, +LR,3.49).
Conclusions: BAP may be a clinically useful bone formation marker to predict the BMD reduction in MetS patients.Further investigations with larger patient groups are required to confirm our results.

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Introduction

Serum alkaline phosphatase plays a role in vascular calcification (1). It is found in various tissues, whereas bone-spesific alkaline phosphatase (BAP) more specifically reflects mineral metabolism. The relationship of BAP with metabolic syndrome (MetS) is largely unknown. A number of studies have investigated the association between serum alkaline phosphatase and diabetes / MetS owing to its role as a hepatobiliary marker (2). However, serum alkaline phosphatase consists of several alkaline phosphatase isoforms from various tissues, such as liver, bone, and kidney, with a large proportion derived from liver and bone. Previous studies showed that BAP reflects mineral metabolism with a higher sensitivity and specificity than total alkaline phosphatase (3). Bone alkaline phosphatase (BAP) is an alkaline phosphatase (ALP) isoenzyme (EC 3.1.3.1). ALP is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules. Different ALP isoenzymes have been identified and are encoded by different genes. BAP is an isoform of the tissue non-specific ALP gene (4). For the studies that evaluate bone turnover, BAP is one of the most commonly measured parameters. The serum BAP level reflects the metabolic status of osteoblasts (5).

The aim of our study was to assess whether BAP could represent a novel, sensitive marker of in MetS patients and to determine the optimal cut off level for BAP to predict bone mineral disease (BMD) in MetS patients.

Materials and methods

80 metabolic sydrome patients (57 female and 23 male) and 50 healty individuals (33 female, 17, male) were included in this study.Experiments were undertaken with the understanding and written consent of each subject. Demographic data for the patient and control groups are shown in Table 1.

The present study was designed as a prospective, cross sectional study. The study included 80 consecutive patients who were admitted to the Cardiology and Internal Diseases Polyclinic and were diagnosed with MetS between December 2012 and January 2014. 50 patients who were of similar age and demographics and who were excluded from the MetS diagnosis were included in the control group. Metabolic syndrome was diagnosed based on the International Diabetes Federation (IDF) Criteria 2005 [12]. Metabolic syndrome was defined as having at least two of the following criteria in addition to the abdominal obesity criterion that was defined as a waist circumference > 94 cm in men and >88 cm in women: (1) elevated levels of triglycerides: ≥150mg/dL (1.7 μmol/L); (2) reduced levels of HDL cholesterol: <40mg/dL (1.03 μmol/L) in men and 50 mg/dL (1.29 μmol/L) in women; (3) elevated blood pressure: systolic blood pressure ≥ 130mmHg or diastolic blood pressure ≥ 85mmHg; (4) elevated fasting plasma glucose: elevated fasting plasma glucose ≥ 100mg/dL (5.6 μmol/L).

The exclusion criteria were as follows: diabetes mellitus or the use of oral antidiabetics, coronary artery disease, congestive heart failure, atrial fibrillation, congenital heart disease, valvular heart disease, myocarditis, pericarditis, cardiomyopathy, impaired renal functions (creatinine > 1.5 mg/dL), neoplasia, autoimmune disease, chronic inflammatory disease, active infection, chronic hepatic disease, and antioxidant vitamins or medications.

The waist circumference of the patients was measured parallel to the floor from the narrowest distance between the lowest costa and processus spinosus as the patients were standing and the belly was opened. The body mass index was calculated using the weight (kg)/height (m2) formula.

Blood Sample Collection. Venous blood samples were collected in tubes from the antecubital vein, followed by overnight fasting. The tubes were centrifuged at 4000 rpm (10 min) to remove the serum. The serum samples were kept at −80∘C until analysis of BAP. BAP levels were measured using on Ostease Kit (catalog number:37300)(Beckman Coulter , California, USA) .The BAP concentration was reported as the microgram per liter (µg/L). The reported with –in run CV was 1.9% and with-in day CV was 4.8% .

Other variables; Serum glucose, urea, creatinine, total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride were determined by AU 5800 otoanalyzer system and ınsulin was detected DXI 800 Beckman Coulter System and using commercial kits (Beckman Coulter, USA).

Statistics

Statistical calculations were performed with NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA). Besides standard descriptive statistical calculations (mean, standard deviation) independent t test was used to compare groups.

Receiver operating curve (ROC) analysis was performed to determine sensitivity and specificity of different cut off points for BAP, HOMA-IR,insuline to predict MeS. The most appropriate cutoff point was chosen according to ROC analysis, and the area under the curve (AUC) was calculated. Statistical significance level was established at $p<0.05$.

Results

The descriptive values of BAP and other parameters levels obtained for the control, and metabolic syndrome groups in Table 1.

Table 1. The demographic values of control and patient group and descriptive values (mean, standart deviation) of studied parameters.

		Control		Met S		p
Age, years		40,9±9,63		40,15±11,74		0,705
Gender	Male	17	34,00%	23	28,75%	
	Female	33	66,00%	57	71,25%	0,528
Height,cm		164,54±8,91		163,81±10,15		0,678
Weight,kg		73,48±14,39		92,37±18,09		0,0001
BMI kg/m2		27,11±4,65		34,51±6,56		0,0001
Waist circumferences,cm		92,76±13,56		110,4±13,17		0,0001
Hypertension,n		4	8,00%	49	61,25%	0,0001
Hyperlipidemia,n		4	8,00%	14	17,50%	0,127
Smoking, n		13	26,00%	32	40,00%	0,103
Family History,n		15	30,00%	49	61,25%	0,001
Systolic blood pressure, mmHg		113,7±13,08		133,44±20,83		0,0001
Diastolic blood pressure, mmHg		72,8±9,27		83,71±12,16		0,0001
Fasting Blood Glucose, mg/dl		87,86±9,62		96,63±11,81		0,0001
T.CHOL,mg/dl		192,42±44,19		187,13±38,78		0,474
LDL,mg/dl		115,58±38,67		110,04±29,95		0,361
HDL,mg/dl		49,98±11,72		43,36±9,76		0,001
TG,mg/dl		124,92±75,92		180,49±89,29		0,0001
Urea,mg/dl		25,82±6,78		27,56±10,31		0,292
Creatinine ,mg/dl		0,72±0,47		0,69±0,17		0,678
Insulin, mIU/mL		6,99±3,29		20,82±9,13		0,0001
HOMA-IR		1,42±0,66		4,87±2,15		0,0001
BAP (µg/L)		22,48±13,12		11,78±5,38		0,0001

There was no statistically significant difference in age, gender, height, hyperlipidemia, smoking ratio between the MetS group and control group (p>0.05 for all). Weight, BMI, waist circumference , hypertension, family history, systolic and diastolic blood pressure were statistically significant higher in MetS group compared to the control group (p=0.0001 for all).

When the laboratory parameters compared between the patients and control groups ; there was no significant differences for T. Chol, LDL, Urea, Creatinine (p>0.05 for all). There was significant differences between patients and control group for, HDL, fasting blood glucose ,TG, Insuline, HOMA-IR and BAP (p=0.001, p=0.0001 respectively for all)

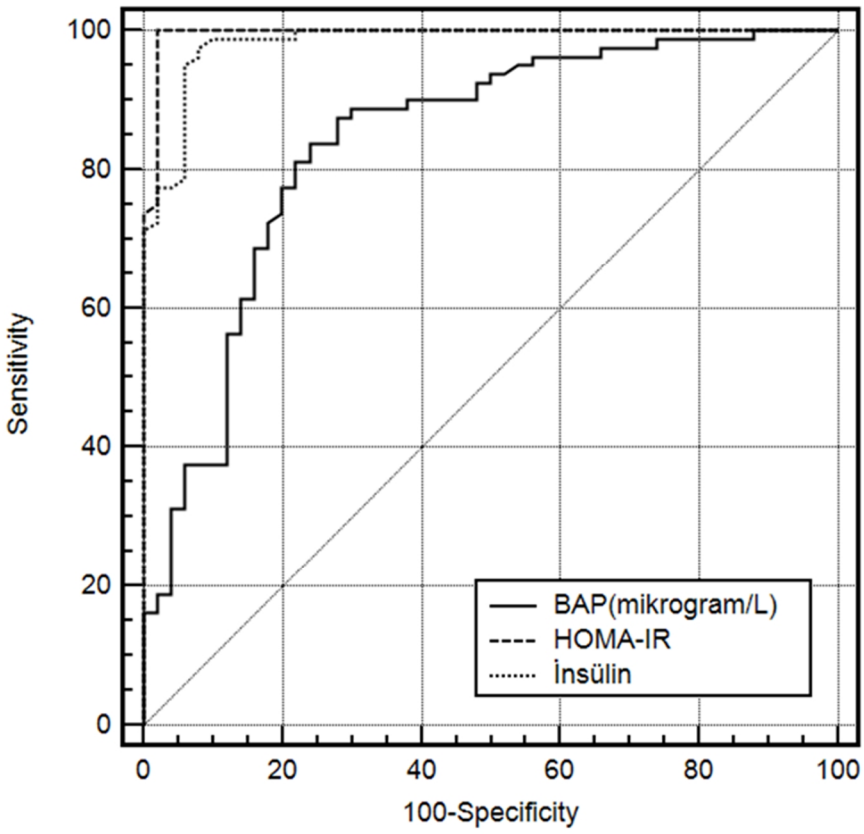

Fig1. Receiver operating characteristics curve (ROC) of BAP, HOMA-IR and Insuline

According to ROC analysis, the optimal cutoff level was ≤15,1 µg/L for BAP to predict BMD in MetS patients.Using a acut off of 15,1 µg/L for BAP for diagnosis of BMD in MetS, sensivity and specivity were 83,75% and 76%, respectively (**Fig.1**). When BAP is higher than this cutoff value , the possibility of BMD in MetS increases three- four times (positive likelihood ratio 3,49).Positive predictive value was 84.4, negative predictive value was 74.5. AUC was 0.839 for BAP. These data suggesting that BAP is good marker to diagnose BMD in MetS. According to ROC analysis, the optimal cutoff level was >2,49 for HOMA-IR to predict MetS.Using a acut off of >2,49 for HOMA-IR for diagnosis of MetS, sensivity and specivity were 100% and 98%, respectively**(Fig.1).** When HOMA-IR is higher than this cutoff value , the possibility of MetS increases fifty times (positive likelihood ratio 50.00).Positive predictive value was 98.8, negative predictive value was100.0 AUC was 0.994 for HOMA-IR . These data suggesting that HOMA-IR is excellent marker to diagnose MetS.According to ROC analysis, the optimal cutoff level was >11,4 for insuline to predict MetS.Using a acut off

of >11,4 for insuline for diagnosis of MetS, sensivity and specivity were 97.5% and 98.00 %, respectively **(Fig1).** When insuline higher than this cutoff value , the possibility of MetS increases twelve times (positive likelihood ratio 12.19).Positive predictive value was 95.1, negative predictive value was 95.8. AUC was 0.986 for insuline . These data suggesting that insulin is excellent marker to diagnose MetS. **Table 2.**

	AUC	SE	95% CI
BAP (µg/L)	0,839	0,038	0,764 - 0,890
HOMA-IR	0,994	0,007	0,958 - 0,998
Insulin (mIU/mL)	0,986	0,009	0,946 - 0,998

Pairwise comparison of ROC curves	p
BAP (µg/L) / HOMAIR	0,001
BAP (µg/L) / Insulin	0,001
HOMAIR / Insulin	0,106

	Cut Off	Sensitivity	Specificity	PPV	NPV	+LR
BAP (µg/L)	≤15,1	83,75	76,00	84,8	74,5	3,49
HOMA-IR	>2,49	100,00	98,00	98,8	100,0	50,00
Insulin (mIU/mL)	>11,4	97,50	92,00	95,1	95,8	12,19

Table 2. The results of ROC analysis for BAP, Insuline and HOMA-IR

Discussion

In conclusion ,we observed that BAP levels were significantly lower in the metabolıc syndrome groups than the control group.These results suggest that osteoblast activity is suppressed during metabolic syndrome , thus causing the bone to enter a non –dynamic state. Bone is a dynamic tissue that is continuously remodeling itself through osteoclastic bone resorption and osteoblastic bone formation.A bone biopsy is the gold standart for the diagnosis of osteopathology but this method is invasive and ,thus, is not preferred.The measurement of bone mineral density is non –invasive and may provide useful information. During osteoclastic bone resorption ,collogen type 1 breakdown into

fragments and released into the circulation and excreted in the urine and can be measured in the serum or urine as bone resorption markers. Pyridinoline , deoxypryridinoline , crossslinked N-terminal telopeptides of type 1 collogen and C-terminal telopeptides of type 1 collogen are markers used to evaluate bone resorption. BAP reflects an increase in bone turnover and is useful for monitoring bone formation(6).

In our study, BAP showed good diagnostic performance (AUC=0.839), the critical value was (15.1). Estimated sensitivity (true positivity through patient population) was 83.75, specifity was (wrong negativity through healthy subjects) 76.00. Because of using these levels at patient population , high sensitivity were important.

C.Cheung at all evaluated the relationship between glucose metabolism, MetS, alkaline phosphatase, and BAP in a large nationally representive population.Their study sheds light on the mechanism of elevated BAP. Although insulin resistance is known to be a risk factor for vascular calcification , the mechanism is not completely understood. They showed that multiple HOMA-IR indices and insulin levels are robustly associated with BAP.In addition , their study also showed that BAP is associated with multiple components of MetS, which suggested that hyperinsulinemia, insulin resistance, and MetS could lead to serum BAP elevation (1).

In contrast to this literature we found lower BAP levels in our metabolic syndrome group than control group. There have only been a few studies of BAP with insulin resistance and MetS. In agreement with a cross –sectional study carried out in 328 type 2 diabetes patients , they also observed no significant association between BAP and fasting plasma glucose and HbA1c (7). In a study of 54 healty postmenopausal women , there was no correlation between BAP and any components of MetS (8), which could be due to the small sample size. On the other hand, elevated BAP levels could be due to the precence of fatty liver and also high CRP levels could be effect BAP levels. But we excluded all these patients in our study to find real BAP levels in MetS patients (9,10,11).

Previous studies showed that BAP reflects mineral metabolism with a higher sensitivity and specifity (3).

BAP may be the link between insulin resistance and vascular calcification, cardiovascular disease or even mortality. In conclusion , serum BAP is a clinically useful bone formation marker for predicting reduction of BMD in MetS patients. Future studies on clinical outcomes should measure BAP in addition to other parameters. Further investigations with larger patient groups are required to confirm our results.

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