RESEARCH   **Open Access**

Could procollagen type I N-terminal propeptide (PINP) and bone alkaline phosphatase (B-ALP) be valid alternative diagnostic markers to dual X-ray absorptiometry (DEXA) in elderly females with osteoporosis? An Egyptian radiological and laboratory monocentric study

Marwa A. Saad[1]* , Reham A. Aboelwafa[2] and Eman H. Elsayed[1]

Abstract

Background: Osteoporosis is a major health problem of elders. Dual X-ray absorptiometry (DEXA) is the commonly used modality for diagnosis osteoporosis; serum markers have been suggested for predicting osteoporosis and discriminate osteoporotic from healthy subjects. We aimed to analyze the status of some bone turnover biochemical markers namely PINP, B-ALP, estrogen, and progesterone in the elderly osteoporotic and osteopenic women as probable markers for the discrimination between patients and healthy individual in diagnosing osteoporosis, and also, to detect the impact of osteoporosis on quality of life of patients using assessment of the quality of life for osteoporosis (ECOS-16). Post-menopausal 108 females were involved in the current study, divided into two groups (osteoporotic group (60 with BMD<-2.5), osteopenic group (48 with BMD between − 1 and − 2.5)), and 60 healthy elderly females as control group were involved in the study. Serum levels of procollagen type I N-terminal propeptide (PINP), bone alkaline phosphatase (B-ALP), estrogen, and progesterone were measured by ELISA technique.

Results: PINP and B-ALP significantly differ between studied groups. Also, PINP and B-ALP levels had high sensitivity and specificity to discriminate osteoporotic patients from healthy individuals. PINP and B-ALP significantly correlated with bone mineral density (BMD) and with ECOS-16. Estrogen differs significantly between osteoporotic and osteopenic groups and significantly correlated with bone mineral density of femur (BMD-F) and bone mineral density of spine (BMD-S) in the osteopenic group. Progesterone differed significantly between patients and controls and significantly correlated with BMD-F in the osteoporotic group.

(Continued on next page)

* Correspondence: marwa.saad@alexmed.edu.eg
[1]Internal Medicine Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt
Full list of author information is available at the end of the article

(Continued from previous page)

Conclusion: We can consider PINP and B-ALP as biomarkers for early detection then monitoring of osteoporosis. Measuring these serum markers can replace the assessment of BMD if not available. Also, it could replace the gap between BMD subsequently spaced assessment or could be of value in cases with severe spondylosis, DISH syndrome, old spondylarthritis, and/or previous spinal surgery. Sex hormones could not differentiate the normal from the osteoporotic/osteopenic patients, so they cannot be used as diagnostic or prognostic markers. Validation of this assumption needs large and longitudinal studies.

Keywords: PINP, B-ALP, Sex hormones, ECOS-16, Postmenopausal women, Osteoporosis, DEXA

Background

Osteoporosis is a disorder characterized by decreased bone mass, leading to bone fragility and accordingly increase fracture risk especially in elders [1]. Osteoporosis can lead to significant morbidity and mortality in elders, so screening of women aged 60 years and more for osteoporosis is advisable [2]. Increasing life expectancy worldwide together with an increase in the prevalence of osteoporosis makes osteoporosis a major health problem [3]. In Egypt, about 42% of females and 43% of males aged between 40 and 50 years have a low bone mineral density (BMD), while a third of the elderly population aged between 65 and 80 years were osteoporotic [4]. Decreased bone mineral density among elderly Egyptians, maybe due to multiple factors as smoking, sedentary lifestyle, and increased soft drinks consumption, low calcium, and omega 3 intake [4]. Measuring bone mineral density (BMD) using dual-energy X-ray absorptiometry (DEXA) is the commonly used test for diagnosing osteoporosis and osteopenia [5]. Researchers try to find serum markers to diagnose low bone mineral density and, they found that bone turnover markers may be considered as predictors of osteoporosis and osteopenia [6]. Bone turnover markers are either bone formation markers as bone alkaline phosphatase (B-ALP) and the procollagen type I N-terminal propeptide (PINP) or bone resorption markers as amino-terminal cross-linked telopeptide of type I collagen (NTX) and the carboxy-terminal cross-linked telopeptides of type I collagen [7]. Type I collagen which is formed inside osteoblasts is the main protein in bone. PINP is formed by the effect of proteases on type I procollagen and thus serum PINP concentrations reflect the amount of the newly formed bone [8, 9]. So, serum levels of PINP can be increased in diseases characterized by a high rate of bone turnover as osteomalacia and multiple myeloma [10]. Teriparatide treatment can cause a dramatic increase in PINP levels in the serum [7, 8]. Accordingly, PINP had been suggested as a reference serum marker of bone formation [10].

In adults with normal hepatic functions, about 50% of total alkaline phosphatase (ALP) is produced from the bone in serum. B-ALP is another indicator of osteoblastic activity in premenopausal and postmenopausal women [11]. Data from different studies regarding levels of bone biomarkers in different bone diseases are contradictory [12–15].

Apart from bone markers, sex steroid hormones especially estrogen and progesterone, can influence osteoblast maturation and thus affecting bone mass [16, 17].

The area of research of bone turnover markers and sex hormones in osteoporotic and osteopenic patients compared with the control had contradictory results. So, we aimed to analyze the status of some bone turnover biochemical markers namely PINP, B-ALP, and sex hormones (estrogen and progesterone) in the elderly osteoporotic and osteopenic women as probable markers for the discrimination between patients and healthy individual in diagnosing osteoporosis, and also, to detect the impact of osteoporosis on the quality of life of patients using ECOS-16 scoring questionnaire.

Methods

The current study was held in the period from January 2018 till February 2020. From attendants of the rheumatology, geriatric, and general medicine outpatient clinics during this period, 108 subjects were involved in the current study after performing dual-energy X-ray absorptiometry (DEXA) to confirm the diagnosis of osteoporosis or osteopenia. 60 healthy control selected from attendants of ophthalmology, dermatology, and general surgery outpatient clinics. The protocol of the current study was accepted by the relevant committee of ethics. A detailed explanation of the aim and procedures of the current research was explained to all participants or their caregivers, and a signed consent was obtained.

Women aged 50 years and more were included in the current research who live independently in their home and could take over the questionnaires. Exclusion criteria included type 1 diabetes mellitus, hyperthyroidism, hyperparathyroidism, multiple myeloma, hypovitaminosis D less than 20 ng/ml, and gastrostomy. Patients on the following drugs were excluded from the study, glucocorticoids, bisphosphonate, vitamin D, warfarin, or vitamin K.

All participants were subjected to full history taking, thorough clinical examination, routine laboratory

investigations, complete blood picture, fasting blood glucose, hemoglobin A1c, hepatic panel, renal panel, thyroid function tests, and vitamin D3 level.

Blood samples were collected from all study participants on plain vacutainer tubes after an overnight fast, samples were centrifuged, and serum was stored at − 20 °C till further processing. The levels of Procollagen I N-Terminal Propeptide (PINP) were analyzed using enzyme-linked immunosorbent assay (ELISA) kits (MyBioSource, Inc., San Diego, USA. Catalog No: MBS2504819). B-ALP was also analyzed by ELISA (Wuhan Fine Biotech Co., Ltd., Catalog No: EH2691). Estrogen and progesterone were analyzed measured by enzyme-linked fluorescent assay (ELFA) technique on VIDAS (Biomerieux, Italy).

A central DEXA scan was performed on all participants. According to the World Health Organization (WHO) criteria, all participants by the DEXA test were categorized into three separate groups: osteoporotic, osteopenic, and normal subjects. The results of the DEXA scan were reported as T scores: normal bone: T score above − 1, osteopenia: T score between − 1 and − 2.5, and osteoporosis: T-score of − 2.5 or lower [9].

All participants evaluated regarding the quality of life using the ECOS-16 questionnaire [18].

Statistical analysis
Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov-Smirnov was used to verify the normality of distribution of variables; ANOVA was used for comparing the three studied groups and post hoc test (Tukey) for pairwise comparisons. Kruskal-Wallis test was used to compare different groups for abnormally distributed quantitative variables and followed by post hoc test (Dunn's for multiple comparisons test) for pairwise comparison. Pearson coefficient was used to correlate between two normally distributed quantitative variables. Receiver operating characteristic curve (ROC) is generated by plotting sensitivity (TP) on Y-axis versus 1-specificity (FP) on X-axis at a different cutoff value. The area under the ROC curve denotes the diagnostic performance of the test. Area more than 50% gives acceptable performance and area about 100% is the best performance for the test. The ROC curve also allows a comparison of performance between two tests. The significance of the obtained results was judged at the 5% level.

Results
This cross-sectional, case-control study was held in the period from January 2018 till February 2020 and included 108 female patients with osteoporosis/osteopenia, and 60 age-matched healthy females served as a control

group. The mean age of the osteoporotic group was 65.9 ± 7.6 years, the mean age of the osteopenic patient was 65.3 ± 8.6 years, and the mean age of the healthy controls was 68.1 ± 6.1 years, with no statistically significant difference between the three groups (p = 0.113). According to the bone mineral density of the femur and spine (BMD-F, BMD-S), a high statistically significant difference was detected between the three studied groups (p ‹ 0.001). Estrogen level was significantly higher in the osteoporotic group as compared to the osteopenic group, but no statistically significant difference between both groups and the control group. Both osteoporotic and osteopenic groups have significantly lower levels of progesterone levels as compared to the control group (p ‹ 0.001). PINP levels were significantly lower in the osteoporotic group as compared to osteopenic and control groups, also levels were significantly lower in the osteoporotic group compared to the control group (p ‹ 0.001). Levels of B-ALP were significantly higher in both osteoporotic and osteopenic groups as compared to the control group (p ‹ 0.001) (Table 1).

Table 2 shows the correlations between estrogen and progesterone with different parameters. As shown, only a significant positive correlation was found between estrogen and BMD-F and BMD-S in the osteopenic group (p ‹ 0.001, p ‹ 0.002, respectively). Progesterone level showed a significant positive correlation with BMD-F and BMD-S in the whole sample and only with BMD-F in the osteoporotic group ($p < 0.001$, $p < 0.001$, p = 0.037, respectively). Also, a significant positive correlation was detected between progesterone levels and PINP levels in the whole sample, while it showed a significant negative correlation with B-ALP in the whole sample (p = 0.001, p = 0.003, respectively). ECOS-16 had no significant correlation with either estrogen or progesterone levels (p = 0.299, p = 0.986, respectively).

As shown in Table 3, quality of life as determined by the ECOS-16 questionnaire was strongly associated with the degree of osteoporosis as determined by BMD of the femur and spine in the osteoporotic group (p = 0.036 and p = 0.008, respectively). ECOS-16 is strongly associated with both PINP and B-ALP (p = 0.041 and p = 0.048, respectively). PINP has a significant negative correlation with both BMD-F and BMD-S (p = 0.015 and p = 0.012, respectively). B-ALP also has a statistically significant negative correlation with both BMD-F and BMD-S (p = 0.004 and p = 0.035, respectively).

ROC analysis was performed.

Table 4 showed that PINP at a cutoff value of 44.7 pg/ml has a sensitivity of 75% and specificity of 66.67% in detecting osteopenic patients. B-ALP at a cutoff value ˃

Table 1 Comparison between the three studied groups according to different parameters

	Healthy controls (n = 60)	Osteopenia (n = 48)	Osteoporosis (n = 60)	Test of Sig.	p
Age (years)					
Mean ± SD	68.1 ± 6.1	65.3 ± 8.6	65.9 ± 7.6	F = 2.209	0.113
Median (Min.–Max.)	68 (50–81)	62.5 (55–85)	66 (50–82)		
BMD-F (g/cm^2)					
Mean ± SD	0.1 ± 0.6	− 1.9 ± 0.4	− 3.6 ± 0.8	H = 148.125[*]	< 0.001[*]
Median (Min.–Max.)	0.4 (− 1–0.7)	− 2[a] (− 2.4 to − 1.1)	− 3.4[ab] (− 6.3 to − 2.5)		
BMD-S (g/cm^2)					
Mean ± SD	0.4 ± 0.6	− 1.9 ± 0.4	− 3.6 ± 0.8	H = 147.889[*]	< 0.001[*]
Median (Min.–Max.)	0.5 (− 1–0.9)	− 2[a] (− 2.4 to − 1.1)	− 3.5[ab] (− 6.2 to − 2.4)		
Estrogen (pg/ml)					
Mean ± SD	37.2 ± 5.6	35.1 ± 6.2	39.9[b] ± 10.5	F = 5.024[*]	0.008[*]
Median (Min.–Max.)	37 (25–55)	33 (23–50)	37.5 (21–71)		
Progesterone (nmol/L)					
Mean ± SD	0.20 ± 0.09	0.12[a] ± 0.03	0.14[a] ± 0.05	F = 20.663[*]	< 0.001[*]
Median (Min.–Max.)	0.18 (0.10–0.40)	0.11 (0.10–0.25)	0.13 (0.10–0.30)		
PINP (pg/ml)					
Mean ± SD	49.5 ± 10.2	40.2[a] ± 6.5	35[ab] ± 7.6	F = 46.286[*]	< 0.001[*]
Median (Min.–Max.)	49.5 (33.7–80.2)	40.2 (24.6–56.1)	34.8 (17.5–56.1)		
B-ALP (ng/ml)					
Mean ± SD	33.7 ± 7	52.1[a] ± 9.8	56.3[a] ± 14.6	F = 70.619[*]	< 0.001[*]
Median (Min.–Max.)	33.4 (22.1–50.1)	50.8 (33.2–70.8)	55.6 (30.8–83.5)		
ECOS 16					
Mean ± SD	–	–	56.9 ± 15.3	–	–
Median (Min.–Max.)	–	–	58 (20–75)		

F for ANOVA test, pairwise comparison between every 2 groups was done using post hoc test (Tukey)
H for Kruskal-Wallis test, pairwise comparison between every 2 groups was done using post hoc test (Dunn's for multiple comparisons test)
p p value for comparing between the studied groups.
[a]Significant with healthy controls
[b]Significant with osteopenia
[*]Statistically significant at $p \leq 0.05$

Table 2 Correlation between estrogen with progesterone and different parameters

		Total sample (n = 168)		Total patient (n = 108)		Osteopenia (n = 48)		Osteoporosis (n = 60)	
		r	p	r	p	r	p	r	p
Estrogen (pg/ml) vs.	**BMD-F (g/cm^2)**	− 0.106	0.172	− 0.165	0.088	0.484	< 0.001[*]	− 0.022	0.866
	BMD-S (g/cm^2)	− 0.100	0.196	− 0.139	0.151	0.329	0.022[*]	0.056	0.671
	PINP (pg/ml)	0.050	0.523	− 0.026	0.787	0.147	0.317	0.039	0.768
	B-ALP (ng/ml)	− 0.005	0.945	− 0.040	0.679	− 0.030	0.841	− 0.106	0.421
	ECOS 16							− 0.108	0.412
Progesterone (nmol/L) vs.	**BMD-F (g/cm^2)**	0.314	< 0.001[*]	− 0.185	0.055	0.004	0.981	0.083	0.529
	BMD-S (g/cm^2)	0.360	< 0.001[*]	− 0.201	0.037[*]	− 0.134	0.363	0.067	0.612
	PINP (pg/ml)	0.251	0.001[*]	− 0.053	0.588	0.152	0.303	0.016	0.903
	B-ALP (ng/ml)	− 0.229	0.003[*]	− 0.009	0.922	− 0.106	0.475	− 0.048	0.717
	ECOS 16							− 0.043	0.747

r Pearson coefficient
[*]Statistically significant at $p \leq 0.05$

Saad *et al. Egyptian Rheumatology and Rehabilitation*   (2021) 48:20

Page 5 of 10

Table 3 Correlation between different parameters in osteoporosis group (n=60)

	ECOS 16		PINP (pg/ml)		B-ALP (ng/ml)	
	r	p	r	p	r	p
PINP (pg/ml)	− 0.264	0.041*				
B-ALP (ng/ml)	− 0.256	0.048*				
BMD-F (g/cm²)	0.272	0.036*	− 0.311	0.015*	− 0.364	0.004*
BMD-S (g/cm²)	− 0.339	0.008*	− 0.322	0.012*	− 0.273	0.035*

r Pearson coefficient
*Statistically significant at p ≤ 0.05

44.2 ng/ml has a sensitivity of 89.58% and specificity of 85.0% in detecting osteopenia. Both PINP and B-ALP can significantly discriminate osteopenic patients from healthy controls ($p < 0.001$). (Fig. 1)

Table 5 showed that PINP at a cutoff value of ≤ 40.6 pg/ml had a sensitivity of 86.67% and specificity of 80% in predicting osteoporosis. B-ALP at a cutoff value > 40.3 ng/ml had a sensitivity of 85% and specificity of 83.33% in predicting osteoporosis. Both PINP and B-ALP can significantly discriminate osteopenic patients from healthy controls ($p < 0.001$) (Fig. 2)

Table 6 showed that PINP at a cutoff value of ≤ 37.2 pg/ml had a sensitivity of 71.67% and specificity of 62.50% in discriminating osteoporosis from osteopenia. B-ALP at a cutoff value > 51.2 ng/ml had a sensitivity of 58.33% and specificity of 56.25% in discriminating osteoporosis from osteopenia. Only PINP can significantly discriminate osteopenic from osteoporotic patients ($p < 0.001$) (Fig. 3).

Discussion

Osteoporosis is a critical health problem, especially in elderly patients, which has a great impact on their quality of life. In the current study, we investigated two bone turnover biomarkers namely PINP, B-ALP, and sex hormones (estrogen and progesterone) in 108 elderly females with osteoporosis and osteopenia. Results indicated that both PINP and B-ALP are convenient biomarkers for the detection of osteoporosis in elderly post-menopausal females. Both PINP and B-ALP have a significant negative correlation with bone mineral dentistry (BMD-F and BMD-S). Also, both biomarkers are significantly associated with the quality of life of

osteoporotic patients as determined by the ECOS-16 questionnaire. PINP and B-ALP were negatively correlated with both estrogen and progesterone, but the correlation was not statistically significant neither in the osteoporotic nor in the osteopenic group, but progesterone showed a high significant correlation with both PINP and B-Alp in the whole sample. Tehrani and his coworkers [19] studied serum levels of PINP and B-ALP in 28 osteoporotic and 28 osteopenic patients and compared them to healthy controls; they found that both markers differed significantly between the studied groups; and also, they can discriminate between patients and controls.

In the current study, a statistically significant difference between osteoporotic and osteopenic groups regarding estrogen levels was detected; also, levels of progesterone differed significantly between the osteoporotic group and healthy controls and between the osteopenic group and healthy controls. On contrary to our results, Tehrani and his co-workers [19] found no statistically significant difference between studied groups regarding levels of sex hormones. In osteopenic patients in the Tehrani study [19], progesterone was positively correlated with femoral BMD (BMD-F) and in the healthy group. Also, a positive correlation of B-ALP with estrogen was observed. On contrary, in our study, neither estrogen nor progesterone showed a significant positive correlation with femur and spine BMD in the osteopenic group, while progesterone showed a significant positive correlation with femur BMD only in the osteoporotic group.

Change in hormonal levels plays an important role in the development of osteoporosis in elderly women. Sex hormones, namely, estrogen and progesterone, play essential roles in bone balance. Estrogen slows bone resorption, while progesterone stimulates bone formation [16]. Estrogen deficiency occurs during menopause results in rapid bone loss, and this may explain the increased risk of fragility fractures in elderly postmenopausal women compared to men [20]. During adolescence and early adult life, progesterone plays an important role in the achievement of an ideal peak bone mineral density (BMD) and prevention of bone loss during pre-and perimenopausal periods [21]. Results of the studies are controversial regarding the exact status of

Table 4 Agreement (sensitivity, specificity) for PINP and B-ALP to discriminate osteopenia patients (*n* = 48) from healthy control (*n* = 60)

	AUC	p	95% CI	Cut off#	Sensitivity	Specificity	PPV	NPV
PINP (pg/ml)	0.775	< 0.001*	0.689–0.861	≤ 44.7#	75.0	66.67	64.3	76.9
B-ALP (ng/ml)	0.945	< 0.001*	0.906–0.983	> 41.2#	89.58	85.0	82.7	91.1

AUC area under a curve, *p value* probability value, *CI* confidence intervals, *NPV* negative predictive value, *PPV* positive predictive value
*Statistically significant at *p* ≤ 0.05
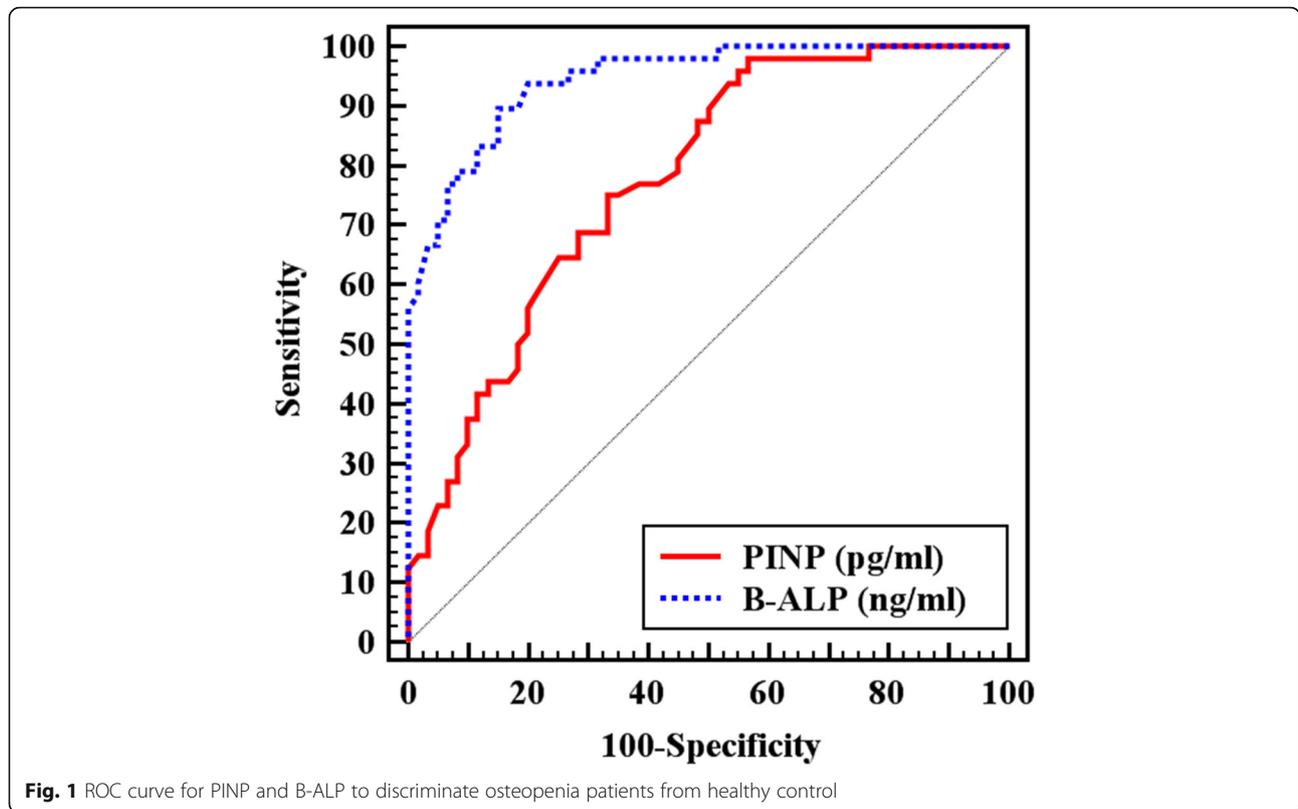#Cutoff was choose according to the Youden index

Fig. 1 ROC curve for PINP and B-ALP to discriminate osteopenia patients from healthy control

these hormones in osteoporotic patients [22, 23]. In a study by Lormeau et al. [24], no significant difference in estradiol levels between osteoporotic patients and the control group was found. Also, they found that estradiol was weakly correlated with BMD-F but not with BMD-S. They concluded that levels of sex hormones in premenopausal women were not correlated with spinal and hip BMD [25]. In our study, progesterone was significantly associated with BMD-F in the osteoporotic group. On contrary, Tehrani and his group detected a significant correlation between progesterone and femoral BMD in osteopenic patients [19].

Our study showed the correlation of estrogen to BMD of femur and spine and the correlation of progesterone to femoral neck BMD in the osteoporotic group, and the ability of estrogen level to differentiate between osteoporotic and osteopenic patients could reflect the impact of the sex hormone on bone mineral density that is

more manifested in the osteoporotic group more than the normal and the osteopenic group. However, these hormones are the main cause of post-menopausal osteoporosis could not differentiate the normal from the osteoporotic/osteopenic patients, so they cannot be used as diagnostic or prognostic markers.

Bone biomarkers are produced by the bone remodeling process which includes bone resorption and bone formation processes [26]. Mechanisms of bone metabolism have been studied with different biomarkers as enzymes, proteins, and by-products during the bone remodeling process [27, 28].

In our study, the levels of procollagen type I N-terminal propeptide (PINP), as a marker of bone formation, differed significantly between studied groups. On contrary to our results, Kharroubi and his colleagues [29] investigated 131 post-menopausal osteoporotic women aged 45 years and more versus 251 who were

Table 5 Agreement (sensitivity, specificity) for PINP and B-ALP to discriminate osteoporosis patients ($n = 60$) from healthy control ($n = 60$)

	AUC	p	95% CI	Cutoff[#]	Sensitivity	Specificity	PPV	NPV
PINP (pg/ml)	0.892	< 0.001[*]	0.835–0.949	≤40.6[#]	86.67	80.0	81.2	85.7
B-ALP (ng/ml)	0.925	< 0.001[*]	0.881–0.969	> 40.3[#]	85.0	83.33	83.6	84.7

AUC area under a curve, *p value* probability value, *CI* confidence intervals, *NPV* negative predictive value, *PPV* positive predictive value

[*]Statistically significant at $p \leq 0.05$
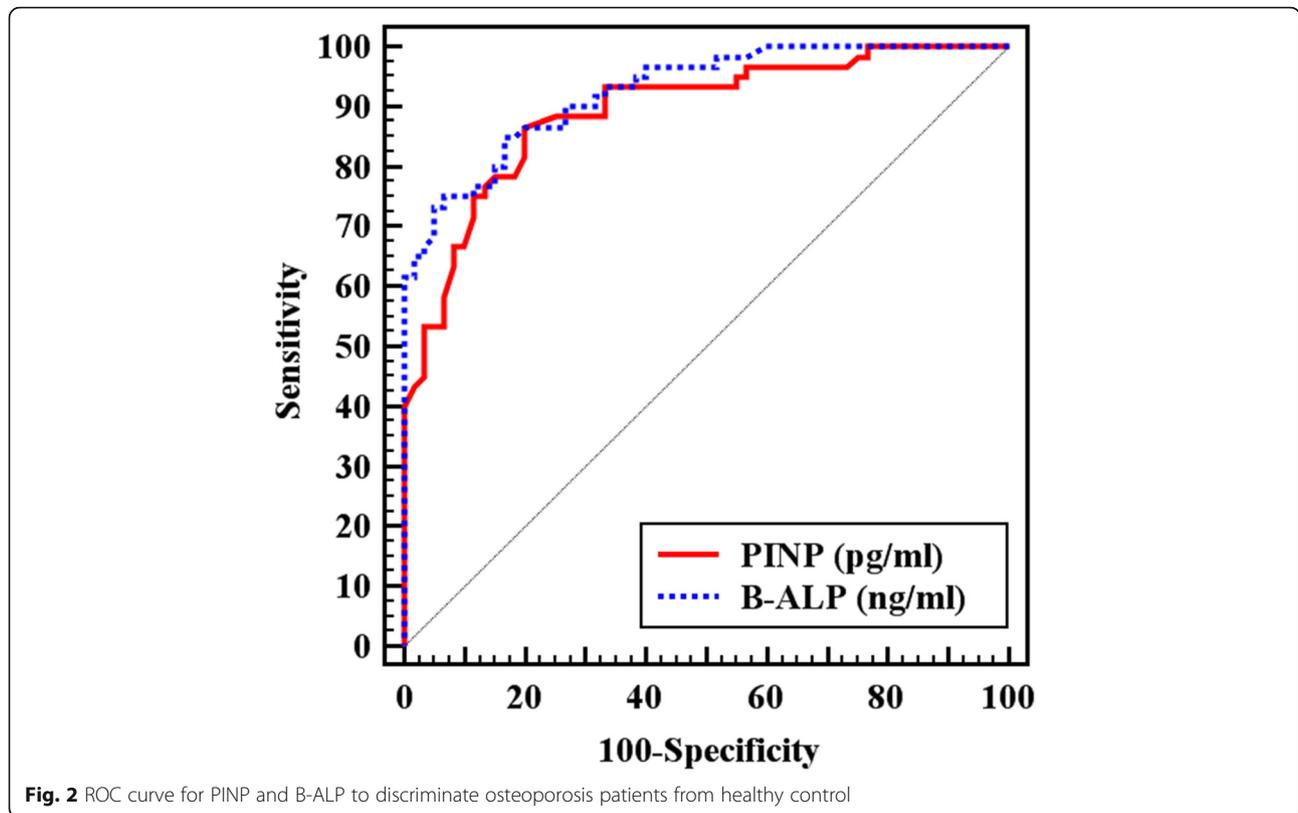
[#]Cutoff was choose according to the Youden index

Fig. 2 ROC curve for PINP and B-ALP to discriminate osteoporosis patients from healthy control

normal women and found no significant differences between osteoporotic and healthy women regarding serum levels of PINP. Also, serum PINP level was not correlated with BMD, while we detected a highly significant correlation between PINP and BMD of femur and spine. Per our findings, Tehrani et al. [19] found that PINP levels differd significantly between the studied groups, and serum PINP level was negatively correlated with both BMD-F and BMD-S.

PINP is a sensitive biomarker of bone turnover. Tähtelä et al. [30] investigated 59 post-menopausal osteopenic women, and they detected a correlation between PINP and BMD. Also, they stated that PINP can be used to assess the response of osteoporosis to treatment [31].

B-ALP is a bone-specific isoform of serum alkaline phosphatase and has been found on the surface of osteoblasts [32]. In bone remodeling, if bone resorption rate

is greater than the bone formation rate, it will be expected that bone loss will occur leads to osteoporosis and related fractures [32]. In our study, levels of B-ALP differed significantly between studied groups. Also, it showed a high significant correlation with BMD-F and BMD-S. In contrary to our results, Zhao et al. [33] examined 22 osteoporotic females versus 73 healthy controls; they found that the levels of B-ALP did not differ significantly between patients and controls. But as our results, they found that BMD-F values were negatively correlated with the B-ALP levels in the osteoporotic group. In the study by Tehrani and his colleagues [19], levels of B-ALP differed significantly between patients (osteoporotic/osteopenic), but not between patients and controls. Lumatchi et al. [11] investigated 48 osteoporotic women divided into two groups according to their age: group A: women aged less than 59 years, and group B: women aged more than 59 years. Patients in women

Table 6 Agreement (sensitivity, specificity) for PINP and B-ALP to discriminate osteoporosis patients ($n = 60$) from osteopenia patients ($n = 48$)

	AUC	p	95% CI	Cut off	Sensitivity	Specificity	PPV	NPV
PINP (pg/ml)	0.721	< 0.001[*]	0.625–0.818	≤ 37.2	71.67	62.50	70.5	63.8
B-ALP (ng/ml)	0.584	0.137	0.475–0.692	> 51.2	58.33	56.25	62.5	51.9

AUC area under a curve, *p value* probability value, *CI* confidence intervals, *NPV* negative predictive value, *PPV* positive predictive value
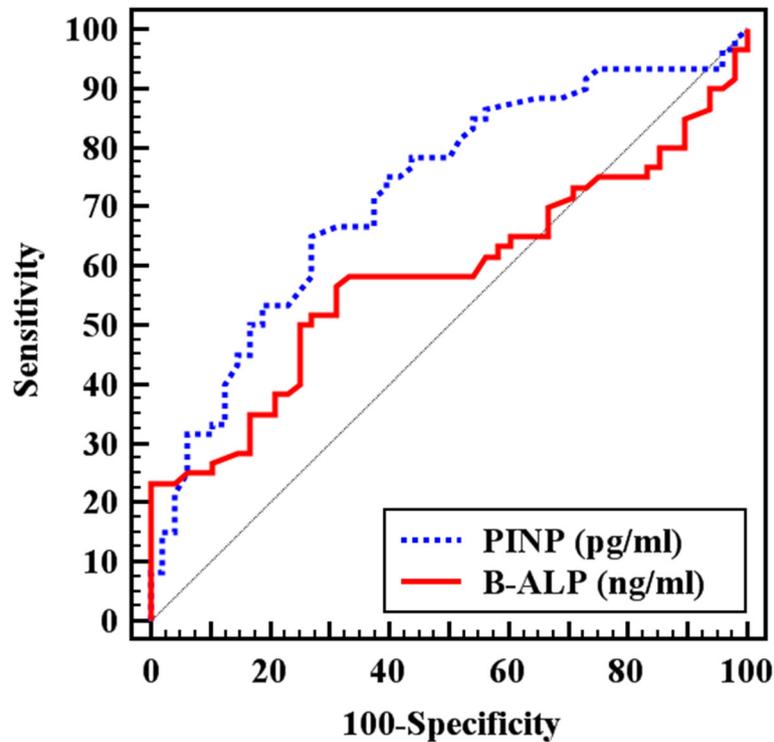[*]Statistically significant at $p \leq 0.05$

Fig. 3 ROC curve for PINP and B-ALP to discriminate osteoporosis patients from osteopenia patients

aged more than 59 years had higher levels of B-ALP. They concluded that high levels of BTM in postmenopausal women may be linked with osteoporosis.

On contrary to our results, Li et al. [34] found that serum B-ALP levels differ significantly between patients (osteoporosis/osteopenia) and the control group.

We observed a highly significant negative correlation between B-ALP and BMD of the femur (BMD-F) and spine (BMD-S); this inverse correlation is due to that B-ALP reflects the surface skeletal activity of bone mass. Other studies fail to find a significant correlation between B-ALP and bone mineral density. They also detected a strong positive correlation between serum estrogen and B-ALP level only in the healthy group, but not in osteoporotic patients [11, 19]. In the current research, we did not find a significant correlation between estrogen and B-ALP in any of the studied groups.

The main limitation of our work was the inability to investigate other bone turnover biomarkers due to financial issues and lack of funds. Also, the lack of follow-up for the patients to reflect the ability of bone turnover markers to change with time and in response to anti-osteoporotic treatment. Future studies are recommended to overcome these limitations. The strength of our work is the type of study, as it is 3-year case-control cross-sectional study, and compares patients to healthy controls.

Conclusion

According to our findings, we can consider PINP and B-ALP as biomarkers for early detection then monitoring of osteoporosis. Measuring these serum markers can replace the assessment of BMD if not available. Also, it could replace the gap between BMD subsequently spaced assessment or could be of value in cases with severe spondylosis, DISH syndrome, old spondylarthritis, and/or previous spinal surgery. Sex hormones could not differentiate the normal from the osteoporotic/osteopenic patients, so they cannot be used as diagnostic or prognostic markers. Validation of this assumption needs large and longitudinal studies.

Authors' contributions
M.S. contributed substantially to the conception and design of this study and acquisition of data, analyzed and interpreted the data, and drafted the manuscript. E.E. contributed substantially to its critical revision. R.A.

Saad *et al. Egyptian Rheumatology and Rehabilitation*   (2021) 48:20

Page 9 of 10

contributed substantially to the laboratory investigations and analysis of the patients' results. All the authors approved the final version submitted for publication and take responsibility for the statements made in the published article.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The proposal of the current study was approved by the ethical committee of the Faculty of Medicine-Alexandria University in January 2018 (approval number 214/2018). An informed written consent was signed by all participants or their care givers prior to the study. The committee's reference number is not available. The current study is original and has not been published elsewhere in any form or language (partially or in full). Results of the current study were presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. No data, text, or theories by others are presented as if they were the author's own ("plagiarism").

Consent for publication
Not applicable

Competing interests
The authors have each completed and submitted an International Committee of Medical Journal Editors Uniform Disclosure Form for Potential Conflicts of Interest. Neither of the authors discloses any potential or actual conflict of interest. No financial or nonfinancial benefits have been or will be received from any party related directly or indirectly to the subject of this article.

Author details
[1]Internal Medicine Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt. [2]Clinical Pathology Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt.

References
1. Handout on Health: "Osteoporosis". NIAMS. 2014. Archived from the original on 18 May 2015. Retrieved 16 May 2015.
2. Johnston CB, Dagar M (2020) Osteoporosis in Older Adults. Med Clin North Am 104(5):873–884
3. Kinsella K, Wan H (2009) An aging world: 2008. U.S. Census Bureau. Int Population Rep P95:9–11
4. Hanan S, Amal T (2018) Knowledge and perception of women at risk for osteoporosis. Educ Intervention 15(2):169–177
5. Beck TJ, Christopheo BR, Wadeu KE, Scott WW, Roa GV (1990) Predicting femoral neck strength from bone mineral data: a structural approach. Investig Radiol 25:6–18
6. Eastell R, Hannon RA (2008) Biomarkers of bone health and osteoporosis risk: symposium on 'diet and bone health'. Proc Nutr Soc 67:157–162
7. Naylor K, Eastell R (2012) Bone turnover markers: use in osteoporosis. Nat Rev Rheumatol 8:379
8. Terreni A, Pezzati P (2012) Biochemical markers in the follow-up of the osteoporotic patients. Clin Cases Min Bone Metab 9(2):80–84
9. Orion Diagnostica. UniQ™ PINP RIA: intact N-terminal propeptide of type I procollagen radioimmunoassay kit. 2008. Catalogue Number 67034. Accessed 3 June 2013.
10. Vasikaran S, Eastell R, Bruyère O et al (2011) Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for internatiosnal reference standards. Osteoporos Int 22(2):391–420
11. Lumachi F, Ermani M, Camozzi V, Tombolan V, Luisetto G (2009) Changes of bone formation markers osteocalcin and bone-specific

12. Donescu O, Battie M, Videman T, Risteli J, Eyre D (2006) The predictive role of bone turnover markers for BMD in middle-aged men. Aging Male 9:97–102
13. Yoshimura N, Muraki S, Oka H, Kawaguchi H, Nakamura K, Akune T (2011) Biochemical markers of bone turnover as predictors of osteoporosis and osteoporotic fractures in men and women: 10-year follow-up of the Taiji cohort. Mod Rheumatol 21:608–620
14. Kharroubi A, Saba E, Smoom R, Bader K, Darwish H (2020) Serum 25-hydroxyvitamin D and bone turnover markers in Palestinian postmenopausal osteoporosis and normal women. Arch Osteoporos. 2017; 12:13. Asian Biomed (Res Rev News) 14(3):97–106
15. Hlaing TT, Compston JE (2014) Biochemical markers of bone turnover– uses and limitations. Ann Clin Biochem 51:189–202
16. Seifert-Klauss V, Prior JC (2010) Progesterone and bone: actions promoting bone health in women. J Osteoporos 2010:845180
17. Cauley JA (2015) Estrogen and bone health in men and women. Steroids. 99:11–15
18. Lee JS, Son SM, Goh TS, Kim TH, Noh EY (2016) Validation of the ECOS-16 Questionnaire in Koreans with Osteoporosis. Asian Spine J 10(5):877–885
19. Tehrani SS, Moallem M, Ebrahimi R, Hosseini SR, Nooreddini H, Parsian H (2020) Status of circulating bone turnover markers in elderly osteoporosis/ osteopenia patients in comparison with healthy subjects. Asian Biomed (Res Rev News) 14(3):97–106
20. Prior JC, Langsetmo L, Lentle BC et al (2014) Ten-year incident osteoporosis-related fractures in the population-based Canadian Multicentre Osteoporosis Study - comparing site and age-specific risks in women and men. Bone 71:237–243
21. Prior JC (2018) Progesterone for the prevention and treatment of osteoporosis in women. Climacteric 21(4):358–365
22. Clapauch R, Mattos TM, Silva P, Marinheiro LP, Buksman S, Schrank Y (2009) Total estradiol, rather than testosterone levels, predicts osteoporosis in aging men. Arq Bras Endocrinol Metabol 53:1020–1025
23. Gillberg P, Johansson AG, Ljunghall S (1999) Decreased estradiol levels and free androgen index and elevated sex hormone-binding globulin levels in male idiopathic osteoporosis. Calcif Tissue Int 64:209–213
24. Lormeau C, Soudan B, d'Herbomez M, Pigny P, Duquesnoy B, Cortet B (2004) Sex hormone-binding globulin, estradiol, and bone turnover markers in male osteoporosis. Bone. 34:933–939
25. Lu L-JW, Nayeem F, Anderson KE, Grady JJ, Nagamani M (2009) Lean body mass, not estrogen or progesterone, predicts peak bone mineral density in premenopausal women. J Nutr 139:250–256
26. Ferreira A, Alho I, Casimiro S, Costa L (2015) Bone remodeling markers and bone metastases: from cancer research to clinical implications. BoneKEy reports 4
27. Shi X, Li C, Liang B, He K, Li X (2014) Weak cation magnetic separation technology and MALDI-TOF-MS in screening serum protein markers in primary type I osteoporosis. Genet Mol Res 14:15285–15294
28. He W-T, Wang B, Li M, Liang B-C, Shi Y-Z, Li X-Y et al (2016) Weak cation exchange magnetic beads coupled with MALDI-TOF-MS in screening serum markers in perimenopausal women with osteopenia. Int J Clin Exp Med 9: 8136–8144
29. Kharroubi A, Saba E, Smoom R, Bader K, Darwish H (2017) Serum 25-hydroxyvitamin D and bone turnover markers in Palestinian postmenopausal osteoporosis and normal women. Arch Osteoporos 12:13
30. Tähtelä R et al (2005) Serum tartrate-resistant acid phosphatase 5b in monitoring bisphosphonate treatment with clodronate: a comparison with urinary N-terminal telopeptide of type I collagen and serum type I procollagen amino-terminal propeptide. Osteoporos Int 16: 1109–1116
31. Miyauchi A et al (2010) Effects of teriparatide on bone mineral density and bone turnover markers in Japanese subjects with osteoporosis at high risk of fracture in a 24-month clinical study: 12-month, randomized, placebo-controlled, double-blind and 12 month open-label phases. Bone 47:493–502
32. Bonnick S, De Villiers T, Odio A et al (2013) Effects of odanacatib on BMD and safety in the treatment of postmenopausal women previously treated with alendronate: a placebo-controlled trial. J Clin Endocrinol Metab 98: 4727–4735

33. Zhao D, Wang J, Liu Y, Liu X (2015) Expressions and clinical significance of serum bone Gla-protein, bone alkaline phosphatase and C-terminal telopeptide of type I collagen in bone metabolism of patients with osteoporosis. Pak J Med Sci 31:91

34. Li N, Zheng Y-B, Han J, Liang W, Wang J-Y, Zhou J-R et al (2013) Lower circulating preptin levels in male patients with osteoporosis are correlated with bone mineral density and bone formation. BMC Musculoskelet Disord 14:49

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.