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Status of circulating bone turnover markers in elderly osteoporosis/osteopenia patients in comparison with healthy subjects

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Abstract

Background: In the aging individuals, osteoporosis is a major health problem. Due to the various limitations of dual X-ray absorptiometry (DEXA) for diagnosis osteoporosis, serum-based biochemical markers have been suggested for the discrimination between the patients and healthy subjects.
Objective: To investigate the serum levels of bone turnover markers in elderly osteoporosis patients.
Methods: The serum samples from elderly subjects (osteoporosis (n = 28), osteopenia (n = 28), and healthy ones (n = 28) were collected from Amirkola Health and Ageing Project study. Furthermore, serum levels of bone formation and bone resorption markers as well as estrogen and progesterone were measured by enzyme-linked immunosorbent assay. Kruskal–Wallis test and receiver operating characteristic curve analysis were used for statistical analysis using SPSS.
Results: Levels of bone alkaline phosphatase (B-ALP) and procollagen type I N-terminal propeptide (PINP) differed between groups ($P = 0.003$ and 0.009, respectively). Furthermore, PINP and B-ALP levels had the best area under the curve, sensitivity, and specificity for the discrimination between patients with osteoporosis and healthy individuals.
Conclusion: In conditions in which we are not able to assess the bone mineral density by DEXA, analysis of the B-ALP and PINP levels may be a helpful tool.

Keywords: bone density; bone remodeling; osteoporosis

Osteoporosis is a common metabolic bone disorder characterized by low bone mass and deterioration of bone tissue [1]. The diagnosis of osteoporosis and osteopenia is possible with bone mineral density (BMD) assessment by dual X-ray absorptiometry (DEXA) technique considered as "gold standard" method [2]. Researchers are trying to find the blood-based method for clinical monitoring and diagnosis of osteoporosis and osteopenia. This method suffers from various limitations, for example, obesity or thinness, previous fractures, and pregnancy can change the results of bone density [3]. Recently, attentions have turned to the role of bone turnover markers (BTMs) as predictors of osteoporosis and osteopenia

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[4]. BTMs have been described as those markers that are able to highlight the skeletal pathology. To some extent the list of these markers is long, but we can categorize them into two large categories: bone formation markers and bone resorption markers [5]. Among the bone formation markers, bone alkaline phosphatase (B-ALP) and the procollagen type I N-terminal propeptide (PINP) are the well-known markers. Amino-terminal cross-linked telopeptide of type I collagen (NTX) and the carboxy-terminal cross-linked telopeptides of type I collagen (CTX or ICTP) are the common markers of bone resorption derived from degradation of type I collagen [6].

There are various controversial reports about the exact status of these markers in bone disorders. As we know, PINP is a propeptide released in the tissue and blood. It seems that in bone disorders, PINP levels change [7, 8]. Yoshimura et al. showed that serum PINP level in men and women is correlated to spinal osteoporosis [9]. Kharroubi et al. did not observe a significant difference between the mean serum values of PINP in osteoporosis and normal groups [10].

B-ALP is an enzyme that plays an important role in bone formation and mineralization [11]. Lumachi et al. reported that the B-ALP levels in osteoporotic women with the age of 49–59 years were significantly higher than those with age above 59 years. In addition, they could not find a significant relationship between B-ALP and BMD in both groups [12]. Zhao et al. reported that the levels of B-ALP did not differ between osteoporosis patients and healthy group [13]. In another study, it has been shown that B-ALP status in osteoporosis patients was lower than osteopenia patients and interestingly the control group had lower B-ALP levels than the osteopenia patients [14].

NTX that is derived from the degradation of type I collagen is a biochemical marker that can show the status of bone resorption. It seems that this molecule can discriminate between women with bone disorders (osteoporosis and osteopenia) and normal postmenopausal subjects [14, 15]. ICTP is a useful marker of the bone remodeling in different pathological conditions, such as bone metastasis in different cancers [16]. This degraded product of type I collagen has been introduced as a diagnostic biochemical marker in several systemic metabolic bone disorders, such as postmenopausal osteoporosis [17].

It seems that other factors, such as sex steroid hormones especially estrogen and progesterone, can influence the number and maturation process of osteoblast and this manner can mediate to reach the optimum bone mass [18, 19]. Estrogen has a direct effect on osteoblasts, osteoclasts, and osteocytes [19–21]. There are studies that reported postmenopausal osteoporosis is associated with decreasing serum levels of estrogen [22]. Moreover, it was reported that serum level of estradiol has a strong relationship with osteoporosis and BMD [23].

According to controversial reports with regard to the status of BTM and sex hormones in osteoporotic and osteopenic patients compared with the control, the main aims of this study are to analyze the status of bone turnover biochemical markers and estrogen and progesterone in the elderly osteoporotic and osteopenic patients as probable markers for the discrimination between patients and healthy individual.

Materials and methods

This study was approved by the Institutional Review Board, Babol University of Medical Sciences (certificate of approval no. 9032417).

Study population

From 1,616 elderly individuals in the Amirkola Health and Ageing Project (AHAP) as an epidemiologic project, we selected 84 subjects according to the inclusion and exclusion criteria to eliminate the effects of interfering factors on bone metabolism and its related biochemical markers [24]. The study population consists of the elderly (osteoporosis (n = 28; male (14, 32.6%), female (14, 34.1%)), osteopenia (n = 28; male (14, 32.6%), female (14, 34.1%)), and healthy ones (n = 28; male (15, 34.9%), female (13, 31.7%)) of the AHAP.

The subjects were included in this study if they were above 60 years, live in their home, walk without any aid, could answer the questionnaires, and give written consent for voluntary participation in the study. The individuals with a present or past disease (type 1 diabetes mellitus, connective tissue disease, hyperthyroidism, parathyroidism, gastrostomy, and prostate cancer) who are taking medications that could affect bone metabolism, such as glucocorticoids (>5 mg/day for more than 3 months), the use of bisphosphonate for ≥6 months, consumption of vitamin D for a period exceeding 2 years, and current warfarin use or vitamin K supplementation and longtime bed rest were excluded.

A BMD test will provide a photograph of bone health. The test can detect osteoporosis, assess the risk for fractures, and identify the response to osteoporosis treatment. The most commonly used BMD test is named a central DXA test. Most commonly, the results of the BMD test were compared with the BMD of healthy young adults, giving a *T*-score. A *T*-score between +1 and −1 is regarded normal. A *T*-score between −1 and −2.5 shows that the individual has low bone mass. A *T*-score of −2.5 or lower demonstrates that the individual

has osteoporosis. In the present study, according to the World Health Organization (WHO) criteria, all participants by the DEXA test were categorized into three separate groups: osteoporotic, osteopenia, and normal subjects. The results of the DEXA scan were reported as *T*-scores:

1. Normal bone: *T*-score above –1
2. Osteopenia: *T*-score between –1 and –2.5
3. Osteoporosis: *T*-score below –2.5 [25].

Biochemical analysis

Fasting blood sample was collected for analysis of biochemical markers in the morning (after 12-hour fasting) and the serum was separated and stored at -80°C until final analysis. The levels of BTMs were analyzed by enzyme-linked immunosorbent assay (ELISA) kits (Cusabio Company, China): B-ALP (code no.CBS-E09033h), NTX (code no. CBS-E09233h), ICTP (code no. CBS-E10363h), and PINP (code no. CBS-E11226h). Moreover, estrogen and progesterone were measured by the ELISA kit (code no. CSB-E07286h and CSB-E07283h, respectively). To analyze the serum levels of B-ALP, PINP, ICTP, and NTX, quantitative sandwich enzyme immunoassay technique was used according to the manufacturer's instruction. Briefly, in this technique, antibody specific for these markers has been precoated onto a microplate. Standards and samples were pipetted into the wells and any B-ALP, PINP, ICTP, and NTX present was bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for these four markers was added to the wells. After washing, avidin-conjugated horseradish peroxidase (HRP) was added to the wells. Following a wash to remove any unbound avidin–enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of B-ALP, PINP, ICTP, and NTX bound in the initial step. Color development was stopped and the intensity of the color was measured. For the analysis of estrogen and progesterone, we used the competitive inhibition enzyme immunoassay technique. In this technique, the microtiter plates were precoated with the goat–anti-rabbit antibody. Standards or samples were added to the appropriate microtiter plate wells with the antibody specific for estrogen and progesterone and HRP conjugated with estrogen and/or progesterone. The competitive inhibition reaction was launched between HRP labeled with these markers and unlabeled estrogen and progesterone with the antibody. A substrate solution was added to the wells and the color developed in opposite to a number of sex hormones in the sample. Color development was stopped and the intensity of the color was measured by an ELISA reader awareness (USA).

Statistical analysis

Statistical data were analyzed by SPSS software (version 24.0; SPSS Inc., Chicago, IL, USA), and a $P < 0.05$ was considered significant. Data were reported as the mean ± standard error of the mean (SEM). For comparisons, Kruskal–Wallis test was used. The diagnostic accuracies of the BTMs for discrimination of healthy controls and osteoporosis/osteopenia patients were analyzed by receiver operating characteristic (ROC) curves [26]. The optimal cutoff values were selected based on the maximum value of sensitivity and specificity. The CAT maker software (version 1.1, the center for Evidence-based Medicine) was used for calculating the positive and negative likelihood ratios (LR^+ and LR^-), positive predictive value (PPV), and negative predictive value (NPV). Analysis of the correlation was also done between various variables and BTMs.

Results

According to the BMD results and with the consideration of the inclusion and exclusion criteria, 28 healthy persons and 56 patients (osteoporosis/osteopenia) were enrolled in this cross-sectional study. As we expected, the mean BMD results in healthy individuals were more than the two patient groups (osteoporosis and osteopenia) and the differences between these groups were statistically significant ($P < 0.001$).

The mean serum levels of the PINP and B-ALP were statistically different between these three groups ($P = 0.003$ and 0.009, respectively). A similar statistical difference was not observed for other parameters including estrogen, progesterone, ICTP, and NTX in the mentioned groups (**Table 1**). Additionally, significant differences were observed in B-ALP levels ($P = 0.012$) between osteopenic patients and healthy subjects, and between osteoporotic patients and control group in B-ALP and PINP levels ($P = 0.005$ and 0.007, respectively), while among the analyzed variables only serum PINP levels had a significant difference between osteopenic and osteoporotic patients.

According to the results of Pearson's correlation analysis, NTX was negatively correlated with estrogen in osteoporotic patients ($r = –0.4$, $P = 0.036$). Interestingly in osteopenic patients, progesterone was positively correlated with femoral BMD ($r = 0.437$, $P = 0.02$). In healthy group, a positive correlation between the progesterone levels with ICTP and spinal BMD ($r = 0.47$, $P = 0.01$ and $r = 0.42$, $P = 0.027$, respectively), as well as B-ALP with estrogen was also observed ($r = 0.7$, $P = 0.001$) (**Figure 1**).

To estimate the diagnostic accuracy of these parameters for distinction between normal subjects and the patient groups,

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Table 1. Comparison of mean values of clinical and biochemical parameters between included persons in this study

Parameters	Healthy persons (mean ± SD)	Osteopenia (mean ± SD)	Osteoporosis (mean ± SD)	*P*
BMD-S (g/cm²)	1.12 ± 0.13	0.85 ± 0.03	0.63 ± 0.08	<0.001
BMD-F (g/cm²)	1.06 ± 0.11	0.84 ± 0.05	0.65 ± 0.08	<0.001
Age	65.61 ± 5.11	66.32 ± 5.21	68.07 ± 5.46	0.11
Estrogen (pg/ml)	79.6 ± 42.96	68.10 ± 13.91	66.53 ± 32.40	0.183
Progesterone (ng/ml)	0.11 ± 0.22	0.12 ± 0.21	0.13 ± 0.42	0.547
ICTP (ng/ml)	438.2 ± 209.81	409.7 ± 177.3	396.8 ± 197.53	0.736
PINP (pg/ml)	57.1 ± 17.53	57.13 ± 12.66	68.5 ± 12.96	0.003
NTX (nmol BCE/L)	15.5 ± 11.71	21.1 ± 12.03	19.06 ± 11.33	0.089
B-ALP (ng/ml)	3.40 ± 4.72	5.90 ± 4.58	5.91 ± 5.17	0.009

Data are presented as mean ± standard deviation (SD), as well as the median and compared by Kruskal–Wallis test, followed by the Mann–Whitney test. BMD-F and BMD-S, bone mineral density of femur and spine; B-ALP, bone alkaline phosphatase; PINP, procollagen type I N-terminal propeptide; NTX, N-terminal cross-linked telopeptide of type I collagen; ICTP, C-terminal telopeptide of type I collagen.

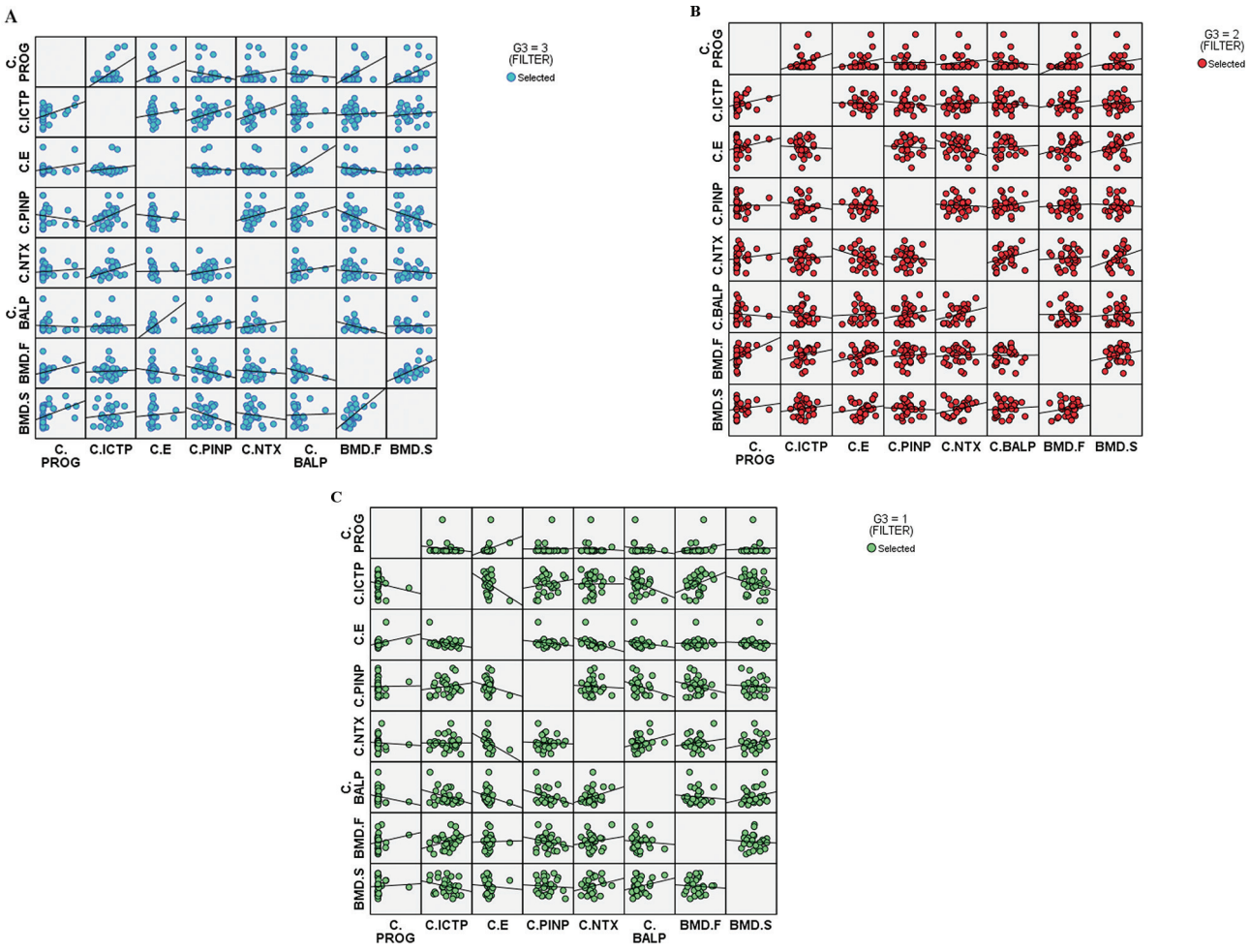

Figure 1. Correlation analysis results between analyzed biochemical parameters in included groups in this study. **A:** normal group, **B:** osteopenia group, **C:** osteoporosis group. A positive correlation between the progesterone levels with ICTP and spinal BMD ($r = 0.47$, $P = 0.01$ and $r = 0.42$, $P = 0.027$, respectively), as well as B-ALP with estrogen was also observed ($r = 0.7$, $P = 0.001$) in healthy individuals. Progesterone was positively correlated with femoral BMD ($r = 0.437$, $P = 0.02$) in osteopenic patients. NTX was negatively correlated with estrogen in osteoporotic patients ($r = –0.4$, $P = 0.036$). ICTP, C-terminal telopeptide of type I collagen; BMD, bone mineral density; B-ALP, bone alkaline phosphatase; BMD-F and BMD-S, bone mineral density of femur and spine; B-ALP, bone alkaline phosphatase; PINP, procollagen type I N-terminal propeptide; NTX, amino-terminal cross-linked telopeptide of type I collagen; ROC, receiver operating characteristics.

Table 2. Comparison of diagnostic accuracies of biochemical parameters by ROC analysis for discrimination of the healthy persons than the two other groups

Parameters	AUC (CI)	*P*	Cutoff point	Sen	Sp	PPV	NPV	LR^+	LR^-
NTX (nmol BCE/L)									
Normal vs osteoporosis	0.63 (0.48–0.78)	0.090	13.57	75	61	65	70	1.92	0.40
Normal vs osteopenia	0.65 (0.5–0.79)	0.050	13.91	71	61	64	68	1.16	0.47
Normal vs patient	0.64 (0.51–0.77)	0.036	13.57	73	61	78	53	2.51	0.44
B-ALP (ng/ml)									
Normal vs osteoporosis	0.72 (0.58–0.86)	0.005	2.10	82	54	63	75	1.78	0.33
Normal vs osteopenia	0.71 (0.55–0.84)	0.012	3.23	71	75	74	72	2.84	0.38
Normal vs patient	0.70 (0.59–0.83)	0.002	3.03	70	68	81	54	2.18	0.44
PINP (pg/ml)									
Normal vs osteoporosis	0.71 (0.57–0.85)	0.007	62.12	71	65	67	70	2.02	0.44
Normal vs osteopenia	0.49 (0.34–0.65)	0.987	46.00	85	33	55	69	1.26	0.45
Normal vs patients	0.58 (0.471–0.738)	0.121	58.85	58	58	73	41	1.32	0.72

AUC, area under the curve; CI, confidence interval; Sen, Sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; LR^+ and LR^-, positive and negative likelihood; B-ALP, bone alkaline phosphatase; PINP, procollagen type I N-terminal propeptide; NTX, amino-terminal cross-linked telopeptide of type I collagen

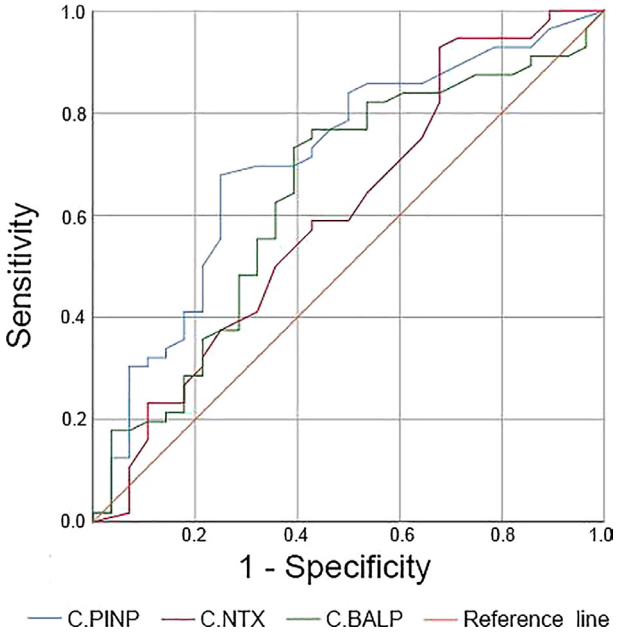

Figure 2. Comparison of diagnostic accuracies of biochemical parameters by ROC curve analysis discriminate for patient groups (osteoporosis and osteopenia) and the healthy subjects. C.PINP, procollagen type I N-terminal propeptide; C.NTX, amino-terminal cross-linked telopeptide of type I collagen; C.BALP, bone alkaline phosphatase.

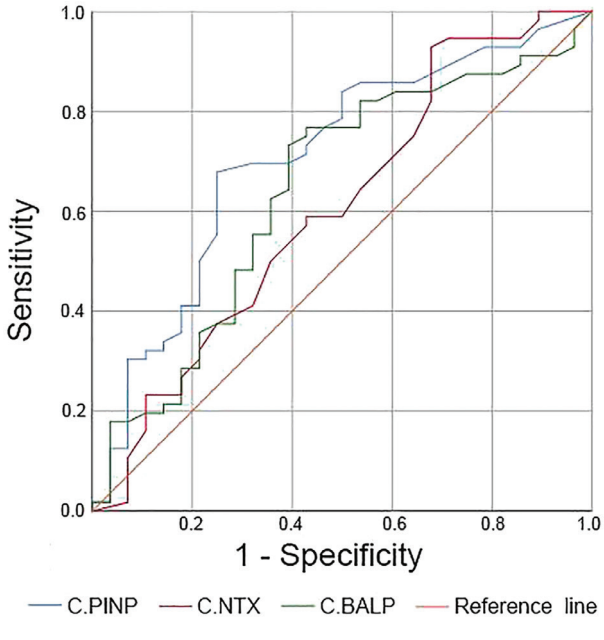

Figure 3. Comparison of diagnostic accuracies of biochemical parameters by ROC curve analysis for discrimination of the healthy persons versus osteoporosis patients. C.PINP, procollagen type I N-terminal propeptide; C.NTX, amino-terminal cross-linked telopeptide of type I collagen; C.BALP, bone alkaline phosphatase.

ROC analysis was performed and the results were presented in **Table 2**. According to the ROC results, for discrimination between the patient groups (osteoporosis and osteopenia) and the healthy subjects, B-ALP and NTX had the highest diagnostic accuracy (**Figure 2**). Besides, for the discrimination between osteoporotic patients and the healthy subjects, PINP and B-ALP showed the best diagnostic accuracy (**Figure 3**). Finally, the results showed that B-ALP is able to discriminate

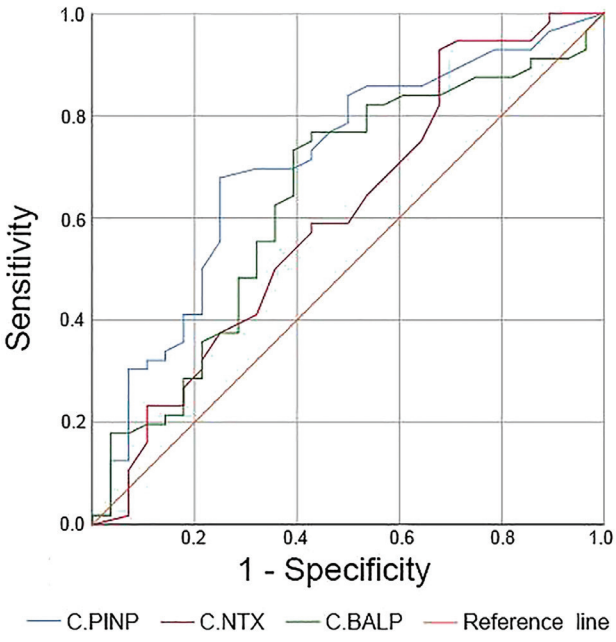

Figure 4. Comparison of diagnostic accuracies of biochemical parameters by ROC curve analysis for discrimination of the healthy persons versus osteopenic patients. C.PINP, procollagen type I N-terminal propeptide; C.NTX, amino-terminal cross-linked telopeptide of type I collagen; C.BALP, bone alkaline phosphatase.

between osteopenia patients and healthy persons with a reasonable area under the curve (AUC), sensitivity, and specificity, PPV, NPV, LR^+, and LR^- (**Figure 4**).

Discussion

The result of our study indicated that B-ALP is a convenient biomarker for the detection of osteoporosis in patients above 59 year-old. Also we discovered a significant relationship between estradiol and B-ALP in osteoporosis patients, and estradiol has a vital function on bone metabolism and estrogen deficiency and it leads to an increase in the osteoclast formation and bone resorption, as well as release of B-ALP to the serum. Based on the result of this study, PINP can be used for prediction of spinal and femoral osteoporosis. Additionally, the serum level of NTX was increased in the patient group compared with the control group. Bone disorders including osteopenia and osteoporosis are critical health problems, especially in the elderly patients. In this study, we analyzed the serum status of BTMs and sex hormones in an elderly population of the AHAP study [27, 28]. Various studies reported the diagnostic accuracy of BTMs for discrimination between healthy individuals and osteoporosis/osteopenia patients. Furthermore, it was indicated that the serum levels of B-ALP are different in various disorders [29]. Zhao et al. measured the B-ALP levels in osteoporosis patients. They observed no significant differences between serum levels of B-ALP in osteoporosis patients in comparison with the healthy control. Besides, they reported a negative correlation between femoral neck BMD values with the B-ALP levels [13]. The relationship between B-ALP with BMD and age in postmenopausal women was studied by Lumachi et al. They elucidated that patients aged over 59 years had a higher level of B-ALP compared with patients aged 49–59 years. In addition, a significant relationship between age and serum levels of B-ALP in patients over 59 years was reported in this study [30]. Furthermore, Li et al. demonstrated that serum B-ALP level in osteoporosis patients was lower than the osteopenia patients. Such a pattern was also observed in osteopenia patients in comparison with normal group [14]. In our study, we did not observe a significant correlation between B-ALP and BMD, similar to the previous study [30]. This inverse correlation between BMD and B-ALP because of this B-ALP largely reflects surface skeletal activity of bone not bone mass. The serum level of B-ALP significantly increased in our patient groups similar to that studied by Li et al. A strong positive correlation was observed between serum estradiol and B-ALP level only in our healthy group, but not in patient groups. It was found a similar correlation between serum estradiol and B-ALP level in the healthy and patient groups [31].

Bone remodeling was assessed by the measurement of specific BTMs such as serum PINP in the present study [32]. Kharroubi et al. reported no significant differences between the mean values of PINP between osteoporosis patients with the normal group. Besides, serum PINP level was not correlated with BMD, as well as the serum levels of PINP and CTX were positively correlated in osteoporosis patients [10]. In this manner, it was revealed that serum levels of PINP in men and women were significantly associated with the occurrence of spinal osteoporosis. Indeed, PINP can be used for the prediction of spinal osteoporosis in women independent of BMD [9]. In the current study, the levels of PINP were significantly different between the three mentioned groups. Based on our knowledge that serum concentration of PINP reflected the amount of bone turnover and formation, multiple disease such as osteoporosis, Paget, and osteopenia have high bone turnover and would be expected to be associated with high PINP serum concentration [33]. In addition in the patient groups, serum PINP level was negatively correlated with spinal and femoral BMD.

ICTP is another important bone remodeling parameters, which can be used as a probable clinical diagnostic marker. It was illustrated that the serum level of the ICTP was significantly higher in men with incident fractures than the

men without fractures, and these patients had lower BMD at baseline [34]. In another study in which the patients were followed up for 5 years (men aged 35–69 years), researchers observed that the change in the ICTP status was not correlated with spine or femoral neck BMD changes [8]. In our study, we observed a slight decrease in the status of ICTP of normal persons toward osteoporosis patients in crude data, but this difference was not statistically significant.

Measurement of NTX in the urine and serum had been developed since 1990, in which the experimental research showed the potency of this biochemical marker as a bone resorption index [35]. Moreover, NTX was considered as a marker of bone resorption with high sensitivity and specificity [36, 37]. Schneider et al. measured the status of urine NTX in older subjects with normal hip bone density, osteopenia, and osteoporosis. They reported that the urine levels of NTX increased slightly with age in men and women who receive estrogen and more dramatically in non-estrogen user women. In addition, the urine level of NTX was able to discriminate between normal, osteopenia, and osteoporotic patients. They suggested that NTX levels could be regarded as a novel marker for prediction of osteoporosis in older men and women [38]. Scariano et al. demonstrated that BMD was associated with serum levels of NTX, and serum NTX levels were significantly elevated in osteopenic women [39]. Contrast to the study [38], they reported that NTX levels significantly decreased in women who received estrogen replacement therapy. In the current study, the mean serum value of NTX was increased in patient groups in comparison with healthy subjects. NTX is another bone resorption marker that has high sensitivity and specificity for this phenomenon. This factor can discriminate osteoporosis, osteopenia, and normal subject. Patients with osteoporosis and osteopenia especially in postmenopausal women have high rate of bone turnover that increases the level of NTX in blood stream. Moreover, in osteoporosis group, only a negative significant correlation was observed between NTX and estrogen levels. This result was predictable because, as the age rises, the amount of estrogen decreased, resulting in increased bone fractures leading to more NTX release similar to the previous study [39].

Accumulating evidence showed that increased fracture risk is along with other biological variables, such as diurnal variation and physical activity, which may inversely affect the balance and result in an increased risk of bone fracture. Additionally, it was indicated that disruptions in the physiology of sleep and circadian rhythmicity may affect bone health [40–42]. On the other hand, the effect of BMI on BMD is variable in different ethnic groups. Several studies have demonstrated an association of low BMI with low BMD and fractures, while several other studies showed a protective effect of higher BMI on BMD [43, 44]. Furthermore, many lifestyle factors affect BMD. Lifestyle and dietary behavior are important factors for the health of bone [45]. The deficiency of calcium and vitamin D contributes to the alterations of bone remodeling and bone integrity. Indeed, dietary calcium has a remarkable positive correlation with higher BMD [46, 47]. Similarly, smokers have three times more probability of having osteoporosis than nonsmokers, which is supported by different studies. In this context, smoking declines calcium absorption. Thus, it decreases BMD and elevates the risk of sustaining the fractures or tendon injury [48, 49]. Alcohol consumption shows protective effects on bone health from the bivariate analysis. Participants who consumed moderate levels of alcohol had a lower risk of hip fracture. Although, high consumption of alcohol increases in loss of calcium in urine which reduces the bone mass [50, 51]. Moreover, a number of studies revealed that osteoporosis was not associated with daily exercise [52, 53].

Sex steroid hormones such as estrogen and progesterone are involved in the development and maintenance of the skeleton in both women and men. Indeed, total estradiol levels <5 pg/ml were correlated with a 2.5-fold increase in fractures in older women. Interestingly, a similar correlation was found in men [19]. Estrogens are decreased with elevating age in both men and women and likely contribute to bone loss and fracture. It should be noted that more studies have focused on the correlation between serum levels of estrogen with osteoporosis in both men and women [54, 55], and only a few studies reported the association between progesterone concentrations and osteoporosis in both men and women. On the other hand, estrogen slows bone resorption as the primary reason for BMD loss and progesterone activates bone formation. Remarkably, bone remodeling/renovation is a two-part, balanced process: bone resorption is fast while formation is slow [18, 54]. Although, in our study, the levels of these hormones had no significant difference between osteoporosis, osteopenia, and healthy groups.

As we know, hormonal changes are vital factors for osteoporosis development especially in elderly women. There are controversial studies regarding the exact status of this important hormone in osteoporotic patients [56, 57]. Nguyen et al. showed that the serum levels of estradiol are important for bone mass development in both men and women [23]. Lormeau et al. demonstrated that there was no significant difference in estradiol levels between osteoporosis patients and control group. Besides, estradiol was weakly correlated with BMD at the femoral neck, but not in the lumbar spine [31]. It was reported that in premenopausal women, serum concentrations of estradiol and progesterone were not predictors of BMD and were not correlated with spinal and hip BMD [58]. Our results

showed that progesterone was significantly correlated with femoral BMD in osteopenia patients.

As an attempt to discriminate between the patients and healthy controls by serum BTMs, we determined the diagnostic accuracy of the measured parameters by ROC analysis and extracted the related cut points and data. The results showed that serum B-ALP and NTX had reasonable AUCs for the discrimination between patients (osteoporosis and osteopenia) and healthy subjects. Furthermore, for the discrimination between healthy individual and osteoporosis patients, B-ALP and PINP showed the best diagnostic accuracy according to the results of AUC, sensitivity, and specificity (**Table 2**). Cabrera et al. showed that serum the levels of B-ALP and PINP had the highest diagnostic accuracy (AUC, sensitivity, and specificity of 0.780, 65%, 94% and 0.86, 73%, 94%, respectively, for B-ALP and PINP) for the discrimination between the osteoporosis patients and healthy controls. They suggested that B-ALP and PINP are good diagnostic markers for the discrimination between osteoporotic patients and healthy subjects [59]. In another study, Yılmaz et al. indicated the AUC of 0.570 for cutoff point 6.5 U/l for B-ALP as a helpful diagnostic tool for the discrimination of postmenopausal osteoporosis patients and control (sensitivity and specificity of 92% and 28%, respectively) [60].

BTMs are valuable parameters for highlighting, monitoring, and early detection of bone disorders. But until now, they have only a limited application in the clinic. We can suggest that in conditions in which the assessment of BMD is not possible by DEXA, analysis of BTMs maybe a helpful tool. Definitely evaluating the diagnostic accuracy of these molecules in large and longitudinal studies can open a new window for possible more applications of these molecules in the clinic. The main limitation of our work was lack of access to samples, and some of the samples were omitted from our study due to the incomplete demographic information. The strength of our work is the type of study, which is a cross-sectional and participants were monitored for a long time. Also we try to introduce the NTX and B-ALP as new marker for discrimination between patients and healthy subjects. From laboratory experiments to clinical trials, many studies have investigated the application of various bone biomarkers to assess bone disorders. Meanwhile, the BTMs still have limited clinical utility for diagnosis of osteoporosis or osteopenia compared with DXA or FRAX score and should not be considered alone in clinical use.

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Author contributions. SST contributed substantially to the conception and design of this study. MM contributed substantially to the acquisition of data. RE, SRH, HN, and HP analyzed and interpreted the data. SRH, HN, MM, and RE drafted the manuscript. SST and HP contributed substantially to its critical revision. All the authors approved the final version submitted for publication and take responsibility for the statements made in the published article.

Conflict of interest statement. 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 non-financial benefits have been or will be received from any party related directly or indirectly to the subject of this article.

Data sharing statement. The datasets generated or analyzed during the current study are available from the corresponding author on reasonable request.

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