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Research Article
Serological Markers: Are they Reliable for the Diagnosis of Low Bone Mass Density?

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Abstract

Background and Objective: Bone remodeling includes balanced bone formation and resorption and low bone mass density (BMD) occurs when there is a higher rate of resorption. Osteoporosis is a chronic asymptomatic disease with bone fragility that increases an impending risk of bone fracture caused by minor trauma. Calcium and vitamin D are critical for bone mineralization and health. Bone-specific alkaline phosphatase (ALP) and osteocalcin (OC) are markers of bone formation. This study was conducted to test the utility of serological parameters as reliable markers in the diagnosis of low BMD compared with BMD measurements. **Materials and Methods:** In this study 715 Saudi students of Umm Al-Qura University aged 19-22 years were assessed for serum levels of calcium, vitamin D, ALP and OC. The BMD was measured by dual-energy X-ray absorptiometry (DEXA) and the results were statistically analyzed. **Results:** We found that 26.57% of the studied cases had low BMD and showed significantly higher levels of serum calcium, bone-specific ALP and OC with significantly lower serum levels of vitamin D compared to control cases. There was no correlation between serum markers and BMD measurements in cases of low BMD. **Conclusion:** Serum markers may be useful for screening and predicting people who are at risk of developing BMD as well as for assessing responses to osteoporosis therapy. Low BMD is better diagnosed by a combination of serum markers and measurements of BMD.

Key words: Osteoporosis, bone mass density, calcium, vitamin D, alkaline phosphatase, osteocalcin, bone turn over

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

Correction was incorporated on July 23, 2021, after online publication.
1. The corresponding author has been changed. Now the corresponding author is Osama A. Shaikhomar.
2. Acknowledgment is added.

INTRODUCTION

Bones are the hardest connective tissue in the body because they store calcium. Bones are continuously changing through a mechanism called 'bone remodeling', which occurs through balanced bone resorption by osteoclasts and bone formation by osteoblasts[1,2]. Imbalance of this tightly coupled process may alter the bone state and lead to low bone mass density (BMD)[3]. Osteoblasts lay down uncalcified bone matrix, which calcifies and hardens by deposition of calcium crystals. Thus, calcium intake ensures bone rigidity and inadequate dietary calcium leads to bone destruction by osteoclasts and subsequent mobilization of calcium from bones to the blood[4,5].

While calcium is a key building block in bones, adequate levels of vitamin D are also important for bone health by increasing the intestinal absorption of calcium[6,7]. Despite the fact that 90% of vitamin D requirements are achieved from sun exposure, the remaining 10% come from dietary sources and supplementation, which are poorly obtained by most people[8,9].

Osteoporosis is a chronic, progressive disease characterized by bone fragility due to low BMD. It is an asymptomatic disease that predisposes patients to fracture after minimal trauma. Moreover, it constitutes a major health problem because of its long-term morbidity and high medical costs[10,11].

Certain enzymes or markers are released by osteoblasts during bone formation, such as bone-specific ALP and OC[12,13]. The crystallization of calcium during bone formation requires an alkaline medium and bone-specific ALP helps in this process. OC reflects osteoblastic activity, adds to bone mineralization and plays a key role in the mechanical properties of bones[14-16]. In osteoporosis and osteopenia, which are the most prevalent disorders of low BMD, there is high bone turnover and an attempt of bone formation to combat the high bone destruction[17]. BMD is currently best measured by dual-energy X-ray absorptiometry (DEXA), which is an enhanced form of X-ray technology that provides an easy, simple and non-invasive estimate of the amount of bone[18].

This study was conducted to test the reliability of using serological parameters in the diagnosis of cases of low BMD through a comparison of the BMD estimate measured by DEXA.

MATERIALS AND METHODS

This cross-sectional study was conducted at Umm Al-Qura University, Holly Makkah, KSA.

Materials and research tools: This study enrolled 715 Saudi students of Umm Al-Qura University aged 19-22 years. There were 384 males and 331 females.

Experimental design: The reliability of specific serological parameters for the diagnosis of low BMD was assessed by comparing these results with definitive DEXA results.

Data collection: Blood samples were collected in plain tubes stored at 37°C and then centrifuged for 5 min at1500 RPM. The purified serum was then stored at -80°C until the time of analysis.

Parameters measured: The following serological parameters were measured: serum level of calcium by SMAC analysis, which is an automated colorimetric technique that has a reference range of 8.5-10.5[19] mg dL^{-1}; serum level of vitamin D using an enzyme immunoassay technique, which has a reference range of 20-50[20] ng mL^{-1}; serum level of bone-specific ALP using an enzyme immunoassay technique, which has a reference range of 44-147[21] IU L^{-1} and serum level of OC using the ELISA technique, which has a wide reference range but generally 7-14 [22,23] ng mL^{-1}. Extremely high or low readings with inconsistent results were excluded. BMD was measured and expressed as a T-score. According to the World Health Organization (WHO), a T-score of -1.0 or above is considered normal, between -1.0 and -2.5 represents osteopenia and a T-score of -2.5 or below is considered osteoporosis[24,25].

Statistical analysis: The results were tabulated and statistically analyzed using the SPSS program version 25 for determining mean, standard deviation and Chi-square. Data were considered statistically significant at $p<0.05$. A one-way analysis of variance (ANOVA) was used to compare the serum markers of the studied groups. The results of serological tests were assessed for correlation with BMD using the Pearson correlation (r) and a correlation was set at $p<0.05$.

RESULTS

The current study was conducted with 715participants. There were 190 cases (26.57%) with low BMD, 89 cases (12.45%) with osteoporosis and 101 cases (14.12%) with osteopenia (Table 1).

Low BMD occurred significantly more in female participants (48 cases of osteoporosis and 61 osteopenic cases) than male participants (41 cases of osteoporosis and 40 osteopenic cases) (Table 2).

Bone mass density measurements by DEXA: In control cases, the BMD expressed as a T-score ranged from -0.64 to -0.98 with a mean of -0.80±0.09. There was a significant reduction in BMD in cases of osteopenia, ranging from -1.12 to -2.37 with a mean of -1.98±0.27. In cases of osteoporosis, the T-score showed a further significant reduction that ranged from -2.60 to -3.41 with a mean of -2.84±0.19. (Table 3) (Figs. 1-3)

Table 1: Study subjects

	No.	Percentage
Control	525	73.43
Osteopenia	101	14.12
Osteoporosis	89	12.45

Table 2: Sex differences of BMD among study subjects

	Male (N = 384)		Female (N = 331)	
BMD	No.	Percentage	No.	Percentage
Control	303	42.38	222	31.05
Osteopenia	40	5.59	61	8.54
Osteoporosis	41	5.73	48	6.71
Total number	384	53.70	331	46.30

Table 3: Results of DEXA radiological examination

	Control (N = 525)	Osteopenia (N = 101)	Osteoporosis (N = 89)
Minimum	-0.64	-1.12	-2.60
Maximum	-0.98	-2.37	-3.41
Mean±SD	-0.80±0.09	-1.98±0.27	-2.84±0.19
F-test		4.648	0.001
Sig.		1.442	0.151

A p-value <0.05 was considered statistically significant. *p≤0.05: significant difference, **p≤0.01: Highly significant difference

B- Serological results

Serum level of calcium: In control cases, the mean serum calcium level was 8.83±0.45. There was a significant increase in calcium levels in participants with osteopenia (mean: 10.86±0.28) and a further significant increase in participants with osteoporosis (mean: 11.66±0.40) (Table 4).

There was no correlation between serum calcium level and BMD in control cases (r = 0.070, p = 0.112), osteopenic participants (r = 0.044, p = 0.202) and osteoporotic participants (r = 0.156, p = 0.058).

Serum level of vitamin D: The control cases showed a mean vitamin D level of 27.38±4.29. There was a significant decrease in the vitamin D level in participants with osteopenia (mean: 15.47±0.77) and a further significant reduction in participants with osteoporosis (mean: 9.96±0.67) (Table 5).

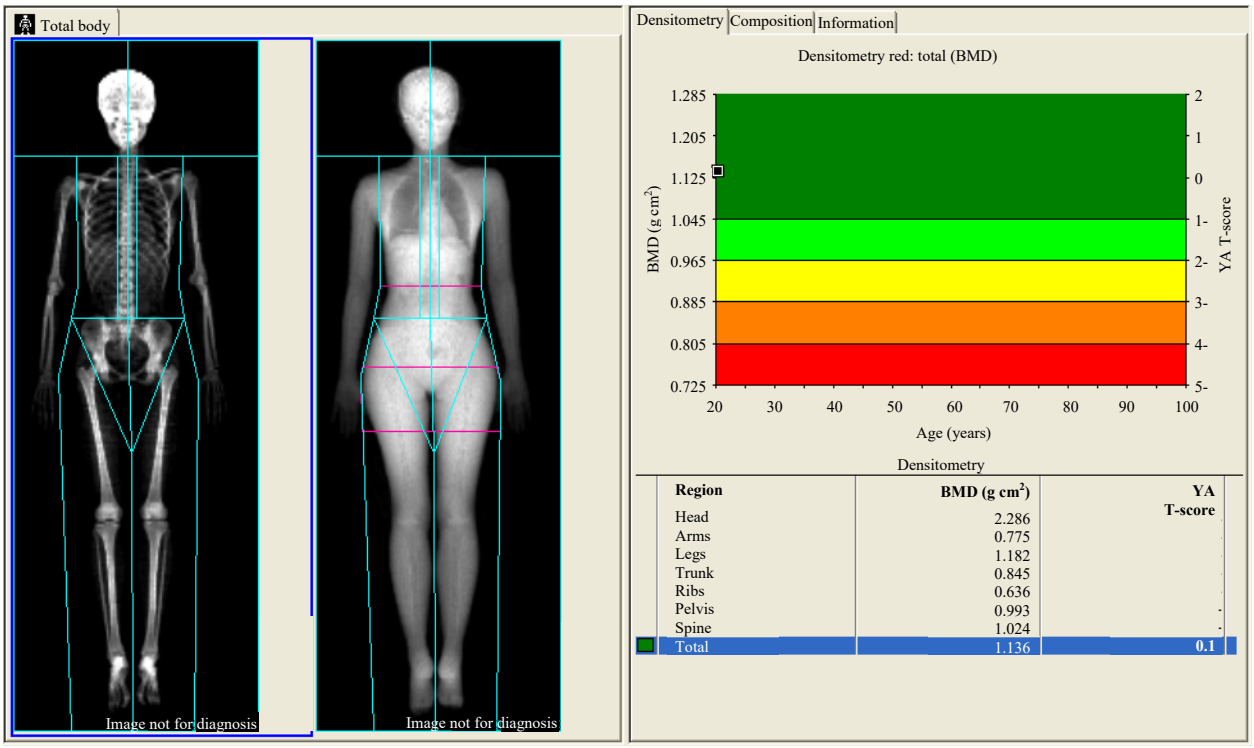

Fig. 1: Representative DEXA for total region of a study subject with normal BMD. The diagram shows a representative result for normal BMD with T-score = 1

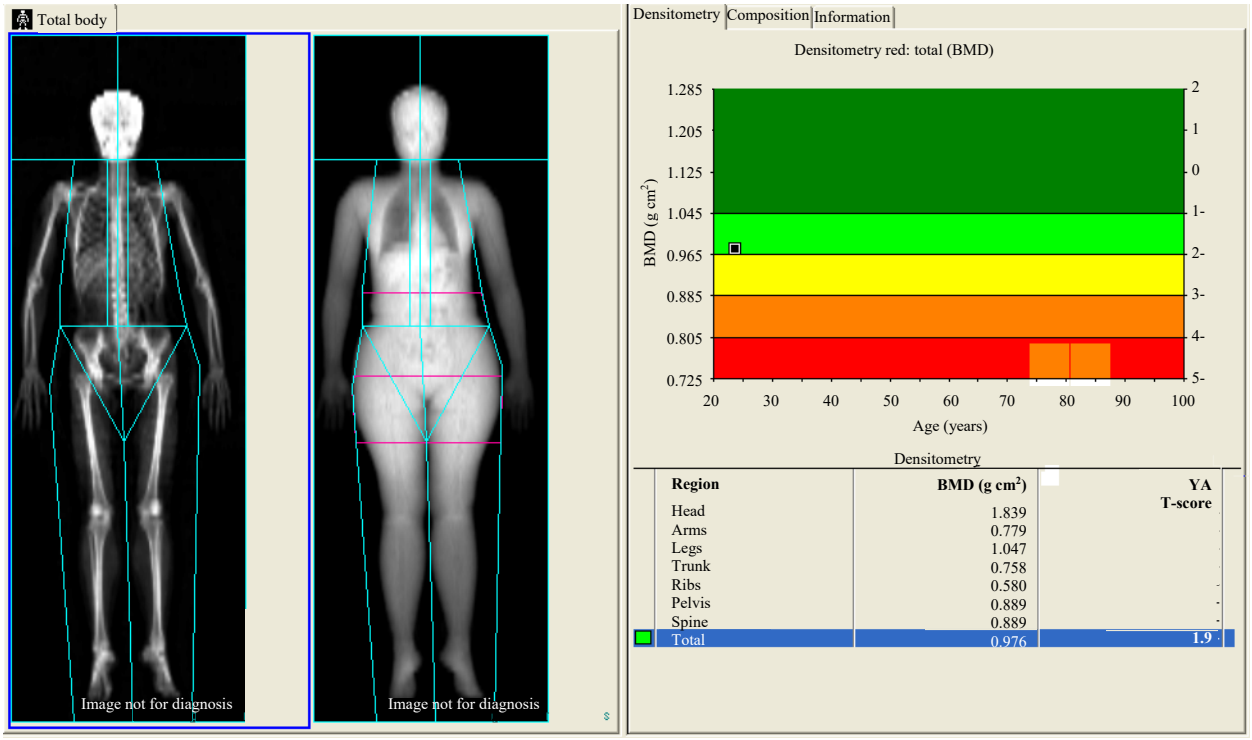

Fig. 2: DEXA for total region of a subject with osteopenia. The diagram shows a representative case of osteopenia with low BMD (T-score = -1.9)

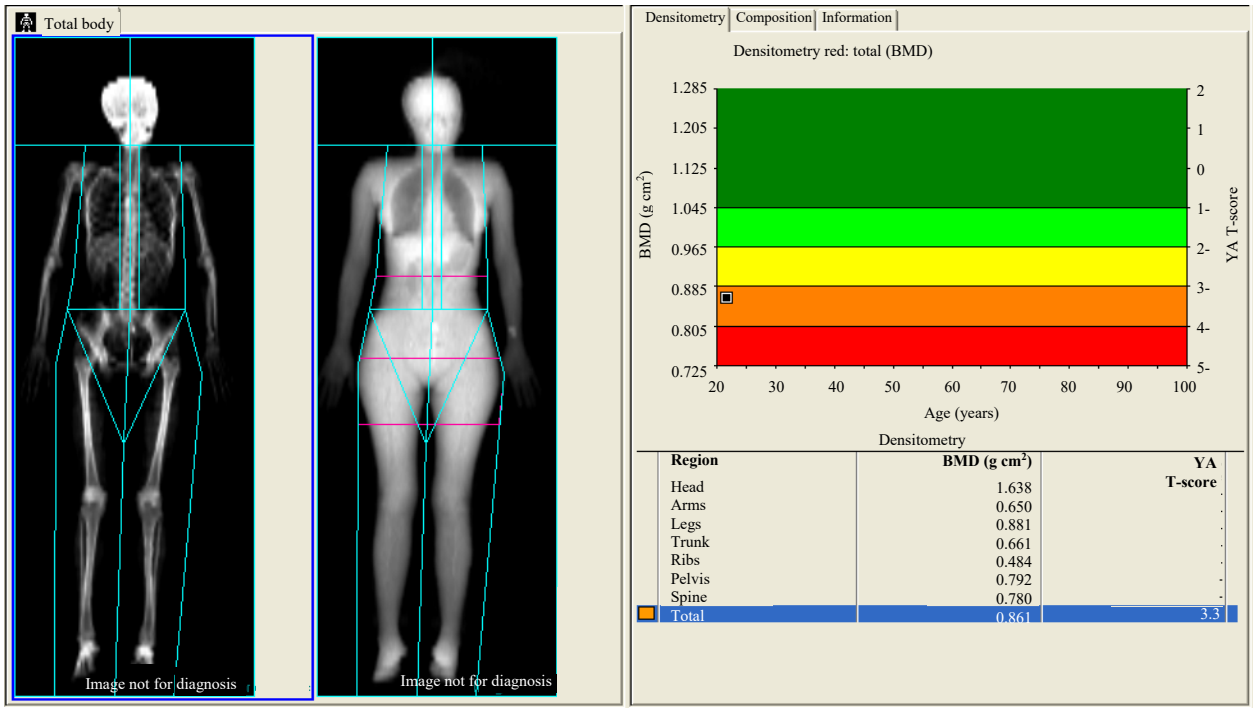

Fig. 3: DEXA for total region of a subject with osteoporosis. The diagram shows a case of osteoporosis with low BMD (T-score = -3.3)

Table 4: Serum calcium level (mg dL^{-1}) in study subjects

	Control (N = 525)	Osteopenia (N = 101)	Osteoporosis (N = 89)
Minimum	6.97	8.77	10.12
Maximum	8.62	9.86	11.76
Mean±SD	7.83±0.45	9.260±0.28	10.66±0.40
F-test		1.293	0.967
Sig.		0.206	0.513

A p-value <0.05 was considered statistically significant. *p≤0.05: significant difference, **p≤0.01: highly significant difference

Table 5: Serum vitamin D levels (ng mL^{-1}) among study subjects

	Control (N = 525)	Osteopenia (N = 101)	Osteoporosis (N = 89)
Minimum	21.55	13.88	8.99
Maximum	38.61	17.76	12.39
Mean±SD	27.38±4.29	15.47±0.77	9.96±0.67
F-test		9.046	1.105
Sig.		0.001	0.369

A p-value <0.05 was considered statistically significant. *p≤0.05: Significant difference, **p≤0.01: Highly significant difference

Table 6: Serum alkaline phosphatase level (IU L^{-1}) in study subjects

	Control (N = 525)	Osteopenia (N = 101)	Osteoporosis (N = 89)
Minimum	115.00	149.000	278.000
Maximum	147.00	261.000	335.000
Mean±SD	135.17±9.21	202.040±33.89	311.360±13.96
F-test		0.931	1.580
Sig.		0.552	0.084

A p-value <0.05 was considered statistically significant. *p≤0.05: Significant difference, **p≤0.01: Highly significant difference

Table 7: Serum osteocalcin levels (ng mL^{-1}) in study subjects

	Control (N = 525)	Osteopenia (N = 101)	Osteoporosis (N = 89)
Minimum	8.99	15.500	24.800
Maximum	15.70	23.700	43.500
Mean±SD	11.73±1.84	18.930±2.08	31.810±4.35***
F-test		0.636	3.582
Sig.		0.868	0.001

A p-value <0.05 was considered statistically significant. *p≤0.05: Significant difference, **p≤0.01: Highly significant difference

There was a correlation between the serum level of vitamin D and BMD in control cases (r = 0.158, p = 0.001) but no correlation was found in both osteopenic participants (r = 0.191, p = 0.056) and osteoporotic participants (r = 0.178, p = 0.095).

Serum level of bone-specific ALP: The mean serum level of bone-specific ALP was 135.17±9.21 in control cases, while participants with osteopenia and osteoporosis showed a significant increase (mean: 202.04±33.89 and 311.36±13.96, respectively) (Table 6).

There was no correlation between serum bone-specific ALP level and BMD in control cases (r = 0.001, p = 0.990), osteopenia participants (r = 0.066, p = 0.539) and osteoporosis participants (r = 0.186, p = 0.081).

Serum level of OC: Participants with osteopenia and osteoporosis exhibited significant increases in mean OC serum levels compared to control cases (18.93±2.08 and 31.81±4.35 vs. 11.73±1.84, respectively; Table 7) In addition, a significant correlation was found between the serum level of OC and BMD in control cases (r = 0.248; p = 0.001). However, no correlation was observed between OC serum levels and osteopenic participants (r = 0.040, p = 0.691) or osteoporotic participants (r = 0.044, p = 0.680).

DISCUSSION

Low BMD and its severe form, osteoporosis, is a silent disorder with no obvious specific symptoms. It is frequently misdiagnosed and often only accurately managed in reaction to harmful fractures[26,27]. Therefore, early diagnosis of osteoporosis is critical for efficient treatment and identifying patients at risk of fracture[28,29].

This study sought to assess the utility of using serum markers for the early diagnosis of low BMD instead of DEXA. The DEXA approach is a simple and accurate method for diagnosing low BMD but it remains too expensive for widespread use, particularly in poor communities. This study aimed to determine the accuracy of serum markers previously unexplored to potentially identify reliable and inexpensive biomarkers for diagnosing these patients. For this study, we assessed calcium, vitamin D, bone-specific ALP and OC in relation to BMD.

We found that although participants with low BMD had increased serum calcium levels, there was no correlation between this biomarker and BMD. Calcium homeostasis may vary under different situations. Under physiological conditions, bone remodeling involves the orchestrated coupling of bone resorption and synthesis that generates equal amounts of destroyed and newly formed bone with no net calcium inflow from the bone pool to serum[30,31].

On the other hand, low BMD is always associated with insufficient dietary calcium but serum calcium levels often remain high, since this situation drives increase bone destruction and the subsequent mobilization of calcium from bone to maintain physiologic calcium levels at the expense of bone health[32,33]. Calcium delivered to the circulation is actively reused by osteoblasts to maintain bone but surprisingly the bone remains weak[34,35]. Therefore, although calcium is a key component of bone, the serum levels do not reflect the state of bone health and cannot be used alone as an indicator of low BMD. Nevertheless, calcium levels may be useful in predicting an underlying problem that could develop into osteoporosis if left untreated[32,33].

In the current study, participants with low BMD showed elevated serum calcium levels, while serum levels of vitamin D were far below normal. It is not surprising that some participants with low BMD may have the same dietary calcium intake as participants without low BMD, since the underlying cause may be due to low vitamin D from either dietary insufficiency or lower sun exposure[36,37]. It has been shown that vitamin D insufficiency is highly prevalent among the Saudi population and is attributed to poor exposure to sunlight due to excessive heat[36]. Insufficient vitamin D levels reduce the intestinal absorption of calcium, which drives the movement of calcium from bone into circulation[7].

Bone turnover markers have been extensively evaluated in bone remodeling for both physiological and pathological situations[28,38]. The current study demonstrated that participants with low BMD had bone-specific ALP serum levels that varied widely from near the upper normal limit to far above the normal limit with no correlation with BMD. This might be explained by the high bone turnover associated with low BMD that is driven to maintain bone remodeling close to the normal state. Thus, higher levels of bone-specific ALP are expected since it is a highly specific marker of bone formation[39-41]. This is in agreement with the results of other studies, where serum bone-specific ALP levels in these patients reached values that were double or triple the normal value[42,43]. Interestingly, some studies have shown that patients with high serum levels of bone-specific ALP had no disturbance in the bone remodeling process, which was attributed to hypophosphatesia[44,45]. On the other hand, other studies have shown low levels of ALP in patients with low BMD, which was attributed to generalized malnutrition, especially with regard to zinc and magnesium, which increase and stimulate ALP, respectively[46,47]. Moreover, Lumachi *et al.*[48] and Zhou *et al.*[49] unexpectedly found no relationship between ALP and BMD. Thus, due to the wide range of serum levels of ALP and the lack of a correlation with BMD in the current study, bone-specific ALP might not be a reliable marker for diagnosis of low BMD.

We also found that participants with low BMD had OC serum levels near the upper limit of normal and increased high above normal with no correlation to BMD. This marker remains controversial, as some studies have shown significantly high serum levels of OC in patients with low BMD patients, others have shown no significant differences and some have found low serum levels in a group of patients with histomorphometrically proven osteoporosis[34,39,50]. Serum OC is a dynamic bone formation marker and has long been considered to be specific for high bone turnover, which is in agreement with the observation of high OC levels in patients with low BMD[39]. However, unlike ALP, genetic evidence has revealed that OC is produced late in the mineralization process and thus it has a minor role in bone mineralization[15,16]. Therefore, the serum level of OC might not be used alone as a marker of low BMD.

Although the high serum levels of bone formation markers indicate newly synthesized bone, they are less mineralized, which may negatively affect the bone microarchitecture and integrity and contribute to the risk of fracture[38-40]. Therefore, serum markers alone may not be sufficient for diagnosing low BMD. Instead, they may be useful for predicting and identifying people at risk of developing fractures, especially in postmenopausal women and assessing the response to osteoporosis therapy[28,29]. On the other hand, a combination of these markers with a BMD measurement by DEXA may provide comprehensive information for the early diagnosis of osteoporosis[51,52].

The use of serum markers for the diagnosis of low BMD will require further research on a larger number of cases and a wider range of ethnic populations. In addition, studies should be conducted on different age groups as well.

CONCLUSION

Serum markers could be useful for screening purposes and assessment of responses to therapy in patients with low BMD. In addition, these biomarkers may provide abetter diagnosis in combination with DEXA.

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