International Journal of General Medicine

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Diagnosis of Low Bone Mass Density: Serological versus Radiological Methods

Osama A Shaikhomar[1], Abdelghnay H Abdelghnay[2,3], Haitham MH Qutob[4]

[1]Physiology Department, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia; [2]Faculty of Medicine, University of Alexandria, Alexandria, Egypt; [3]Faculty of Applied Medical Sciences, Umm Al-Qura University, Makkah, Saudi Arabia; [4]Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences-Rabigh, King Abdulaziz University, Jeddah, Saudi Arabia

Correspondence: Osama A Shaikhomar, Tel +966 555568569, Email oashaikhomar@uqu.edu.sa

Context: Bone remodeling comprises balanced coupling of bone formation and resorption, and low bone mineral density (BMD) demonstrates high rates of bone resorption. Osteoporosis is a chronic asymptomatic disease with fragile bones and impending risk of fractures mediated by minor trauma. Whereas bone mineralization and integrity are determined by calcium and vitamin D, specific serum markers such as bone specific alkaline phosphatase (ALP) and osteocalcin (OC) play a vital role in bone formation.

Materials and Methods: Serum calcium, vitamin D, ALP, and OC levels were measured in 2,145 Saudi students aged 18–22 years at Umm Al-Qura University. The BMD was measured by dual-energy X-ray absorptiometry (DEXA), and the findings were statistically evaluated. The following statistics were utilized in the analysis: the SPSS software was used to record, tabulate, and statistically evaluate the results.

Results: Low BMD cases accounted for 27.46% of all cases investigated, with considerably higher serum calcium, bone-specific ALP, and OC levels compared to control cases, but significantly lower serum vitamin D levels. In low BMD instances, there was no association between serum markers and DEXA findings.

Conclusion: Serum indicators by themselves may be useful for screening and predicting patients at risk of osteoporosis, as well as assessing treatment response. The combination of serum markers and DEXA measures is more effective in detecting low BMD.

Keywords: bone mass density, osteoporosis, calcium, vitamin D, dual-energy X-ray absorptiometry

Introduction

Bones store calcium and, therefore, are considered the hardest tissue in the body. Bone remodeling means a continuous change of the structure of bones, and this comprises a tightly balanced process of bone resorption and bone formation.[1,2] Low bone mineral density (BMD) results from an imbalance of this coupled process.[3]

Bone hardness results from the deposition of calcium crystals. The osteoblasts, bone-forming cells, lay down an unossified boney matrix followed by the deposition of calcium. Therefore, bone rigidity is intimately related to dietary calcium intake, so inadequate calcium intake stimulates osteoclasts, cells responsible for bone destruction, with mobilization of calcium from the bones to the blood.[4,5] So long as calcium is a key mineral for bone rigidity, vitamin D is equally important for bone health as it increases the intestinal absorption of calcium.[6,7] Sun exposure provides more than 90% of vitamin D requirements as the dietary sources and vitamin D supplementation are extremely limited.[8,9]

Osteoporosis is a chronic asymptomatic, progressive illness characterized by low BMD and bone fragility, which predisposes to catastrophic bone fractures even with minor trauma. It is also a worldwide health issue because of the long-term morbidity and medical costs.[10,11]

The osteoblasts release certain markers or enzymes during bone formation, such as bone-specific alkaline phosphatase (ALP) and osteocalcin (OC).[12,13] Bone-specific ALP serves for the crystallization of calcium and its deposition in bones; this process necessitates an alkaline medium. OC represents a reflection of osteoblastic activity and, thus, plays a vital role in the mechanical properties of bones and also adds to bone mineralization as well.[14–16] Interestingly, in the most frequent conditions of low BMD, osteoporosis and osteopenia, there is a high rate of bone formation as a trial to cope

Received: 20 January 2022
Accepted: 22 June 2022
Published: 1 July 2022

with the huge bone destruction.[17] Osteoporosis has been diagnosed by serum levels of calcium, ALP, and OC for the low cost and ease of those laboratory methods despite the fact that they are better used to roll out secondary causes of osteoporosis.[4,7] In the same context, radiological diagnosis of low BMD could be best measured by Dual-Energy X-ray Absorptiometry (DEXA), which is an enhanced form of X-ray technology. This method is considered a simple, easy, and non-invasive way to estimate the real content of bone.[18]

This study was conducted to measure the reliability of diagnosing low BMD by using serological tests in comparison to radiological diagnosis by DEXA.

Subjects and Methods

This cross-sectional study was conducted on 2,145 Saudi students aged 18–22 years in Umm Al-Qura University, Holly Makkah, KSA. From them, 1,084 students were males while 1,061 were females. The participation of the studied cases in the current study complies with ethical principles of the Declaration of Helsinki for medical research.

Before the study, ethical approval was obtained from the Institutional Review Board (IRB) committee at UQU [HAPO-02-K-012-2021-02-136]. The participants were oriented about the study purpose, benefits, risks, and their rights to withdraw from the study.

The study was designed to test the reliability of using a serological marker to diagnose low BMD and whether this method will give real results as given by the more accurate method, DEXA. Blood samples were collected in plain tubes left at 37°C, then centrifuged for 5 minutes at 1,500 R/min, and serum samples were separated and stored in –80°C freezers until the time of measurements.

Some serological markers were measured; serum calcium level (reference range is 8.5–10.5 mg/dL) was measured by SMAC analysis, an automated colorimetric technique.[19] Vitamin D concentration in the blood using the Enzyme Immunoassay technique, with a reference range of 20–50 ng/mL.,[20] serum level of bone-specific ALP using the Enzyme Immunoassay technique with a reference range of 44–147 IU/L,[21] and serum level of OC by ELIZA technique with a reference range of 7–14 ng/mL.[22,23] Inconsistent results were ruled out that were either too low or too high.

DEXA was used to determine BMD, which was expressed as a T-score. A T-score of −1.0 or higher is regarded as normal by the World Health Organization (WHO); between −1.0 and −2.5 is considered as osteopenia, while *T*-scores of −2.5 or lower are diagnostic for osteoporosis.[24,25]

Statistical Analysis

Results were recorded, tabulated, and statistically evaluated using the SPSS program version 26 for the mean, standard deviation, and Chi-square tests. $P<0.05$ was used to determine the significance of the findings. The serum indicators of the studied groups were compared using a one-way ANOVA-test, with a *P*-value of less than 0.05 considered statistically significant. Following that, using Pearson correlation (r), the findings of serological tests were compared to BMD assessed by DEXA, and a correlation was found at $P<0.05$.

Results

A total of 2,145 persons were studied in this study. There were 589 cases of low BMD (27.46%), 282 cases of osteoporosis (13.15%), and 307 cases of osteopenia (14.31%) (Table 1). The cases of low BMD included 334 female

Table 1 Distribution of Studied Cases According to Bone Mineral Density (BMD)

BMD	No	%
Control	1556	72.54
Osteopenia	307	14.31
Osteoporosis	282	13.15

students (175 cases of osteopenia and 159 osteoporotic cases) that were significantly higher than 255 male cases (132 of osteopenia and 123 osteoporotic cases) (Table 2).

DEXA Measurements of Bone Mineral Density

The BMD was expressed as a *T*-score. *T*-scores in the control group varied from −0.67 to −0.96, with a mean of −0.79018. BMD was significantly reduced in cases with osteopenia, ranging from −1.19 to −2.40, with a mean of −1.960.73. In cases of osteoporosis, the BMD decreased even more, with *T*-scores ranging from −2.68 to −3.47, with a mean of −2.900.11 (Table 3; Figures 1–3).

Serological Results

Serum Level of Calcium

The mean serum calcium level showed a significant difference, so that, in control cases, it was 8.85±0.75 that showed a significant increase in cases of osteopenia where the mean was 10.85±0.78. A further significant increase was found in cases of osteoporosis with a mean of 11.66±0.41 (Table 4).

In control cases, there was no link between serum calcium levels and BMD (r=0.068, *P*-value=0.114), in osteopenia (r=0.047, *P*-value=0.207), and also in osteoporosis (r=0.149, *P*-value= 0.059).

Serum Level of Vitamin D

In control cases, it showed a mean of 29.88±3.41, but a significant reduction was seen in cases of osteopenia, with a mean of 15.19±0.62, and a further significant decrease in cases of osteoporosis, with a mean of 9.81±0.57 (Table 5).

In control cases, a correlation was detected between vitamin D levels in the blood and BMD (r= 0.161, *P*-value=0.001) but no correlation was found in cases of osteopenia (r=0.169, *P*-value=0.059) or osteoporosis (r=0.161, *P*-value=0.088).

Table 2 Sex Differences of the Studied Cases

BMD	Male (N=384)		Female (N=331)		Chi-square	
	No.	%	No	%	Value	*P*-value
Control	829	38.65	727	33.89	11.810a	0.001*
Osteopenia	132	6.15	175	8.16		
Osteoporosis	123	5.74	159	7.41		
Total number	1,084	50.54	1,061	49.46		

Note: *$P \leq 0.05$ was considered statistically significant.

Table 3 Radiological Examination by DEXA

	Control (N=525)	Osteopenia (N=101)	Osteoporosis (N=89)	ANOVA	
				F	*P*-value
Mean±SD	−0.79±018	−1.96±0.73	−2.90±0.11	8,968.6	0.000*
Minimum	−0.67	−1.19	−2.68		
Maximum	−1.00	−2.40	−3.47		

Note: *$P \leq 0.05$ was considered statistically significant.

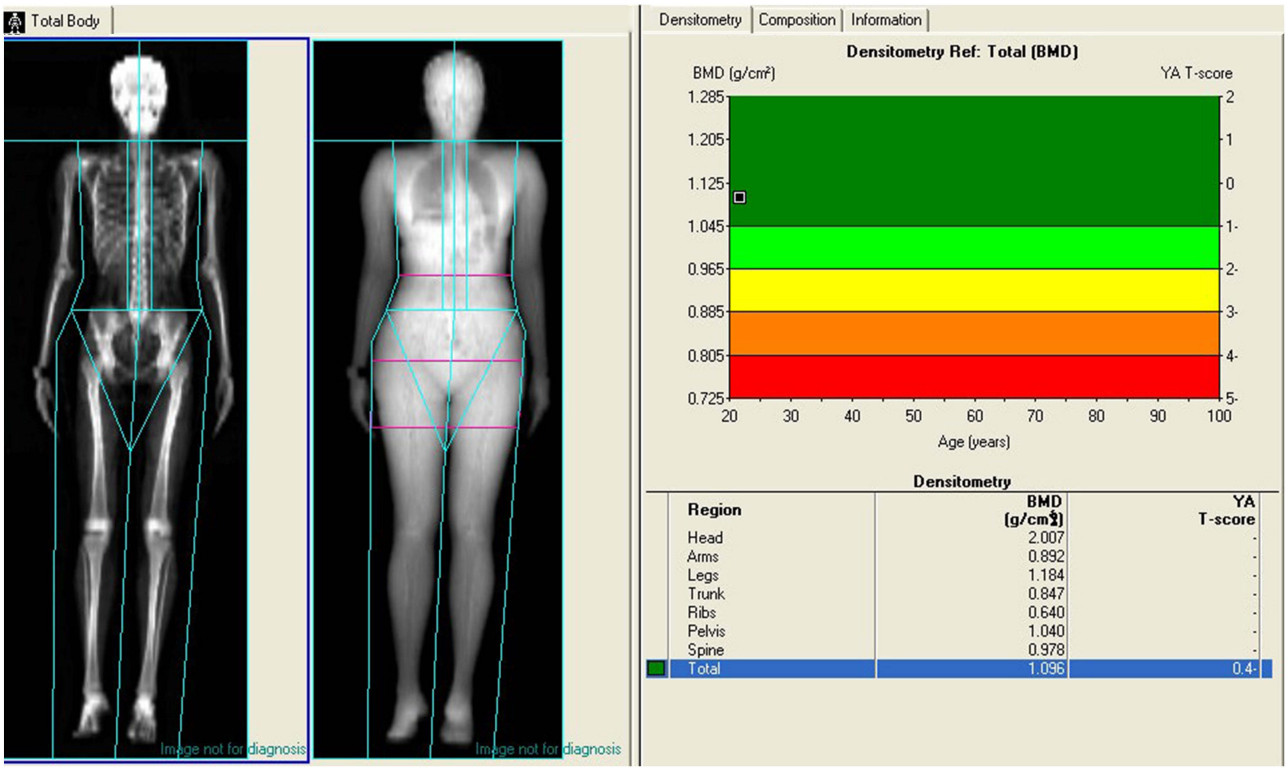

Figure 1 DEXA for normal bone mineral density with T-score=0.4.

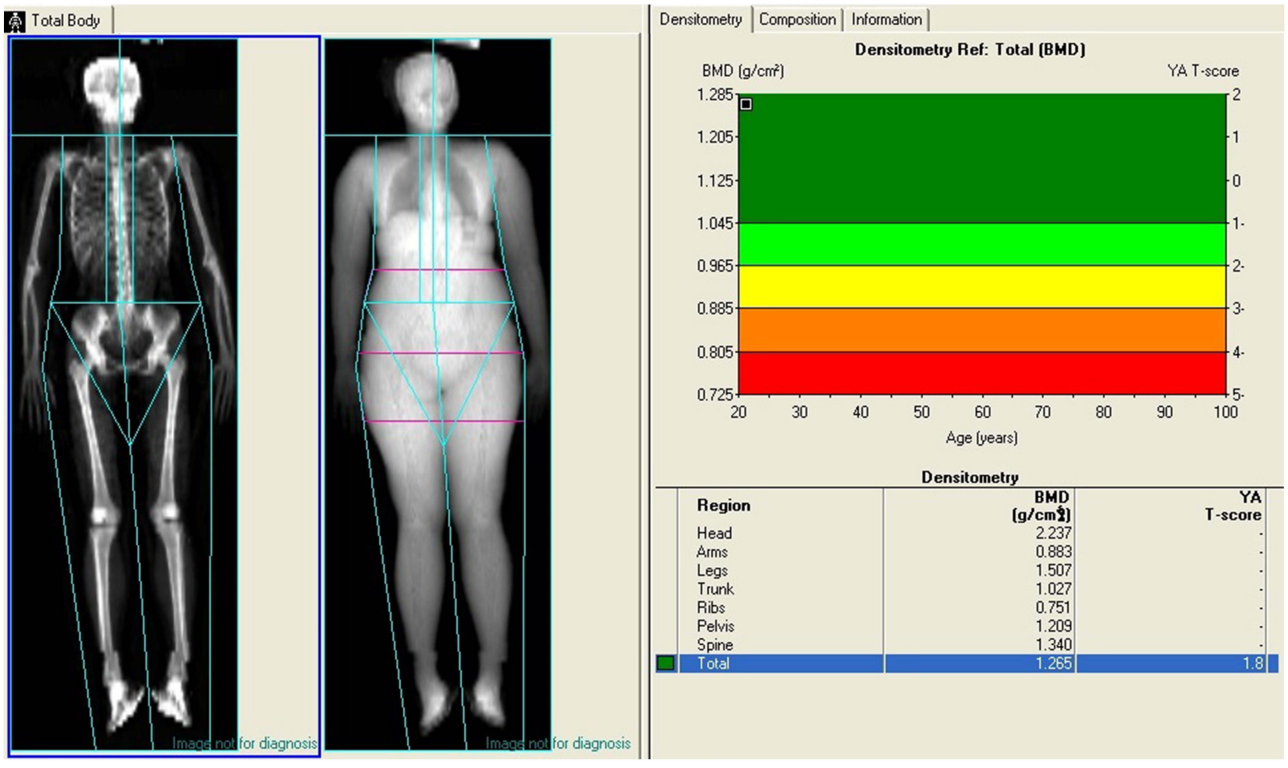

Figure 2 DEXA for a case of osteopenia with T-score=−2.2.

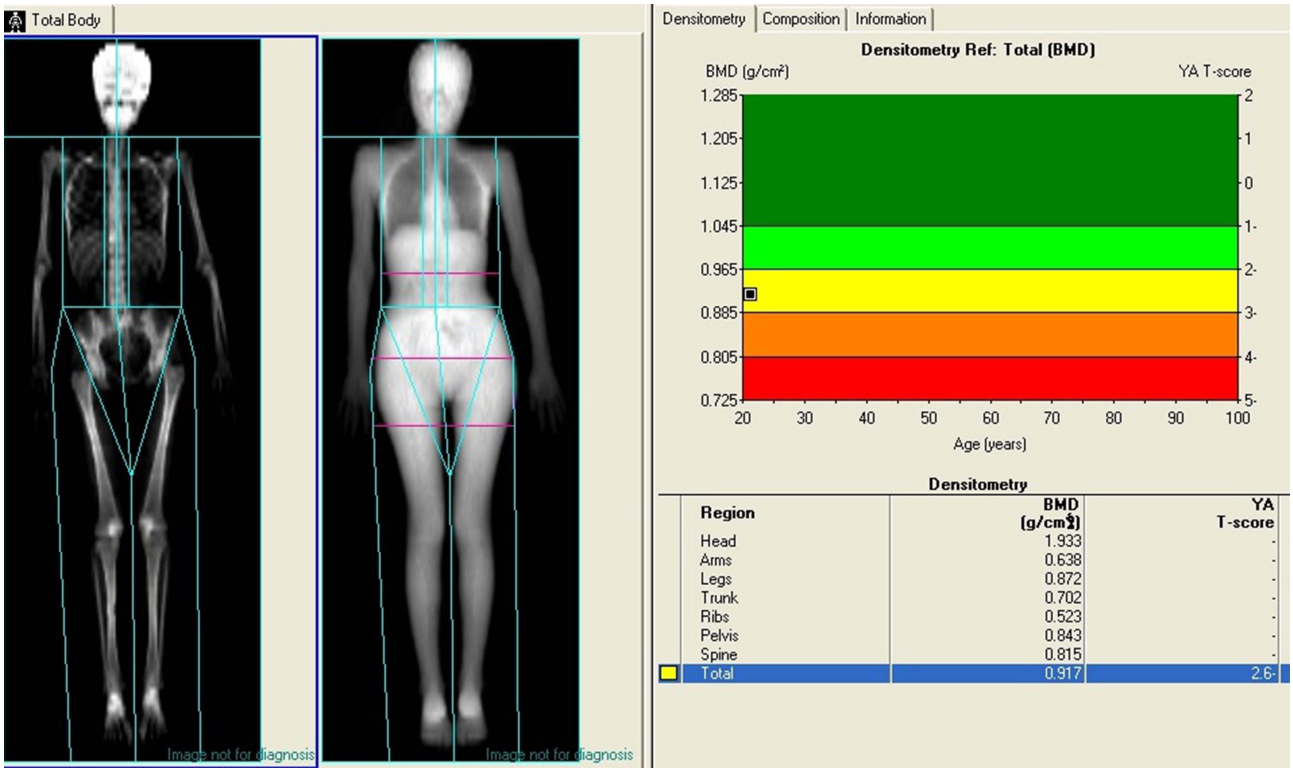

Figure 3 DEXA for a case of osteoporosis with T-score=−3.1.

Serum Level of Bone-Specific ALP

Control cases showed a mean value of 137.21±8.65, while in cases of osteopenia, there was a significant increase with a mean of 203.91±31.29 with a further significant increase found in cases of osteoporosis where the mean value was 312.15±12.19 (Table 6).

Table 4 Serum Level of Calcium (mg/dL) in the Studied Cases

	Control (N=525)	Osteopenia (N=101)	Osteoporosis (N=89)	ANOVA	
				F	*P*-value
Mean±SD	8.85±0.75	10.85±0.78	11.66±0.41	1,644.5	0.000*
Minimum	7.89	9.39	11.30		
Maximum	9.67	11.41	12.88		

Note: *$P \leq 0.05$ was considered statistically significant.

Table 5 Serum Level of Vitamin D (ng/mL) in the Studied Cases

	Control (N=525)	Osteopenia (N=101)	Osteoporosis (N=89)	ANOVA	
				F	*P*-value
Mean±SD	29.88±3.41	15.19±0.62	9.81±0.57	1,037.5	0.000*
Minimum	22.76	13.49	8.91		
Maximum	39.58	17.25	12.18		

Note: *$P \leq 0.05$ was considered statistically significant.

Table 6 Serum Level of Alkaline Phosphatase (IU/L) in the Studied Cases

	Control (N=525)	Osteopenia (N=101)	Osteoporosis (N=89)	ANOVA	
				F	*P*-value
Mean±SD	137.21±8.65	203.91±31.29	312.15±12.19	5,101.6	0.000*
Minimum	117	149	276		
Maximum	145	262	332		

Note: *$P \leq 0.05$ was considered statistically significant.

Table 7 Serum Level of Osteocalcin (ng/mL) in the Studied Cases

	Control (N=525)	Osteopenia (N=101)	Osteoporosis (N=89)	ANOVA	
				F	Sig.
Mean±SD	11.913±1.04	18.87±2.15	31.11±4.78	2709.7	0.000*
Minimum	8.95	15.48	24.79		
Maximum	15.09	23.19	43.10		

Note: *$P \leq 0.05$ was considered statistically significant.

In control cases, there was no correlation between serum ALP levels and BMD (r=0.001, *P*-value=0.974), in cases of osteopenia (r=0.059, *P*-value=0.516) and in cases of osteoporosis (r=0.186, *P*-value=0.090).

Serum Level of OC

The mean value found in control cases was 11.913±1.04, while in cases of osteopenia, it showed a significant increase with a mean of 18.87±2.15. Cases of osteoporosis showed a further significant increase, with a mean of 31.11±4.78 (Table 7).

In control cases, there was a correlation between OC serum levels and BMD (r=0.237, *P*-value=0.001) while no correlation was found in either osteopenia (r=0.042, *P*-value=0.686) or osteoporosis (r=0.049, *P*-value=0.671).

Discussion

Osteoporosis, which is the severe form of low BMD, is a disorder with no obvious specific symptoms and is usually misdiagnosed so that accurate dealing with osteoporosis is often reactionary to harmful fractures.[26,27] So, accurate diagnosis of osteoporosis is the key stone for treatment and discovering patients vulnerable to fractures.[28,29]

This work studies the possibility to use serum markers for early diagnosis of low BMD instead of measuring the bone minerals by DEXA, which is an accurate simple means of diagnosis but is too costly to be widely recommended and is not available, especially in poor communities. This study will help to explore the accuracy of serum markers so that a new reliable and cheap tool for diagnosing such patients may be suggested.

In the current study, the accuracy of certain serological markers; calcium, vitamin D, bone-specific ALP, and OC were investigated.

The current study revealed that cases of low BMD showed unexpectedly elevated serum calcium levels with no correlation to BMD. Calcium homeostasis may show variations under different situations so that, under physiological conditions, bone remodeling involves orchestrated coupling of bone resorption and bone synthesis so that the amounts of destroyed and newly-formed bones are equal without inflow of calcium from the bones to the serum.[30,31]

On the other hand, although low BMD is always associated with dietary calcium insufficiency, serum calcium level remains high due to the increased bone destruction and mobilization of calcium from the bones to keep its serum level within the physiological range at the expense of bone health.[32,33] Calcium delivered to the circulation is continuously reused by the osteoblasts to maintain the bones as much as possible but, surprisingly, bones are still weak due to their

continuous resorption.[34,35] Therefore, although calcium is the key stone of bone structure, its serum level does not reflect the state of bone health and might not be used alone as an indicator of low BMD, but may indicate an underlying problem that may develop to osteoporosis if untreated.[32,33]

In the current study, cases of low BMD exhibited elevated serum calcium levels while serum vitamin D levels were far below normal values. It is not surprising that some cases of low BMD may have the same dietary calcium intake as those with healthy bones, as the underlying cause might be less vitamin D either due to dietary insufficiency or less sun exposure.[36,37] It has been found that vitamin D insufficiency is highly prevalent among the Saudi population, attributed to poor exposure to sunlight due to the excessive heat which makes sun exposure a real risk of heat stroke.[36] Inavailability of vitamin D reduces the intestinal absorption of calcium and this causes the body to move calcium from the bones.[7]

Bone turnover markers have been extensively used to study and evaluate bone remodeling in both physiological and pathological situations and the studies examining their benefits in predicting bone loss are increasing.[28,38]

The current study demonstrated that cases of low BMD had serum levels of bone-specific ALP widely ranging from near the upper normal limit to far higher above normal with no correlation with BMD. This might be explained by the high bone turnover associated with low BMD to keep bone remodeling much nearer to the normal state. Thus, higher levels of bone-specific ALP are expectedto be 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 reached up to double or triple the normal value.[42,43] Interestingly, some authors proved high serum levels of bone-specific ALP with no disturbance in the bone remodeling process and this was attributed to hypophosphatasia.[44,45] On the other hand, other studies mentioned low levels of ALP in patients of low BMD and attributed that to the associated generalized malnutrition, especially for zinc and magnesium, as zinc raises the activity of ALP while magnesium is an important stimulator of this marker.[46,47] Moreover, Lumachi et al,[48] and Zhou et al,[49] unexpectedly proved no relationship between serum levels of ALP and BMD.

Therefore, based on the results of the current study beside other studies and due to the wide range of serum levels of ALP and absence of correlation with BMD, bone-specific ALP might not be considered a reliable marker for the diagnosis of low BMD.

Regarding the second marker of osteoblastic activity, the current study demonstrated that cases of low BMD had serum levels of OC near the upper normal limit and increased high above normal values with no correlation to BMD.

A controversy was demonstrated as, while some researchers found significant high serum levels of OC in low BMD patients, others showed no significant difference compared with normal cases, while other studies surprisingly found low serum levels in a group of patients diagnosed as osteoporosis.[34,39,50] Serum OC, being a dynamic marker of bone formation, has long been considered specific for high bone turnover and this explains its high levels with low BMD.[39] Unlike bone-specific ALP, genetic evidence has proved that OC is produced late in the process of bone mineralization and, thus, it shares a minor role in the mineralization process.[15,16] So, the serum level of OC could not be used alone as a marker in the diagnosis of low BMD.

Although the high serum levels of bone formation markers indicate newly-formed bones, those bones were found to be less mineralized which might negatively alter the bone microarchitecture and strength and predispose to the risk of fracture.[38,40]

Therefore, serum markers might not be used for the diagnosis of low BMD. Instead, they may be used for prediction of people at risk of developing fractures, especially in postmenopausal women and for evaluation of the response to osteoporosis therapy.[28,29] On the other hand, a combination of these serum markers together with BMD measurement by DEXA might provide comprehensive information for the diagnosis of low BMD at it early stages.[51,52]

Finally, the use of serum markers for diagnosis of low BMD needs future research work on a larger number of cases of different age groups as well as a wider range of ethnic populations.

Conclusion

Serum markers alone, although easy and cheap, might not be used for diagnosis of low BMD, but they may help in screening purposes and for assessment of the response of the patients to therapy. Instead, accurate diagnosis might be better based on DEXA evaluation, despite its high cost, and if possible by a combination of serum markers together with DEXA.

Acknowledgments

The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project number UQU- 43309024. I want to extend my sincere thanks and gratitude to Umm-Al-Qura University, Makkah, Saudi Arabia for providing me with the platform in fulfilling this research.

Finally, I give my deep gratitude and sincere thanks to my family for their continuous and unparalleled support and love. I am deeply indebted to my dear parents for providing me with opportunities and encouragement to make me who I am today.

Disclosure

The authors declare no conflicts of interest in relation to this work.

References

1. Gkiatas I, Lykissas M, Kostas-Agnantis I, Korompilias A, Batistatou A, Beris A. Factors affecting bone growth. *Am J Orthop*. 2015;44:61–67.
2. Haglin JM, Jain S, Eltorai AEM, Daniels AH. Bone growth stimulation: a critical analysis review. *JBJS Rev*. 2017;5(8):e8–e8. doi:10.2106/JBJS.RVW.16.00117
3. Chen X, Wang Z, Duan N, Zhu G, Schwarz EM, Xie C. Osteoblast–osteoclast interactions. *Connect Tissue Res*. 2018;59(2):99–107. doi:10.1080/03008207.2017.1290085
4. Fang A, Li K. Calcium deficiency: where does the diagnostic criterion come from and by what is bone health influenced? *Chin Med J*. 2014;127:4161–4163.
5. Kovacs CS. Calcium, phosphorus, and bone metabolism in the fetus and newborn. *Early Hum Dev*. 2015;91(11):623–628. doi:10.1016/j.earlhumdev.2015.08.007
6. Spustová V, Dzúrik R. Vitamin D: synthesis, metabolism, regulation, and an assessment of its deficiency in patients with chronic renal disease. *Vnitřní lékařství*. 2004;50:537–543.
7. Reid IR. Vitamin D effect on bone mineral density and fractures. *Endocrinol Metab Clin North Am*. 2017;46(4):935–945. doi:10.1016/j.ecl.2017.07.005
8. Paschalis EP, Gamsjaeger S, Hassler N, et al. Vitamin D and calcium supplementation for three years in postmenopausal osteoporosis significantly alters bone mineral and organic matrix quality. *Bone*. 2016;95:41–46. doi:10.1016/j.bone.2016.11.002
9. Bolland MJ, Grey A, Reid IR. Should we prescribe calcium or vitamin D supplements to treat or prevent osteoporosis? *Climacteric*. 2015;18:22–31. doi:10.3109/13697137.2015.1098266
10. Gozashti MH, Shahesmaeili A, Zadeh NA. Is opium addiction a risk factor for bone loss? *Iran Red Crescent Med J*. 2011;13:464–468.
11. Main RP. Osteocytes and the bone lacunar-canalicular system: insights into bone biology and skeletal function using bone tissue microstructure. *Int J Paleopathol*. 2017;18:44–46. doi:10.1016/j.ijpp.2017.05.002
12. Li Q, Wu J, Xi W, et al. Ctrp4, a new adipokine, promotes the differentiation of osteoblasts. *Biochem Biophys Res Commun*. 2019;512(2):224–229. doi:10.1016/j.bbrc.2019.03.053
13. Oury J, Oury F. [Osteocalcin, a key molecule for bone endocrine functions]. *Med, Sci*. 2018;34(1):54–62. In French. doi:10.1051/MEDSCI/20183401014
14. Grote-Koska D, Klauke R, Brand K, Schumann G. Alkaline phosphatase activity - PH impact on the measurement result. *Clin Chem Lab Med*. 2016;55(7):e146–e149. doi:10.1515/cclm-2016-0771
15. Bailey S, Karsenty G, Gundberg C, Vashishth D. Osteocalcin and osteopontin influence bone morphology and mechanical properties. *Ann N Y Acad Sci*. 2017;1409:79–84. doi:10.1111/nyas.13470
16. Moser SC, van der Eerden BCJ. Osteocalcin — a versatile bone-derived hormone. *Front Endocrinol*. 2019;9:794. doi:10.3389/fendo.2018.00794
17. Oladipo OO, DeCrescenzo AJ, Marquez CP, Okorodudu AO. Increased alkaline phosphatase in a child. *Clin Chem*. 2017;63(6):1174–1175. doi:10.1373/clinchem.2016.268904
18. Van Dort MJ, Romme EAPM, Smeenk FWJM, Geusens PPPM, Wouters EFM, van den Bergh JP. Diagnosis of vertebral deformities on chest CT and DXA compared to routine lateral thoracic spine X-ray. *Osteoporos Int*. 2018;29(6):1285–1293. doi:10.1007/s00198-018-4412-1
19. Clark EP, Colip JB. A study of the Tisdall method for the determination of blood serum calcium with a suggested modification. *J Biol Chem*. 1925;63(1):461–464. doi:10.1016/S0021-9258(18)85009-8
20. Bayard F, Bee P, Louvet JP. Measurement of plasma 25-hydroxycholecalciferol in man. *Eur J Clin Invest*. 2008;2(4):195–198. doi:10.1111/j.1365-2362.1972.tb00644.x
21. Behr W, Barnert J. Quantification of bone alkaline phosphatase in serum by precipitation with wheat-germ lectin: a simplified method and its clinical plausibility. *Clin Chem*. 2020;32:1960–1966. doi:10.1093/clinchem/32.10.1960
22. Wenz I, Reissmann R, Bornig H. Determination of osteocalcin in serum by an ultramicro-ELISA with alkaline phosphatase as marker enzyme. *Biomed Biochim Acta;*. 1991;50:145–149.
23. Hannemann A, Friedrich N, Spielhagen C, et al. Reference intervals for serum osteocalcin concentrations in adult men and women from the study of health in Pomerania. *BMC Endocr Disord*. 2013;13(1). doi:10.1186/1472-6823-13-11
24. Kung AW, Fan T, Xu L, et al. Factors influencing diagnosis and treatment of osteoporosis after a fragility fracture among postmenopausal women in Asian countries: a retrospective study. *BMC Womens Heal*. 2013;13(1):7. doi:10.1186/1472-6874-13-7
25. Cortet B, Biver E, Borg S, et al. Management of male osteoporosis: lessons for clinical practice. *Joint Bone Spine*. 2011;78:S208–S210. doi:10.1016/s1297-319x(11)70006-9
26. Hlaing TT, Compston JE. Biochemical markers of bone turnover - uses and limitations. *Ann Clin Biochem*. 2014;51(2):189–202. doi:10.1177/0004563213515190

27. Yedavally-Yellayi S, Ho AM, Patalinghug EM. Update on osteoporosis. *Primary Care: Clinics in Office Pract*. 2018;46:175–190. doi:10.1016/j.pop.2018.10.014
28. Eastell R, Szulc P. Use of bone turnover markers in postmenopausal osteoporosis. *Lancet Diabetes Endocrinol*. 2017;5(11):908–923. doi:10.1016/S2213-8587(17)30184-5
29. Kuo TR, Chen CH. Bone biomarker for the clinical assessment of osteoporosis: recent developments and future perspectives. *Biomark Res*. 2017;5(1). doi:10.1186/S40364-017-0097-4
30. Siddiqui JA, Partridge NC. Physiological bone remodeling: systemic regulation and growth factor involvement. *Physiology*. 2016;31(3):233–245. doi:10.1152/physiol.00061.2014
31. Armas LAG, Recker RR. Pathophysiology of osteoporosis. New mechanistic insights. *Endocrinol Metab Clin North Am*. 2012;41(3):475–486. doi:10.1016/j.ecl.2012.04.006
32. Cano A, Chedraui P, Goulis DG, et al. Calcium in the prevention of postmenopausal osteoporosis: EMAS clinical guide. *Maturitas*. 2018;107:7–12. doi:10.1016/j.maturitas.2017.10.004
33. Chiodini I, Bolland MJ. Calcium supplementation in osteoporosis: useful or harmful? *Eur J Endocrinol*. 2018;178(4):D13–D25. doi:10.1530/EJE-18-0113
34. Weng XM, Pan JP. Bone alkaline phosphatase and N-MID osteocalcin in monitoring of osteoporosis treatment with recombinant human parathyroid hormone. 1–34. *Zhejiang Da Xue Xue Bao Yi 2Xue Ban*. 2013;42:578–582.
35. Diemar SS, Møllehave LT, Quardon N, et al. Effects of age and sex on osteocalcin and bone-specific alkaline phosphatase—reference intervals and confounders for two bone formation markers. *Arch Osteoporos*. 2020;15(1). doi:10.1007/s11657-020-00715-6
36. Al-Daghri NM, Abd-Alrahman SH, Panigrahy A, et al. Efficacy of Vitamin D interventional strategies in Saudi children and adults. *J Steroid Biochem Mol Biol*. 2018;180:29–34. doi:10.1016/j.jsbmb.2017.12.004
37. Garriguet D. Bone health: osteoporosis, calcium and Vitamin D. *Health Rep*. 2011;22:7–14.
38. Shetty S, Kapoor N, Bondu J, Thomas N, Paul T. Bone turnover markers: emerging tool in the management of osteoporosis. *Indian J Endocrinol Metab*. 2016;20(6):846–852. doi:10.4103/2230-8210.192914
39. Kalaiselvi VS, Prabhu K, Ramesh M, Venkatesan V. The association of serum osteocalcin with the bone mineral density in post menopausal women. *J Clin Diagn Res*. 2013;7(5):814–816. doi:10.7860/JCDR/2013/5370.2946
40. Mukaiyama K, Kamimura M, Uchiyama S, Ikegami S, Nakamura Y, Kato H. Elevation of serum alkaline phosphatase (ALP) level in postmenopausal women is caused by high bone turnover. *Aging Clin Exp Res*. 2015;27(4):413–418. doi:10.1007/s40520-014-0296-x
41. Nakamura Y, Suzuki T, Kato H. Serum bone alkaline phosphatase is a useful marker to evaluate lumbar bone mineral density in Japanese postmenopausal osteoporotic women during denosumab treatment. *Ther Clin Risk Manag*. 2017;9(13):1343–1348. doi:10.2147/TCRM.S142828
42. Tariq S, Tariq S, Lone KP, Khaliq S. Alkaline phosphatase is a predictor of bone mineral density in postmenopausal females. *Pakistan J Med Sci*. 2019;35(3):749–753. doi:10.12669/pjms.35.3.188
43. Alonso N, Larraz-Prieto B, Berg K, et al. Loss-of-function mutations in the ALPL gene presenting with adult onset osteoporosis and low serum concentrations of total alkaline phosphatase. *J Bone Miner Res*. 2020;35(4):657–661. doi:10.1002/jbmr.3928
44. Hicks RW, Umnitz L, Seibert DC. Hypophosphatasia: a rare disorder. *J Am Assoc Nurse Pract*. 2018;30(11):600–602. doi:10.1097/JXX.0000000000000149
45. Tsiantouli E, Trombetti A, Ferrari S. Hypophosphatasia. *Rev Med Suisse*. 2017;13:855–858.
46. Ray CS, Singh B, Jena I, Behera S, Ray S. Low Alkaline Phosphatase (ALP) in adult population an indicator of Zinc (Zn) and Magnesium (Mg) deficiency. *Curr Res Nutr Food Sci*. 2017;5:347–352.
47. Arise RO, Davies FF, Malomo SO. Independent and interactive effect of Mg2+ and Co2+ on some kinetic parameters of rat kidney alkaline phosphatase. *Res Ess*. 2008;3:488–494.
48. Lumachi F, Ermani M, Camozzi V, Tombolan V, Luisetto G. Changes of bone formation markers osteocalcin and bone-specific alkaline phosphatase in postmenopausal women with osteoporosis. *Ann N Y Acad Sci*. 2009;1173:SUPPL.1. doi:10.1111/j.1749-6632.2009.04953.x
49. Zhou XW, Wu XY, Luo L, et al. The relationship between bone turnover markers and BMD decreasing rates in Chinese middle-aged women. *Clin Chim Acta*. 2011;412(17):1648–1657. doi:10.1016/j.cca.2011.05.020
50. Liu Z, Chen R, Jiang Y, et al. A meta-Analysis of serum osteocalcin level in postmenopausal osteoporotic women compared to controls. *BMC Musculoskelet Disord*. 2019;20(1). doi:10.1186/s12891-019-2863-y
51. Bauer DC. Clinical use of bone turnover markers. *JAMA*. 2019. doi:10.1001/jama.2019.9372
52. Jain S, Camacho P. Use of bone turnover markers in the management of osteoporosis. *Curr Opin Endocrinol Diabetes Obes*. 2018;25(6):366–372. doi:10.1097/MED.0000000000000446