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Role of serum levels of bone alkaline phosphatase and osteocalcin in thalassemia induced osteoporosis in a cohort of Egyptian females with Beta thalassemia major.

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ABSTRACT:
Thalassemias are heterogenous disorders caused by defects in production of globin chain of the hemoglobin tetramer. The mainstay of treatment depends on blood transfusion resulting in iron excess, blood borne infections, and alloimmunization. Osteoporosis is a prominent cause of morbidity in thalassemia. Serum Osteocalcin can be Considered as a specific marker of osteoblast function as its levels have been shown to correlate with bone formation rates. BAP is a highly specific marker of the bone-forming activity of osteoblasts.
This study aims to determine the serum level of both BAP and OC in TDTM female patients and to find the relationship between them.
Methods: CBC, CRP, ferritin, HCV screening by ELISA, BAP was measured by immunoenzymatic assay, vitamin D was measured immunoenzymatically, OC was by fully automated spectrophotometer, and DEXA scan was done
Results: There is positive correlation between PLR and BAP. A positive correlation was found between BAP and decreased T score of patients. There was a positive relation between splenectomized patients and increased PLR, and BAP. Moreover there was a positive relation between decreased T score and BAP, PLR, and increased platelets in patients.
Conclusion: BAP and OC are surrogates of osteoporosis in thalassemic patients. BAP and OC have an inverse relationship with BMD, PLR is considered discriminative in patients with low BMD which makes it a valuable screening tool.

Keywords: thalassemia, osteocalcin, osteoporosis

INTRODUCTION

Thalassemia syndromes are heterogenous groups of disorder caused by defects in production of one or more of the globin chains of the hemoglobin (Hb) tetramer (Angelucci et al., 2014). Thalassemia major represents a significant health concern in Egypt; the carrier rate reaches about 9-10% (El-Shanshory et al., 2023).
In patients with beta-thalassemia major, the synthesis of beta-globin chain subunits in the hemoglobin tetramer is deficient. This leads to disproportionate accumulation of alpha chains, causing ineffective erythropoiesis and varying levels of hemolytic anemia. (Thein, 2013).
Blood transfusions, the primary therapeutic intervention for thalassemia, are frequently associated with adverse outcomes, including iron overload, blood-borne infections, and alloimmunization. Therefore, it is critical to implement routine serum ferritin monitoring and iron chelation therapy to manage iron accumulation in these patients (Borgna-Pignatti et al., 2004; Cohen et al., 2008). Advances in transfusion protocols and iron chelation have significantly extended the lifespans of individuals with thalassemia. Despite these improvements, iron overload continues to pose a substantial risk of morbidity in this population (Borgna-Pignatti et al., 2004; Pinto & Forni, 2020).
The pathogenesis of thalassemia-induced osteoporosis (TIO) is multifactorial, encompassing ineffective erythropoiesis, bone marrow expansion, endocrine dysfunctions, complications of iron overload , vitamin D deficiency, and reduced physical activity (Haidar et al., 2011). Haidar et al, found that thalassemic patients receiving suboptimal transfusions having lower bone mass compared to others receiving regular transfusions. Yet, there is a lack of evidence-based data on the advantage of maintaining elevated hemoglobin levels for preservation of bone mass in thalassemic patients (Dede et al., 2016; Haidar et al., 2011).
Serum osteocalcin serves as a specific marker of osteoblast function, with its levels correlating with bone formation rates (Greenspan et al., 2005; Zoch et al., 2016)
Traditionally, bone strength and fracture risk prediction have relied on densitometry. Recently, bone turnover biomarkers, including osteocalcin—also known as bone gamma-carboxyglutamic

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acid-containing protein (BGLAP)—have gained prominence for assessing bone turnover rates and monitoring osteoporosis treatment (Ali, 2020; Civitelli et al., 2009; Hamdi, 2013). Secreted by osteoblasts during bone formation, osteocalcin is calcium-dependent and binds strongly to the bone matrix. In osteoporosis, reduced hydroxyapatite crystal formation leads to elevated serum osteocalcin levels due to calcium and phosphorus deficiencies (Filip & Zagórski, 2004; Jagtap et al., 2011).

Bone-specific alkaline phosphatase (BAP) is a reliable marker for osteoblast activity related to bone formation. Moderate elevations in BAP levels are seen in osteomalacia and return to normal with vitamin D therapy. In osteoporosis, BAP activity is typically normal, whereas in rickets, BAP levels can increase 2 to 4 times, normalizing with treatment.

A transient increase in BAP activity is often observed during the healing of bone fractures (Bansal et al., 2020).The Hawaii Osteoporosis Study cohort identified a strong correlation between BAP levels and rapid bone loss as for BAP levels 2 SD above the mean, the probability of rapid bone loss was 80%; in contrast, the probability was only 20% at 2 SD below the mean. This relationship is comparable to the association between bone mineral density (BMD) and fracture risk, suggesting that BAP could serve as a valuable biomarker for assessing fracture risk and monitoring bone health (Masrour Roudsari & Mahjoub, 2012; Ross & Knowlton, 1998)

Aim:

Aim of study to evaluate the Correlation between serum levels of bone Alkaline Phosphatase and Osteocalcin and to study their role in thalassemia induced osteoporosis in a cohort of Egyptian females with Beta thalassemia major.

Patients:

Study population:

This study protocol received approval from the Medical Research Institute's Ethics Committee at Alexandria University, adhering to the World Medical Association's Declaration of Helsinki. Conducted in the Alexandria governorate, this cross-sectional study involved an age-stratified random sample. Written consent was obtained from all participants. The study included 50 transfusion-dependent female patients with Beta-Thalassemia Major from the Hematology Department and 50 healthy female volunteers from the outpatient clinic, medical, nursing, or secretarial staff, or their relatives, at Alexandria University. Participants were aged 18-35 years and were enrolled between April 2022 and November 2022.

Exclusion criteria:

Participants with cancer, chronic diseases, or any medical conditions affecting bone metabolism were excluded from the study. Additionally, subjects who had taken medications influencing bone metabolism such as selective estrogen receptor modulators, phytoestrogens, anticonvulsants, calcitonin, anabolic agents, steroids, vitamin D supplements, hormonal treatments for endocrinopathies, non-steroidal anti-inflammatory drugs (NSAIDs), depo-provera, calcium supplements, serotonin reuptake inhibitors, L-thyroxine, antihypertensive drugs, and antiresorptive treatments, within a year prior to the study, were excluded. Those with a history of smoking, recent fractures, pregnancy, lactation, and renal or endocrine disorders were also excluded.

All participants completed a detailed questionnaire covering demographic data, medical history, lifestyle, and other relevant factors.

Methods:

Patients: The study was conducted on 100 female subjects divided into 2 main groups the cases group compromising 50 female patients suffering from beta-thalassemia major and the second group including 50 apparently healthy female volunteers of comparable age, sex and socioeconomic standards.

A thorough medical and surgical history was recorded for each participant. Complete blood counts (Lewis et al., 2006), C-reactive protein levels, and serum ferritin levels were assessed (Hultin, 2012). Hepatitis C virus antibodies were screened using enzyme-linked immunosorbent assay (Hultin, 2012; "Testing for HCV infection: an update of guidance for clinicians and laboratorians," 2013). Serum bone alkaline phosphatase levels (Saad et al., 2021) were measured, along with serum 25-hydroxyvitamin D levels (Garnett et al., 2019) via immunoenzymatic assay on the Cobas 411e (Roche Diagnostics), with a detection limit of 3.00 ng/mL (7.5 nmol/L). Serum osteocalcin levels (Belaya et al., 2016) were also measured using the Cobas system (Roche Diagnostics), with a detection limit of 5 U/L. Additionally, bone mineral density was evaluated using dual-energy X-ray absorptiometry (DEXA) scans (Messina et al., 2020).

Results

The mean age of the cases and control groups was 26 and 27 years, respectively. As expected, the mean serum level of ferritin was significantly higher among the thalassemic group (3518.2 ng/ml) than the control one (90 ng/ml) with a p value < 0.001. The hemoglobin level was significantly lower in the cases group (8 g/dl) than the control group (11.7 g/dl) with a p value $< .001$. While the WBC, platelet counts, and platelet to Lymphocyte ratio were significantly higher among the thalassemic cases with a p value < 0.001. The mean LDH serum level was significantly higher among the cases group (506 U/L) than the control group (174.8 U/L) with a p value < 0.001. (Table 1)

As regards the bone turnover markers, serum levels of Vitamin D and ionized calcium were significantly lower in the cases group than the control group, while those of bone alkaline phosphatase (BAP) and osteocalcin were significantly higher in the cases group as compared to control one ($p < 0.001$). (Table 1)

As for the clinicopathological features of the studied groups, the prevalence of hepatitis C virus infection among the thalassemic patients was 24%, while 98% suffered from bone pains with 70% of the patients had osteoporosis with a T-score < -2.5 and the remaining 30% were osteopenic with a T-score between -2.5 and -1. (Table 2).

The correlation between several parameters was assessed. There was a significant positive correlation between Bone Alkaline Phosphatase (BAP) and Platelet-Lymphocyte Ratio ($r = 0.533$; $p < 0.001$) and between platelet count and BAP (r

= 0.64, p<0.001). Also, a significant negative correlation was found between osteocalcin and ionized calcium (r = -0.358; p = 0.011) and between lumbar spine T-score and BAP (r = -0.654; p < 0.001). (Table 3)

Moreover, it's worth mentioning that non splenectomized patients were associated with lower platelet counts, lower platelet to lymphocyte ration, and lower BAP levels with a p value < 0.001. On the other hand, they were associated with higher prevalence of osteopenia with a p value < 0.001. (Table 5)

The relationship between bone mineral density and different parameters was assessed, showing increased BAP, platelet count and Platelet/ lymphocyte ratio among osteoporotic patients as compared to osteopenic one with a p value < 0.001. (Table 6)

Table (1):Comparison of Demographic Data and Laboratory Findings Between the Two Study Groups

	Cases (n = 50)	**Control (n = 50)**	**P**
Age (years)			
Mean ± SD.	26 ± 7.3	27 ± 7	0.564
Ferritin (ng/ml)			
Mean ± SD.	3518.2 ± 1426.9	90 ± 19.3	<0.001*
Hemoglobin (g/dl)			
Mean ± SD.	8 ± 1	11.7 ± 0.8	<0.001*
WBCs (10^9/L)			
Mean ± SD.	9.1 ± 3.6	5.5 ± 0.9	<0.001*
Platelets (10^9/L)			
Mean ± SD.	559.3 ± 194.2	279.4 ± 72	<0.001*
Neutrophils (%)			
Mean ± SD.	50 ± 10.1	54.3 ± 5.3	0.011*
Lymphocytes (%)			
Mean ± SD.	39.7 ± 9.5	36.4 ± 5.4	0.038*
Platelet/ lymphocyte ratio			
Mean ± SD.	15.2 ± 7.4	7.9 ± 2.6	<0.001*
CRP (mg/L)			
Mean ± SD.	3.6 ± 3	2.9 ± 0.6	0.467
LDH (U/L)			
Mean ± SD.	506 ± 202	174.8 ± 37.9	<0.001*
Vitamin-D (ng/ml)			
Mean ± SD.	6.9 ± 4.7	29.3 ± 2.1	<0.001*
BAP (IU/L)			
Mean ± SD.	66 ± 16.2	28.6 ± 5.4	<0.001*
Osteocalcin (ng/ml)			
Mean ± SD.	18 ± 6.2	4.4 ± 1.4	<0.001*
Ionized Ca (mg/dL)			
Mean ± SD.	4.1 ± 1.1	4.8 ± 0.2	0.001*

SD: **Standard deviation** p: p value for comparing between the studied groups

*: Statistically significant at $p \leq 0.05$

Table (2):Distribution according to HCV Status, presence of Splenectomy, Bone Pain, and Lumbar Spine T-score in the Patients Group (n = 50)

	No. (%)
HCV	12 (24%)
Splenectomy	15 (30%)
Bone pain	49 (98%)
T-score lumbar spine	
Osteopenia (-2.5 – -1)	15 (30%)
Osteoporosis (< -2.5)	35 (70%)
Mean ± SD.	-4.5 ± 1.6

SD: **Standard deviation**

Table (3):Correlation between different parameters in the Patients group (n = 50)

	Corr. Coeff.	p
Vitamin-D vs. Bone Alkaline Phosphatase	-0.246	0.086
Vitamin-D vs. Osteocalcin	-0.116	0.421
Vitamin-D vs. Ionized Ca#	0.016	0.913
BAP vs. Osteocalcin	0.233	0.103
BAP vs. Ionized Ca	-0.124	0.391
Osteocalcin vs. Ionized Ca	-0.358	0.011*
Ferritin vs. Osteocalcin	0.047	0.744
Hemoglobin vs. Osteocalcin	-0.030	0.838
Platelet/ lymphocyte ratio vs. Osteocalcin	-0.048	0.739
Platelet/ lymphocyte ratio vs. Bone Alkaline Phosphatase	0.533	<0.001*
Platelet/ lymphocyte ratio vs. CRP#	0.211	0.141
LDH vs. BAP	0.092	0.526
LDH vs. Osteocalcin	-0.187	0.194
Platelets vs. BAP	0.654	<0.001*
Platelets vs. Osteocalcin	0.160	0.266
Lumbar Spine T-score vs. BAP	-0.654	<0.001*
Lumbar Spine T-score vs. Osteocalcin	0.081	0.575

Corr. Coeff.: **Correlation coefficient Pearson coefficient**
#: Correlation coefficient Spearman coefficient
*: Statistically significant at $p \leq 0.05$

Table (4):Prognostic performance for Bone Alkaline Phosphatase and Osteocalcin to Differentiate Patients (n = 50) from Controls (n = 50)

	AUC	p	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
BAP	0.982	<0.001*	0.957 – 1.000	>38	94.0	98.0	97.9	94.2
Osteocalcin	0.992	<0.001*	0.980 – 1.000	>7	94.0	98.0	97.9	94.2

AUC: Area Under a Curve p value: Probability value CI: Confidence Intervals
NPV: Negative predictive value PPV: Positive predictive value
*: Statistically significant at $p \leq 0.05$

Table (5):Relation between Spleen condition with different parameters in the Patients group (n = 50)

	Splenectomy		
	Negative (n = 35)	**Positive (n = 15)**	**p**
Platelets (10^9/L)			
Mean ± SD.	661.1 ± 113.2	321.7 ± 120.3	<0.001*
Platelet/ lymphocyte ratio			
Mean ± SD.	18.2 ± 6.5	8 ± 3.2	<0.001*
BAP (IU/L)			
Mean ± SD.	73.6 ± 12.6	48.3 ± 6.8	<0.001*
Osteocalcin (ng/ml)			
Mean ± SD.	18 ± 6.6	18 ± 5.3	0.981
Lumbar Spine T-score			
Osteopenia	0 (0%)	15 (100%)	FEp
Osteoporosis	35 (100%)	0 (0%)	<0.001*
Mean ± SD.	-5.5 ± 0.5	-2.1 ± 0.3	<0.001*

SD: **Standard deviation** FE**: Fisher Exact**
p: p value for comparing between **Negative** and **Positive**
*: Statistically significant at $p \leq 0.05$

Table (6):Relation between Lumbar Spine T-score with different parameters in the Patients group (n = 50)

	Lumbar Spine T-score		
	Osteopenia (n = 15)	**Osteoporosis (n = 35)**	**p**
Platelets (10^9/L)			
Mean ± SD.	321.7 ± 120.3	661.1 ± 113.2	<0.001*
Platelet/ lymphocyte ratio			
Mean ± SD.	8 ± 3.2	18.2 ± 6.5	<0.001*
BAP (IU/L)			
Mean ± SD.	48.3 ± 6.8	73.6 ± 12.6	<0.001*
Osteocalcin (ng/ml)			
Mean ± SD.	18 ± 5.3	18 ± 6.6	0.981

SD: **Standard deviation**
p: p value for comparing between **Osteopenia** and **Osteoporosis**
*: Statistically significant at $p \leq 0.05$

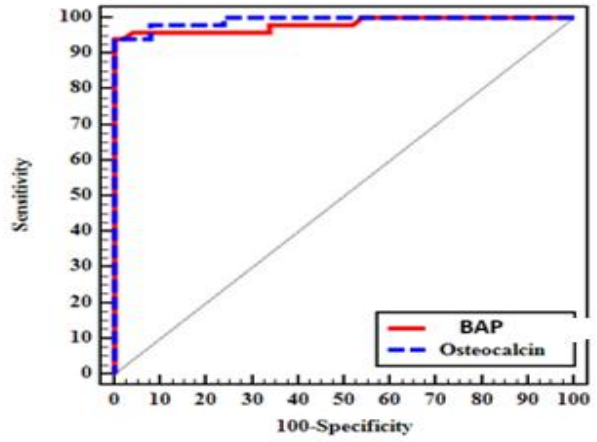

Figure (1):ROC Curve Analysis of Bone Alkaline Phosphatase and Osteocalcin for Differentiating Patients (n = 50) from Controls (n = 50)

Discussion

In this study, serum ferritin levels were significantly higher in patients compared to the control group (P <0.001). The platelet-lymphocyte ratio was elevated among splenectomized patients and correlated significantly with bone alkaline phosphatase levels (P <0.001). This ratio was also higher in osteoporotic patients compared to those with osteopenia (P <0.001), highlighting the role of platelets in osteoporosis. Semra Eroglu et al, also found that higher platelet lymphocyte ratio is considered to be a simple marker to predict postmenopausal osteoporosis (Eroglu & Karatas, 2019)

Bone mineral density (BMD) decreased with higher platelet counts, with cut-off values of 217,000 for osteopenia and 269,000 for osteoporosis. The mean platelet count in osteopenic patients was 321.7 ± 120.3, while in osteoporotic patients it was 661.1 ± 113.2. Supporting our results as Kim et al. identified an inverse relationship between platelet count and bone mineral density (Kim et al., 2020). These findings suggest that a high platelet count could serve as a screening tool for decreased BMD.

Platelets play a crucial role in atherogenesis, inflammation, and thrombosis (Wang & Tang, 2020). Chronic inflammation and platelet activation, along with inflammatory cytokines, modulate bone remodeling through osteoclast activation, leading to bone loss. Platelets release thromboxane A2 and act as factors in bone formation and resorption, influenced by inflammation levels (Maruyama et al., 2020). Increased oxidative stress also links platelets to bone resorption and osteoporosis (Domazetovic et al., 2017), as oxidative stress triggers platelet activation via multiple pathways (Messina et al., 2020).

In this study, serum ferritin levels were significantly higher in patients compared to the control group. Thalassemic patients also exhibited significantly elevated oxidative stress, which plays a crucial role in the pathogenesis of osteoporosis (Shahir et al., 2014). Additionally, there was a significant positive correlation between bone alkaline phosphatase and the platelet-lymphocyte ratio (PLR) (P <0.001), as well as a significant correlation between PLR and bone mineral density (BMD) (P <0.001). Osteocalcin levels were significantly higher in patients than in the control group (P <0.001), likely due to low serum calcium levels reducing hydroxyapatite crystal formation, making osteocalcin more available in circulation (Zoch et al., 2016). This finding aligns with the results of other researchers, who observed elevated osteocalcin levels in osteoporotic postmenopausal women, highlighting its value as a surrogate marker for osteoporosis (Mohammadi et al., 2024; Singh et al., 2015)

However, no correlation between osteocalcin and BAP could be detected in this study. The absence of correlation between BAP and OC could be attributed to non-simultaneous secretion of BAP from osteoblasts vesicles with corresponding synthesis of OC different stages of osteoblastic activity (Díaz Diego et al., 1995). Supporting our findings, Lumachi et al. reported no significant relationship between osteocalcin and bone alkaline phosphatase in elderly men without a history of fractures (Lumachi et al., 2009).

This finding aligns with the results of other researchers, who observed elevated osteocalcin levels in osteoporotic postmenopausal women, highlighting its value as a surrogate marker for osteoporosis (Mohammadi et al., 2024; Singh et al., 2015). Conversely, Lumachi et al. reported no significant relationship between osteocalcin and bone alkaline

phosphatase in elderly men without a history of fractures (Lumachi et al., 2012).

Both osteocalcin and bone alkaline phosphatase are highly informative markers for osteoporosis, demonstrating a sensitivity of 94% and specificity of 98% for both of them. This high diagnostic accuracy suggests that these biomarkers could potentially replace bone mineral density (BMD) measurements obtained by DEXA scans, particularly in resource-limited settings.

Conclusion

1. Bone alkaline phosphatase and osteocalcin are effective surrogate markers for diagnosing osteoporosis in thalassemic patients.
2. Both markers show an inverse relationship with bone mineral density (BMD).
3. The platelet-lymphocyte ratio (PLR) serves as a valuable screening tool, as it effectively discriminates patients with low BMD.

Recommendation

1. Thalassemic patients should be regularly assessed for osteoporosis markers.
2. Platelet counts should be monitored in thalassemic patients as a screening tool for low bone mineral density (BMD).
3. Vitamin D and calcium supplementation should be provided, especially for splenectomized thalassemic patients.

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