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# Serum Tartrate Resistant Acid Phosphatase 5b in Beta Thalassemia Egyptian Patients: Promising Biomarker of Iron Overload Oxidative Stress and Bone Disease

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## Abstract

**Background:** Beta-thalassemia is a genetic disorder characterized by either a reduced or deficiency of  $\beta$ -globin chain which results in imbalance of  $\alpha$ -globin chains and red cell hemolysis. It is a chronic hemolytic anemia that constitutes a global health problem in our country. It presents early in life necessitating blood transfusion from the first year of life with its sequelae of iron overload, oxidative stress and complications of blood transfusion. In the recent years, osteopenia and osteoporosis in thalassaemic patients has attracted much attention because it is a major cause of morbidity despite adequate blood transfusion and iron chelation therapy. Bone mass density is maintained by the equilibrium between bone formation and bone resorption under the control of multiple systemic and local factors such as hormones, Vitamin-D and proinflammatory cytokines. The etiology of osteoporosis in thalassemia is multifactorial and is only partially understood.

**Objective:** The aim of the present work was to evaluate the level of serum TRAP-5b in patients with  $\beta$ -thalassemia and its relation to iron overload and oxidative stress.

**Subjects and methods:** The present study included 90 subjects; 60 patients with  $\beta$ -thalassemia major and 30 healthy individuals served as control. Thalassaemic patients were then divided into, unsplenectomized patients group (n=30) and splenectomized patients group (n=30). Blood samples were collected from all subjects and the level of serum tartrate resistant acid phosphatase 5b (TRACP-5b) activity was measured by enzyme linked immunosorbent assay (ELISA).

**Results:** The median values of serum (TRACP-5b) (U/l) in unsplenectomized and splenectomized patients groups were significantly higher than in normal control group, notably the unsplenectomized group reflecting a state of bone resorption. A statistically significant higher serum

ferritin was evident in patients versus the control especially in the splenectomized. A significantly higher MDA as a marker of oxidative stress was found in patients compared to the control especially in the splenectomized.

**Conclusion:** Estimating the serum tartrate resistant acid phosphatase 5b (TRACP-5b) activity could be considered as informative diagnostic and prognostic markers of oxidative stress and bone disease in thalassaemic patients especially in the early stages of bone resorption.

**Keywords:**  $\beta$ -thalassemia; Tartrate resistant acid phosphatase 5b; Iron overload; Oxidative stress

## Introduction

The thalassemia syndromes are a heterogeneous group of inherited hemolytic anemia characterized by defects in the synthesis of one or more of the globins chains subunits of the hemoglobin tetramer [1]. In  $\beta$ -thalassemia there is inadequate hemoglobin production of the  $\beta$  chain with unbalanced accumulation of  $\alpha$  chains leading to their precipitation leading to an ineffective erythropoiesis and hemolytic anemia [2-4]. The main stay of treatment is palliative by regular blood transfusion which results in iron overload [5], oxidative stress [6,7] and blood-borne infections [5]. Oxidative stress was documented in thalassemia as well as in other hemolytic anemias, the main cause of oxidative stress is iron overload, the free-iron species generate oxygen radicals which damage practically the cell membrane of vital organs. Measurement of malondialdehyde (MDA) is widely used as an indicator of increased levels of lipid peroxidation due to oxidative stress [8,9]. One of the consequences of iron overload is delayed puberty and hypogonadism which is one of the causes of osteoporosis in patients with thalassemia [10].

Osteoporosis is a skeletal disease characterized by low bone mass and micro architectural deterioration with a resulting

increase in bone fragility and consequent susceptibility to fracture [11]. With increased life expectancy, osteopenia/osteoporosis is a major cause of bone pain which may be found in 70-80 % of patients with  $\beta$  thalassemia worldwide, but the underlying pathogenesis is still speculative. The causes of osteopenia/osteoporosis in thalassemia syndrome are multifactorial [12]

Tartrate resistant acid phosphatase (TRAP) expressed by osteoclasts, macrophages and dendritic cells, are a class of metalloenzymes that catalyze the hydrolysis of various phosphate esters and anhydrides under acid reaction conditions [13].

They are glycoproteins which must be cleaved or modified to permit secretion, two isoforms have been identified in human serum termed 5a and 5b, each having different pH optimum. The TRAP gene is located on chromosome 19 (19p13.2-13.3) [13,14].

The aim of the present work was to evaluate the level of serum TRAP-5b in patients with  $\beta$ -thalassemia and its relation to iron overload and oxidative stress.

## Subjects and Methods

Individuals submitted to this study were divided into three groups: Group I: included 30 healthy volunteers clinically free from any disease their mean age was ( $24.67 \pm 0.78$ ) years. They were chosen from the staff members of Medical Research Institute (MRI), Alexandria University and their relatives. Group II: included 30 unsplenectomized patients with  $\beta$  thalassemia major, their mean age was ( $16.80 \pm 1.13$ ) years. Group III: included 30 splenectomized patients with  $\beta$ -thalassemia major, their mean age was ( $23.83 \pm 1.27$ ) years.

Patients in group II and III were of matched age as the control group and were recruited from the children blood transfusion

center, the Egyptian Red Crescent, Bacous branch, Alexandria branch. This work was conducted according to the guidelines of the local Ethical Committee of MRI and an informed consent was taken from all contributors in this study.

1. To all subjects the following investigations were done.
2. Full history recording.
3. Thorough clinical examination, Routine laboratory investigations including complete blood picture (CBC) [15], serum ferritin [16], serum calcium [17] and serum albumin [18], 4- Determination of serum tartrate resistant acid phosphatase 5b (TRACP-5b) activity [19] and serum malondialdehyde (MDA) [20].

Patients with cardiovascular disease, hypertension, thyroid dysfunction and diabetes mellitus which will potentiate the oxidative stress were excluded from the study.

## Hematological Results

The statistical analyses of these results showed that the mean values of hemoglobin concentration (g/dl) in unsplenectomized and splenectomized patients groups were significantly lower than in normal control group ( $p < 0.001$ ,  $p < 0.001$  respectively). With respect to unsplenectomized patients group, the mean value of hemoglobin concentration (g/dl) was significantly lower than in splenectomized patients group ( $p < 0.001$ ).

Also the statistical analyses of these results revealed that the mean value of WBCs ( $\times 10^3/\text{ul}$ ) in splenectomized patients group was significantly higher than in normal control group ( $p < 0.001$ ). On other hand, the mean values of WBCs count ( $\times 10^3/\text{ul}$ ) in unsplenectomized patients group showed insignificant difference from control group ( $p = 0.983$ ), but significantly lower than in splenectomized patients group ( $p < 0.001$ ) (**Table 1**).

**Table 1** Statistical analyses of hematological parameters in control group as well as in unsplenectomized and splenectomized  $\beta$ -thalassemia patients groups.

Hematological parameters	Control group (n=30)	$\beta$ -Thalassemia patients groups	
		Unsplenectomized group (n=30)	Splenectomized group (n=30)
<b>Hb concentration (g/dl)</b>			
Mean $\pm$ SE	13.38 $\pm$ 0.13	6.52 $\pm$ 0.19	7.81 $\pm$ 0.13
P		<0.001*	<0.001*
P1			<0.001•
<b>WBCs (<math>\times 10^3/\text{ul}</math>)</b>			
Mean $\pm$ SE	6.95 $\pm$ 0.25	6.83 $\pm$ 0.32	59.92 $\pm$ 6.52
P		0.983	<0.001*
P1			
<b>Lymphocyte count (%)</b>			
Mean $\pm$ SE	37.40 $\pm$ 0.65	44.36 $\pm$ 1.42	62.87 $\pm$ 1.66

P			
P1			
<b>Platelets count (<math>\times 10^3/\mu\text{l}</math>)</b>			
Mean $\pm$ SE	241.57 $\pm$ 7.49	267.03 $\pm$ 15.43	963.20 $\pm$ 62.76
P		0.633	<0.001*
P1			
P: Values compared to control group. P1: Value compared to unsplenectomized patients group. *: Significantly different from Control group. *: Significantly different from unsplenectomized patients group. Significance was considered at $p \leq 0.05$ .			

Moreover, the statistical analyses of these results showed that the mean values of lymphocyte count (%) in unsplenectomized and splenectomized patients groups were significantly higher than in normal control group ( $p < 0.001$ ,  $p < 0.001$  respectively). With respect to unsplenectomized patients group, the mean value of lymphocyte count (%) was significantly lower than in splenectomized patients group ( $p < 0.001$ ).

In addition the statistical analyses of these results showed that the mean values of platelets count ( $\times 10^3/\mu\text{l}$ ) in splenectomized patients group was significantly higher than in normal control group ( $p < 0.001$ ). On other hand, the mean values of platelets count ( $\times 10^3/\mu\text{l}$ ) in unsplenectomized patients group showed insignificant difference from control ( $p = 0.633$ ) but was significantly less than in splenectomized patients group ( $p < 0.001$ ).

**Table 2** Statistical analysis of serum (MDA) (nmol/ml) in control group as well as in unsplenectomized and splenectomized  $\beta$ -thalassemia patients groups.

Statistical analysis	Control group (n=30)	$\beta$ -Thalassemia patients groups	
		Unsplenectomized group (n=30)	Splenectomized group (n=30)
Mean $\pm$ SE	5.10 $\pm$ 0.29	29.23 $\pm$ 2.48	33.27 $\pm$ 0.2.94
P		<0.001*	<0.001*
P1			0.204
P: Values compared to control group. P1: Value compared to unsplenectomized patients group. *: Significantly different from Control group. Significance was considered at $p \leq 0.05$ .			

### Serum tartrate resistant acid phosphatase 5b (TRACP-5b(U/I))

The statistical analyses of these results showed that the median values of serum (TRACP-5b) (U/I) in unsplenectomized and splenectomized patients groups were significantly higher than in normal control group ( $p < 0.001$ ,  $p < 0.001$  respectively). On the other hand, the median values of serum (TRACP-5b) (U/I)

## Biochemical results

### Serum malondialdehyde (MDA) (nmol/ml)

The statistical analyses of these results showed that the mean values of serum (MDA) in unsplenectomized and splenectomized patients groups were significantly higher than in normal control group ( $p < 0.001$ ,  $p < 0.001$  respectively). On the other hand, the mean values of serum (MDA) in unsplenectomized and splenectomized patients groups showed insignificant difference ( $p = 0.204$ ) (**Table 2**).

in unsplenectomized and splenectomized patients groups showed insignificant difference ( $p = 0.240$ ) (**Table 3**).

### Serum ferritin (ng/ml)

The statistical analyses of these results showed that the mean values of serum ferritin in unsplenectomized and splenectomized patients groups were significantly higher than in normal control group ( $p < 0.001$ ,  $p < 0.001$  respectively).

Moreover, the mean values of serum ferritin in splenectomized patients group was significantly higher than in unsplenectomized patients groups ( $p < 0.001$ ) (Table 4).

**Table 3** Statistical analysis of Serum (TRACP-5b)(U/l) in control group as well as in unsplenectomized and splenectomized  $\beta$ -thalassemia patients groups.

Statistical analysis	Control group (n=30)	$\beta$ -Thalassemia patients groups	
		Unsplenectomized group (n=30)	Splenectomized group (n=30)
Min. – Max.	2.20 – 3.70	2.50 – 170.00	1.70 – 128.00
Median	2.90	4.30	3.70
P		<0.001*	<0.001*
P1			0.240

P: Values compared to control group.  
P1: Value compared to unsplenectomized patients group.  
\*: Significantly different from Control group.  
Significance was considered at  $p \leq 0.05$

**Table 4** Statistical analysis of serum ferritin (ng/ml) in control group as well as in unsplenectomized and splenectomized  $\beta$ -thalassemia patients groups.

Statistical analysis	Control group (n=30)	$\beta$ -Thalassemia patients groups	
		Unsplenectomized group (n=30)	Splenectomized group (n=30)
Mean $\pm$ SE	115.50 $\pm$ 5.15	2517.03 $\pm$ 238.42	4062.50 $\pm$ 348.96
P		<0.001*	< 0.001*
P1			<0.001*

P: Values compared to control group.  
P1: Value compared to unsplenectomized patients group.  
\*: Significantly different from Control group.  
•: Significantly different from unsplenectomized patients group.  
Significance was considered at  $p \leq 0.05$

### Serum calcium (mg/dl)

The statistical analyses of these results showed that the mean values of serum calcium (mg/dl) in unsplenectomized and splenectomized patients groups were significantly lower than in

normal control group ( $p < 0.001$ ,  $p < 0.001$  respectively). On the other hand, the mean values of serum calcium in unsplenectomized and splenectomized patients groups showed insignificant difference ( $p = 0.055$ ) (Table 5).

**Table 5** Statistical analysis of serum calcium (mg/dl) in control group as well as in unsplenectomized and splenectomized  $\beta$ -thalassemia patients groups.

Statistical analysis	Control group (n=30)	$\beta$ -Thalassemia patients groups	
		Unsplenectomized group (n=30)	Splenectomized group (n=30)
Mean $\pm$ SE	9.35 $\pm$ 0.06	8.09 $\pm$ 0.16	8.48 $\pm$ 0.17
P		<0.001*	<0.001*
P1			0.055

P: Values compared to control group.  
P1: Value compared to unsplenectomized patients group.  
\*: Significantly different from Control group.  
Significance was considered at  $p \leq 0.05$ .

### Serum albumin (mg/dl)

The statistical analyses of these results showed that the mean values of serum albumin (mg/dl) in unsplenectomized and splenectomized patients groups were significantly lower than in normal control group ( $p < 0.001$ ,  $p = 0.002$  respectively). On the other hand, the mean values of serum albumin in unsplenectomized and splenectomized patients groups showed insignificant difference ( $p = 0.323$ ) (Table 6).

**Correlation between serum malondialdehyde (MDA) and serum TRACP-5b with hematological parameters and biochemical parameters in patients with  $\beta$ -thalassemia:** The results revealed that serum malondialdehyde (MDA) was positively correlated with white blood cells ( $r = 0.351$ ;  $p < 0.001$ ),

lymphocyte count ( $r = 0.533$ ;  $p < 0.001$ ), platelets count ( $r = 0.391$ ;  $p < 0.001$ ), serum TRACP-5b ( $r = 0.509$ ;  $p < 0.001$ ) and serum ferritin ( $r = 0.643$ ;  $p < 0.001$ ). On the other hand, it was negatively correlated with hemoglobin concentration ( $r = -0.686$ ;  $p < 0.001$ ), serum calcium ( $r = -0.437$ ;  $p < 0.001$ ), and serum albumin ( $r = -0.421$ ;  $p < 0.001$ ).

Also the results revealed that serum (TRACP-5b) was positively correlated with platelets ( $r = 0.250$ ;  $p = 0.017$ ), and serum ferritin ( $r = 0.465$ ;  $p < 0.001$ ), but it was negatively correlated with hemoglobin concentration ( $r = -0.495$ ;  $p < 0.001$ ), serum calcium ( $r = -0.463$ ;  $p < 0.001$ ), and serum albumin ( $r = -0.430$ ;  $p < 0.001$ ) (Table 7).

**Table 6** Statistical analysis of serum albumin (mg/dl) in control group as well as in unsplenectomized and splenectomized  $\beta$ -thalassemia patients groups.

Statistical analysis	Control group (n=30)	$\beta$ -Thalassemia patients groups	
		Unsplenectomized group (n=30)	Splenectomized group (n=30)
Mean $\pm$ SE	4.30 $\pm$ 0.05	3.89 $\pm$ 0.09	3.99 $\pm$ 0.07
P		<0.001*	0.002*
P1			0.323

P: Values compared to control group.  
P1: Value compared to unsplenectomized patients group.  
\*: Significantly different from Control group.  
Significance was considered at  $p \leq 0.05$ .

**Table 7** Correlation between serum malondialdehyde (MDA) and serum TRACP-5b with hematological parameters and biochemical parameters in patients with  $\beta$ -thalassemia.

	Serum (MDA) (nmol/ml)		Serum TRACP-5b (U/l)	
	r	p	r	p
Hb concentration (g/dl)	0.6	<0.001	-0.495*	<0.001
	0.351*	<0.001	0.176	0.096
WBCS count ( $\times 10^3$ /ul)	0.533*	<0.001	0.234*	0.026
	0.391*	<0.001	0.250*	0.017
Lymphocyte count (%)	1.000		0.509*	<0.001
	0.509*	<0.001	1.000	
Platelets count ( $\times 10^3$ /ul)	0.643*	<0.001	0.465*	<0.001
	-0.437*	<0.001	-0.463*	<0.001
Serum (MDA) (nmol/ml)				
Serum TRACP-5b (U/l)				
Serum ferritin (ng/ml)				
Serum calcium (mg/dl)				

Serum albumin (mg/dl)	r	-0.421*	r	-0.430*
	p	<0.001	p	<0.001

r: Spearman correlation Coefficient.

\*: Statistically significant at  $p \leq 0.05$ .

## Discussion

Patients with thalassemia now achieve a longer life than before due to advent of better blood transfusion policies and better chelation. Yet, they are faced with many complications that make them have a lower quality of life.

In the present study, a statistically significant lower hemoglobin concentration was found in patients compared to the control ( $p < 0.001$ ), and in unsplenectomized compared to splenectomized patients. This could be attributed to enhanced hemolysis, ineffective erythropoiesis and hypersplenism.

As regards the total leucocytic count (TLC), a significant increase was found in splenectomized patients due to increased circulating immature forms namely normoblasts, and increased lymphocytic count. The high TLC in patients with thalassemia may be caused by chronic antigenic stimulation from repeated blood transfusions which are the mainstay of treatment in these patients.

The present study revealed a highly significant increase in platelet counts in splenectomized patients versus the control. This is attributed to removal of the platelet pool.

On comparing splenectomized to non-splenectomized patients, no significant difference was found in serum calcium, but the level was significantly low in patients versus the control. This could be explained by calcium chelation by the citrate anticoagulant of the blood unit, or by low dietary calcium intake. Another possible cause for the low calcium is the low albumin level in patient groups as calcium is carried by albumin. Moreover, these patients have hypovitaminosis D due to low exposure to sun and restricted physical activity [3,21,22].

On the other hand, the low calcium level in these patients is due to endocrine disturbances namely hypoparathyroidism and hypogonadism. De Sanctis et al. found hypoparathyroidism in 6.9% of their patients [23]. They conducted a multi-centric study on the prevalence of endocrine complications among their patients and they observed that they differed among centers.

In addition to the known functions of calcium in the body, it has a crucial role in thalassemia major as it guards against the toxic effects of free Hb. Madsen et al. (2004) stated that the haptoglobin hemoglobin receptor (CD 163) which clears free Hb from plasma needs calcium for its signaling [24]. Their data revealed that a higher calcium concentration is required to elicit ligand binding activity of (CD 163) compared to other receptors which bind calcium at very high affinity.

It is tempting to speculate that the low calcium level in thalassemia patients might accentuate the oxidative stress in these patients. In the present study, a statistically negative correlation exists between a low calcium level and a high MDA

level which is true for albumin and MDA ( $r = 0.437$ ,  $p < 0.001$ ,  $r = -0.421$ ,  $p < 0.001$  respectively).

Chronic blood transfusion in thalassemia major is associated with iron overload as each transfused unit adds to the body 200 mg of iron, which together with the intramedullary destruction of RBCs caused by ineffective erythropoiesis leads to liberation of free unbound hemoglobin and iron overload.

In the present study, a statistically significant higher serum ferritin was evident in patients versus the control especially in the splenectomized ( $p < 0.001$ ). This finding emphasizes the role of the spleen as a store of ferritin in its phagocytic system. Hence, after splenectomy, a higher serum ferritin is observed. This agreed with Di-sabatino et al. [25].

Yet, the relationship between body iron as serum ferritin is not always linear, particularly if there is concomitant inflammation or tissue damage. Below 3000  $\mu\text{g/dl}$  values are related to iron stores in the macrophage system, whereas above this value they are determined by ferritin leakage from the liver cells [26]. In addition, SF level and body iron stores may vary with the chelation used.

Iron overload occurs when body iron increases over a sustained period of time either by repeated blood transfusion or by increased iron absorption from the gastrointestinal tract, especially that the body lacks a mechanism of iron excretion [23]. On the other hand, in thalassemia major iron absorption from the gut is accentuated which adds to the burden of iron overload.

Iron chelators balance the rate of iron accumulation by increasing its excretion in urine or feces. Despite the availability of iron chelators, thalassemia patients have iron overload resulting either from non-compliance to the injectable drug or from interrupting the chelator regimen [26].

The degree of adherence to iron chelators is reflected in our patients by the degree of variability of TRACP-5b level in our patients. This shows the role of iron chelators in decreasing TRAP expression, as well as osteoclast activity which confirms our results. Hence, iron chelators produce a reversible inactivation of TRACP-5b either by decreasing the levels of TRACP-5b mRNA, decreasing its transcription or increasing its degradation [27].

Iron is lightly reactive, and it alternates between two states: iron III and iron II in a process resulting in the gain or loss of electrons, and the generation of harmful free radicals that can damage lipid membranes, cell organelles and DNA.

In the present study a significantly higher MDA as a marker of oxidative stress was found in patients compared to the control.

Although it was higher in splenectomized patients yet the difference was not statistically significant.

In agreement with our findings, the higher oxidative stress in splenectomized patients was reported by Ebeid et al. who estimated myeloperoxidase level in these patients. Similarly Cighetti et al. [28] confirmed a higher oxidative stress in beta thalassemia which also agrees with other studies [28-31].

On the other hand Ghone et al. explained the increased MDA level in thalassaemic patients by absence or deficiency of  $\beta$ -globin chains and accumulation of unpaired  $\alpha$ -globin chains as well as iron overload. As we found a positive correlation between serum ferritin and MDA levels ( $r=0.643$ ;  $p<0.001$ ) [32]. In the same line, Mahdi et al. reported that MDA cross links several membrane components, giving the thalassemia red cells a more rigid texture [33].

Free radical production is a normal cellular function however when it exceeds its limit, many diseases occur. Antioxidants interact with free radical to scavenge them and promote their decomposition [34]. The overall activity of antioxidants and antioxidant enzymes constitutes the total antioxidant capacity (TAC) which is depleted by oxidative stress [35].

In agreement to our findings, Bazvand et al. reported an increased level of total antioxidant states in their thalassaemic patients, and they attributed it to enhanced oxidative stress in  $\beta$ -thalassemia [36]. They added that oxidative stress in males was higher than in females and they related this difference to hormonal alterations.

As regards serum TRACP-5b, the present study revealed a highly significant level in patients versus the control, notably the unsplenectomized group reflecting a state of bone resorption. This high level could be explained by the fact that the enzyme is a unique bone resorption marker, increased at lower hemoglobin concentration, which is observed in the unsplenectomized group.

Lower hemoglobin concentration was found in patients with  $\beta$ -thalassemia which is caused by an ineffective erythropoiesis which is considered an important factor in thalassemia-induced osteoporosis (TIO) leading to increased erythropoietic activity and bone marrow cavity expansion, and then ultimately distorted bone architecture. Theoretically, suppression of erythropoietic activity by maintaining hemoglobin levels higher than baseline should decrease bone distortion that leads to cortical bone loss [37]. In the present study, a statistically negative correlation exists between a low hemoglobin concentration level and high TRACP-5b level ( $r=-0.686$ ;  $p<0.001$ ). Similarly, Nakhakes et al. found that patients with severe thalassemia and regular transfusions have greater bone mass density (BMD) than those lacking regular transfusion, which also agrees with other studies [37-39].

The high TRACP-5b in thalassaemic patients is also due to low vitamin C levels in these patients as vitamin C is a strong inhibitor of TRACP [27].

Bone metabolism is a complex process of both osteoblasts as bone forming cells and osteoclasts responsible for bone

resorption. The development of an immunoassay for TRACP-5b has led to unlock the closed concepts of bone metabolism [14].

The process of bone resorption starts by the attachment of osteoclasts to the bone surface, followed by secretion of acid and enzymes into the space separating the osteoclasts from the bone. The acidic environment is created by the enzyme carbonic anhydrase and an  $H^+$  ATPase proton pump. It has been demonstrated that the parathyroid hormone (parathyrin), which is the promoter of bone resorption, stimulated the secretion of TRACP by osteoclasts in the presence of osteoblasts [40].

In the present study, we observed a very wide range of the enzyme level in patients, whether splenectomized or not in comparison to the control group (1.70-128 u/L, 2.50-170 u/L and 2.2-3.7 u/L respectively). This discrepancy could result from the degree of iron overload rather than different polymorphism of TRACP-5b gene as it is encoded by a single gene, ACPS on chromosome 19P (13.3-13.2), and TRACP-5b is solely secreted by osteoclasts.

Iron triggers osteoporosis through several mechanisms, namely lower osteoblast activity, hypogonadism, hypothyroidism and reactive oxidative stress [27].

On the contrary, Binkley et al. did not find any correlation between serum ferritin and TRACP in patients with sickle cell disease [41]. They attributed the high TRACP level to chronic inflammation which prevails in sickle cell patients, but in patients with  $\beta$ -thalassemia we found a positive correlation between serum ferritin and TRACP-5b ( $r=0.465$ ;  $p<0.001$ ).

It could be speculated that, by virtue of its iron content, the enzyme catalyzes iron deposition in bone matrix and impairs osteoid maturation, inhibits bone mineralization and decreases bone metabolism ending in focal osteomalacia.

In addition, Rossi et al. stated that iron overload – induced osteoporosis in thalassemia major patients is mediated by interaction with transient receptors potential vanilloid type 1 (TRPV1) channel [42]. It appears that TRPV- type 1 activation or desensitization influences TRACP expression and activity, and this effect depends on iron, adding a further proof of the role of iron overload in the dysregulation of bone metabolism in these patients. This pathway could be targeted to reduce bone resorption.

Our findings delineate a positive correlation between serum MDA as a marker of oxidative stress and serum levels of TRACP-5b as a marker of bone resorption. We agree with Abdollahi et al. and Jia et al. who reported that oxidative stress, either by itself, or by influencing regulatory cytokines such as TNF and interleukins incriminated in osteoporosis which supports our findings [43,44]. On the other hand, we could explain the higher levels of TRACP-5b in our female patients to lower estrogen levels as most of them did not reach puberty due to hypogonadism. In agreement to our results, Salari et al. advised the use of phytoestrogens to prevent bone resorption in post – menopausal women [45]. This dictates that the low estrogen level constitutes by itself a state of oxidative stress and administration of estrogen could counteract the oxidative stress.

The present study confirmed a significant positive correlation between TRACP-5b and MDA ( $r=0.509$ ;  $p<0.001$ ) reflecting the impact of oxidative stress on enhanced osteoclast activity and subsequent osteoporosis. This finding agrees with Rossi et al. MDA in addition to serving as an index of lipid peroxidation has also served as a measure of osteoclastic activity [42,46,47]. Several studies reported that there is association between malondialdehyde and osteoclasts [48-50]. Sontakke and Tare reported that an increase in malondialdehyde level and a reduction in glutathione peroxidase activity were obtained in osteoporotic people as compared to healthy controls [49,51].

From the clinical perspective, serum TRACP-5b is a good marker for evaluation bone disease in thalassaemic patients especially in the early stages of bone resorption even before it could be detected by DXA. This has been observed in some of our patients who have still normal DXA but an elevated TRACP level.

This is in agreement with Bjarnason et al., Halleen et al., Eastell et al., Bauer et al., Szulc and Ivaska et al., who reported that an elevated TRACP-5b level is an early marker of bone resorption in patients with thalassaemia major [19,52-56].

## Conclusion

From the findings of the present study, we may conclude that: thalassaemic patients have iron overload which is causal in producing osteoporosis. Positive correlation is observed between SF and TRAP-5b. TRACP-5b is significantly elevated in thalassaemic patients and it is a good marker for bone resorption. Positive correlation exists between oxidative stress as measured by MDA and TRACP-5b as a marker of osteoclast activity.

## Recommendations

From the present study the followings are to be recommended:

1. All thalassaemic patients should be screened for bone disease whether osteopenia or osteoporosis, on an annual basis starting at around 8-years-old.
2. TRACP-5b level should be measured to all thalassaemic patients as a part of their regular assessment.
3. DXA study should be done to patients with bone disease and therapy instituted.
4. Chelation therapy should be adhered to in order not to lower iron overload but also to treat osteoporosis in these patients.

## References

1. Weatherall DJ (2010) The inherited diseases of hemoglobin are an emerging global health burden. *Blood* 115: 4331-4336.
2. Birgens H, Ljung R (2007) The Thalassaemia syndromes. *Scand J Clin Lab Invest* 67: 11-25.
3. Cao A, Galanello R (2010) Beta-thalassaemia. *Genet Med* 12: 61-76.
4. Lithanatudom P, Leecharoenkiat A, Wannatung T, Svasti S, Fucharoen S, et al. (2010) A mechanism of ineffective erythropoiesis in beta thalassaemia/Hb E disease. *Haematologica* 95: 716-723.
5. Thuret I, Pondarré C, Loundou A, Steschenko D, Girot R, et al. (2010) Complications of treatment of patients with  $\beta$  - thalassaemia in France: results of the national registry. *Hematologica* 95: 724-729.
6. Fibach E, Rachmilewitz E (2008) The role of oxidative stress in hemolytic anemia. *Curr Mol Med* 8: 609-619.
7. Sehshier SL, Ceutes F, Vernesis M, Ma L, Angelucci E (2003) The role of oxidant injury in the pathophysiology of human thalassaemia. *Redox Rep* 8: 241-245.
8. Roberts RA, Smith RA, Safe S, Szabo C, Tjalkens RB, et al. (2010) Toxicological and pathophysiological role of reactive oxygen and nitrogen species. *Toxicology* 276: 85-94.
9. Romero FJ, Bosch-Morell F, Romero MJ, Jareño EJ, Romero B, et al. (1998) Lipid peroxidation products and antioxidants in human disease. *Environ Health Perspect* 106: 1229-1234.
10. Perrotta S, Cappellini MD, Bertoldo F, Servedio V, Iolascon G, et al. (2000) Osteoporosis in beta-thalassaemia major patients: Analysis of genetic background. *Br J Haematol* 111: 461-466.
11. Sambrook P, Cooper C (2010) Osteoporosis. *Lancet* 367: 2010-2018.
12. Borgna-Pignatti C, Rugolotto S, De-Stefano P, Zhao H, Cappellini MD, et al. (2004) Survival and complications in patients with thalassaemia major treated with transfusion and deferoxamine. *Haematologica* 89: 1187-1193.
13. Oddie GW, Schenk G, Angel NZ, Walsh N, Guddat LW, et al. (2000) Structure, function and regulation of Tartrate resistant acid phosphatase. *Bone* 27: 575-584.
14. Janckila AJ (2011) Development and clinical applications of Tartrate resistant acid phosphatase isoform 5b. *J Med Sci* 31: 211-222.
15. Bain BJ, Bates I (2001) Basic haematological techniques. In: Lewis SM, Bain BJ, Bates I, editors. *Dacie and Lewis Practical Haematology*. 9th edn. Edinburgh Scotland Churchill Livingstone pp: 19-46.
16. Ikram N, Hassan K, Younas M, Amanat S (2004) Ferritin levels in patients of beta thalassaemia major. *Int J Pathol* 2: 71-74.
17. Nada AM, Shaheen D (2017) Cholecalciferol improves glycemic control in type 2 diabetic patients: A 6-month prospective interventional study. *Ther Clin Risk Manag* 13: 813-820.
18. Smith JW, Kroker-Lobos MF, Lazo M, Rivera-Andrade A, Egner PA, et al. (2017) Aflatoxin and viral hepatitis exposures in Guatemala: Molecular biomarkers reveal a unique profile of risk factors in a region of high liver cancer incidence. *PLoS One* 12: e0189255.
19. Halleen JM, Alatalo SL, Suominen H, Cheng S, Janckila AJ, et al. (2000) Tartrate-resistant acid phosphatase 5b: A novel serum marker of bone resorption. *J Bone Miner Res* 15: 1337-1345.
20. Detchaporn P, Kukongviriyapan U, Prawan A, Jetsrisuparb A, Greenwald SE, et al. (2012) Altered vascular function, arterial stiffness, and antioxidant gene responses in pediatric thalassaemia patients. *Pediatr Cardiol* 33: 1054-1060.
21. Galanello R, Origa R (2010) Beta-thalassaemia. *Orphanet J Rare Dis* 5: 1-15.

22. Rund D, Rachmilewitz E (2005) Medical progress-Beta-thalassemia. *Rev Art N Engl J Med* 353: 1135-1146.
23. De Sanctis V, Soliman AT, Elsedfy H, Yassin M, Canatan D, et al. (2013) Osteoporosis in thalassemia Major: An update and the I-CET 2013 recommendations for surveillance and treatment. *Pediatr Endocrinol Rev* 11: 167-180.
24. Madsen M, Moller HJ, Nielsen MJ, Jacobsen C, Graversen JH, et al. (2004) Molecular characterization of the haptoglobin. Hemoglobin receptor CD163. Ligand binding properties of the scavenger receptor cysteine-rich domain region. *J Biol Chem* 279: 51561-51567.
25. Di Sabatino A, Carsetti R, Corazza GR (2011) Post-splenectomy and hyposplenic states. *Lancet* 378: 86-97.
26. Porter J, Viprakasit V (2014) Iron overload and chelation. In: Cappellini MD, Cohen A, Porter J, et al. editors. *Guidelines for the Management of Transfusion Dependent thalassemia (TDT)* [Internet]. 3rd edition. Nicosia (CY): Thalassemia International Federation.
27. Tsay J, Yang Z, Ross FP, Cunningham RS, Lin H, et al. (2010) Bone loss due to iron overload in a murine model: Importance of oxidative stress. *Blood* 116: 2582-2589.
28. Cighetti G, Duca L, Bortone L, Sala S, Nava I, et al. (2002) Oxidative status and malondialdehyde in beta-thalassemia patients. *Eur J Clin Invest* 32: 55-60.
29. Abdalla MY, Fawzi M, Al-Maloul SR, El-Banna N, Tayyem RF, et al. (2011) Increased oxidative stress and iron overload in Jordanian  $\beta$ -thalassemic children. *Hemoglobin* 35: 67-79.
30. Ferro E, Visalli G, Give R (2012) Oxidative damage and feroxidicity biomarkers in transfused and untransfused thalassemic subjects. *Free Radic Biol Med* 3: 1829-1837.
31. Kattamis C, Lazaropoulou C, Delaphorta P, Apostolou F, Kattamis A, et al. (2011) Disturbances of biomarkers of iron and oxidant – antioxidant homeostasis in patients with beta. *Thalassemia Intermedia Pediatr Endocrinol Rev* 8: 256-262.
32. Ghone RA, Kumbar KM, Suryakar AN, Katkam RV, Joshi NG (2008) Oxidative stress and disturbance in antioxidant balance in beta thalassemia major. *Ind J Clin Biochem* 23: 337-340.
33. Mahdi EA (2014) Relationship between oxidative stress and antioxidant states in beta thalassemia major patients. *Acta Chim Pharm Indica* 4: 137-145.
34. Mamtani M, Kulkarni H (2010) Bone recovery after zoledronate therapy in thalassemia-induced osteoporosis: A meta-analysis and systematic review. *Osteoporos Int* 2: 183-187.
35. Al – Mashhedy LA (2007) Total antioxidant capacity as indicative of oxidative stress on  $\beta$ -thalassemia patients. *Med J Babylon* 4: 291-295.
36. Bazvand F, Shams S, Esfahani MB, Koochakzadeh L, Monajemzadeh M, et al. (2011) Total antioxidant status in patients with major  $\beta$ -thalassemia. *Iran J Pediatr* 21: 159–165.
37. Nakhakes C, Jaruluxananan S, Saengsuda Y, Saengsuda S (2015) Risk factors for osteoporosis and the relationship between osteoporosis and hemoglobin level in adult patients with thalassemia in Rajavithi hospital. *Asian Biomed* 9: 169-174.
38. Izadyar S, Fazeli M, Izadyar M, Salamati P, Gholamrezanezhad A (2012) Bone mineral density in adult patients with major thalassemia: Our experience and a brief review of the literature. *Endokrynol Pol* 63: 264-269.
39. Mahachoklertwattana P, Chuansumrit A, Sirisriro R, Choubtum L, Sriphrapradang A, et al. (2003) Bone mineral density, biochemical and hormonal profiles in suboptimally treated children and adolescents with beta-thalassemia disease. *Clin Endocrinol (Oxf)* 58: 273-279.
40. Terpos E, Voskaridou E (2010) Treatment options for thalassemia patients with osteoporosis. *Ann NY Acad Sci* 1202: 237-243.
41. Binkley NC, Schmeer P, Wasnich RD, Lenchik L (2002) What are the criteria by which a densitometric diagnosis of osteoporosis can be made in males and non-Caucasians?. *J Clin Densitom* 5: 19–27.
42. Rossi F, Perrotta S, Bellini G, Luongo L, Tortora C, et al. (2014) Iron Metabolism and its disorders. *Haematologica* 99: 1876-1884.
43. Abdollahi M, Larijani B, Rahimi R, Salari P (2005) Role of oxidative stress in osteoporosis. *Therapy* 2: 787-796.
44. Jia P, Xu YJ, Zhang ZL, Li K, Li B, et al. (2012) Ferric ion could facilitate osteoclast differentiation and bone resorption through the production of reactive oxygen species. *J Orthop Res* 30: 1843-1852.
45. Salari SP, Nikfar S, Abdollahi M (2011) Prevention of bone resorption by intake of phytoestrogens in postmenopausal women: A meta-analysis. *Age (Dordr)* 33: 421-431.
46. Bonjour JP, Ammann P, Rizzoli R (1999) Importance of preclinical studies in the development of drugs for treatment of osteoporosis: A review related to the 1998 WHO guidelines. *Osteoporos Int* 9: 379–393.
47. Sheweita SA, Khoshhal KI, Baghdadi HH (2014) Osteoporosis and oxidative stress – role of antioxidants. *Sys Biol Free Radic Antiox* p: 128.
48. Berger CE, Rathod H, Gillespie JJ, Horrocks BR, Datta HK (2001) Scanning electrochemical microscopy at the surface of bone-resorbing osteoclasts: evidence for steady-state disposal and intracellular functional compartmentalization of calcium. *J Bone Miner Res* 16: 2092-2102.
49. Sontakke AN, Tare RS (2002) A duality in the roles of reactive oxygen species with respect to bone metabolism. *Clin Chim Acta* 318: 145-148.
50. Yan XT, Lee SH, Li W, Sun YN, Yang SY, et al. (2014) Evaluation of the antioxidant and anti-osteoporosis activities of chemical constituents of the fruits of *Prunus mume*. *Food Chem* 156: 408-415.
51. Jahanian E, Karimifar M, Rafeian-Kopaei M (2016) Antioxidants as a novel way to alleviate the adverse effects of oxidative stress in osteoporosis. *J Parathyroid Dis* 4: 60-65.
52. Bauer DC, Black DM, Garnero P, Hochberg M, Ott S, et al. (2004) Change in bone turnover and hip, non-spine, and vertebral fracture in alendronate-treated women: The fracture intervention trial. *J Bone Miner Res* 19: 1250-1258.
53. Bjarnason NH, Sarkar S, Duong T, Mitlak B, Delmas PD, et al. (2001) Six and twelve month changes in bone turnover are related to reduction in vertebral fracture risk during 3 years of raloxifene treatment in postmenopausal osteoporosis. *Osteoporos Int* 12: 922-930.
54. Eastell R, Barton I, Hannon RA, Chines A, Garnero P, et al. (2003) Relationship of early changes in bone resorption to the reduction in fracture risk with risedronate. *J Bone Miner Res* 18: 1051-1056.
55. Ivaska KK, Gerdhem P, Väänänen HK, Akesson K, Obrant KJ (2010) Bone turnover markers and prediction of fracture: A prospective

- follow-up study of 1040 elderly women for a mean of 9 years. *J Bone Miner Res* 25: 393-403.
56. Szulc P, Delmas PD (2008) Biochemical markers of bone turnover: Potential use in the investigation and management of postmenopausal osteoporosis. *Osteoporos Int* 19: 1683-1704.