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Parathormone Hormone Levels, Myocardial Iron Overload and Cardiac Functions in Patients with ß-Thalassaemia Major

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ABSTRACT

Background: Iron overload cardiomyopathy (IOC) represents the leading cause of death in patients with ß-thalassemia major (TM). There is increasing evidence that high parathormone hormone levels are associated with increased cardiac iron content.

Aim of the Work: In this study we aimed to assess the level of serum parathormone and its relation to cardiac functions, cardiac magnetic resonance T2* and liver iron concentration in children and adolescents with ß-thalassemia major.

Patients and Methods: In seventy TM patients and 30 age and sex matched healthy subjects, levels of intact parathormone (iPTH) levels, calcium, phosphorus, and alkaline phosphatase were measured in serum and cardiac functions were assessed by complete M-mode and pulsed Doppler echocardiography and myocardial performance index (MPI) was calculated for both the left and right ventricles. Myocardial iron content and liver iron content were assessed by Magnetic resonance imaging T2* and results were derived from patients' medical records. Average values of serum ferritin were calculated for 12 months prior to the study.

Results: The results of iPTH revealed that 72.5% had normal values, 18.5% had below normal values and 9% had elevated levels. We observed weak positive correlation between iPTH and LVEF and LIC but no correlation was found between iPTH and serum ferritin or cardiac T2*. Cases with increased cardiac iron (T2*<20ms) had insignificantly lower iPTH, higher mean age, more frequent transfusions, higher LIC, higher ferritin and lower calcium. Mean calcium values were significantly lower among cases while serum phosphorus and alkaline phosphatase were significantly higher among the cases compared to the control. There was no statistically significant difference in fractional shortening (FS %), left ventricular ejection fraction (LVEF %), or mean MPI of the left ventricle between the cases and control group. However, the left ventricular diastolic E and E/A indices were significantly higher amongst the cases, indicating restrictive pattern of iron overload cardiomyopathy.

Conclusions: In ß-thalassemia, cardiac iron overload can occur in spite of the low levels of PTH. Subclinical cardiac dysfunction is common in TM patients but not dependant on PTH level. Early and adequate vitamin D and calcium supplementation is mandatory to prevent secondary hyperparathyroidism and to delay the onset of cardiac dysfunctions in pediatric patients with ß-thalassemia major.

Key Words: *Thalassemia major – Magnetic resonance imaging – Cardiac functions – Iron overload – Parathormone.*

INTRODUCTION

ß-Thalassemia is a hereditary anemia resulting from defects in hemoglobin production. ß-thalassemia, which is caused by a decrease in the production of ß-globin chains, affects multiple organs and is associated with considerable morbidity and mortality **[1]**. Heart complications represent the leading cause of mortality in thalassemia major, even though, following the introduction of chelating therapies, an important and progressive increase of life expectancy mainly due to a reduction in mortality due to cardiac dysfunction has been demonstrated **[2]**.

Vitamin D deficiency is quite common in thalassemia major patients' due to increased metabolic demands, chronic medical care, and iron overload **[3,4]**. It was reported that low D25-OH levels produce reciprocal increase in serum parathyroid levels, leading to higher heart rates, higher cardiac intracellular calcium levels, and hypertrophy. However, this data is the first to suggest an association between cardiac iron uptake and vitamin D25-OH deficiency **[5]**.

Both parathyroid hormone and vitamin D1-25OH appear to stimulate transmembrane cal-

cium movement via L-type calcium-dependent channels (LTCC); although the details of this interaction remain poorly characterized **[5]**. Murine data indicate that LTCC are important in transporting non-transferrin bound iron (NTBI) into the myocardium. Thus, LTCC modulation represents the logical link between vitamin D deficiency, cardiac iron, and cardiac function **[6]**. Recent studies have correlated increased myocardial iron content to decreased levels of vitamin D in thalassemic patients and reported increased PTH levels as the major predictor of increased myocardial iron **[7]**.

When putting the aforementioned facts together, it is reasonable to hypothesize that secondary hyperparathyroidism may be common in pediatric patients with TM and could be associated with increased myocardial iron content and a higher incidence of myocardial dysfunction in these patients.

In this study we aimed to assess the level of serum parathormone and its relation to cardiac systolic and diastolic dysfunction, cardiac magnetic resonance T2* and liver iron concentration in children and adolescents with ß-thalassemia major.

PATIENTS AND METHODS

This was a case-control study conducted on 70 children with established diagnosis of ß-thalassemia major and 30 age and sex matched healthy subjects taken as control group, after obtaining consents from their legal guardians. All recruited patients were attending routine follow-up visits at the outpatient clinic, New Children's Hospital, Cairo University, Cairo, Egypt, during the study period (1st August 2011 to 1st February 2012). We excluded patients aged less than 6 years or older than 18 years and those with significant valvular or congenital heart disease. The study protocol was approved by the Institutional Ethical Committee and was conducted in accordance with the Institutional Committee for the Protection of Human Subjects and adopted by the 18th World Medical Assembly, Helsinki, Finland.

Detailed history-taking with emphasis on age, age at diagnosis, splenic status, onset of blood transfusion and transfusion frequency, calcium and vitamin D supplementation and chelation therapy was carried out. Serum ferritin concentrations were derived from patients' records, and mean values were calculated for 1 year prior to the study. Complete blood picture with blood indices was assessed by Coulter Counter (Cell-Dyn® 1700CS; Abbott). Parameters of calcium homeostasis including serum calcium, serum phosphate and alkaline phosphatase (ALP) were measured by conventional laboratory methods. Two ml of whole blood were collected into EDTA-containing tubes and were kept cold throughout the collection and separation process for measurement of intact Parathormone levels (iPTH) by chemiluminescent assay **[8]**. Data of myocardial and Liver iron concentration (LIC) were assessed with magnetic resonance imaging by means of T2* based on a method that has been previously described and validated **[9]**, and data were extracted from patients' medical records. Increased cardiac iron is defined as (T2*<20ms).

Echocardiography examination: Was performed at Echocardiography Laboratory of Abu El-Reesh Hospital, Faculty of Medicine, Cairo University. Transthoracic two dimentional (2D) guided (M Mode) and Doppler echocardiogram was performed with a Hewlett-Packard 5500 SONOS ultrasonic machine phased array sector scanner with the 4 and 8 MHZ probes according to age. Patient's recordings were taken while patients were in supine position without breath holding. M-mode, 2D and Doppler echocardiographic parameters were averaged over 3 cardiac cycles and all echocardiographic measurements were performed according to the guidelines for performance of a pediatric echocardiogram by American Society of Echocardiography. The MPI is a pure number and is calculated from the ratio of time intervals (a-b/b) derived with the aid of pulsed Doppler echocardiography **[10]**.

Statistical analysis: Patients' data were analyzed using SPSS 17.0 for windows 7. Quantitative variables were expressed by mean and SD (Standard deviation), median and range. Comparison of Quantitative variables between groups was done using *t* student test for parametric data or Mann Whitney test for nonparametric data. Correlations were done to test for linear relations between variables. Qualitative variables were expressed as numbers (frequency) and percent and compared between groups using Chi-square test or Fisher exact test for

small values. Receiver's operating characteristics (ROC) curve was made and area under the curve (AUC) was calculated for the ability of elevated PTH to predict cardiac iron overload. All *p*-values are two tailed and considered statistically significant If ≤0.05.

RESULTS

The study group consisted of 41 (58.6%) males and 29 (41.4%) females with male sex predominance (M/F ratio 1.4). Mean patients' age was 14.8±2.9 years (range: 6-18 years). The mean age at diagnosis was 12.5 months and the mean age of first transfusion was 12.6 months. At the time of enrollment; 58 (83%) patients were splenectomized at a median age of 6 years (range 3-17 yrs) and 12 (17%) patients had splenomegaly. All patients received blood transfusions at 4-8 week intervals to maintain a mean hemoglobin level of 7.6±1.6g/dl. Fifty-five (78.6%) patients had begun chelation with deferoxamine in 30 (42.9%) patients, deferiprone in 16 (22.9%) while 9 (12.9%) were currently on deferasirox. Folate, calcium and vitamin D supplementation were prescribed to all patients; no patients were taking cardiac medications.

The study populations were mild to severely iron overloaded with serum ferritin ranging from 263 to 12128ng/ml with a median of 2879.0ng/ml, average liver iron was 25.5±12.8 mg/g dry weight (rang: 2.3-43.0) and mean cardiac T2* was 27.3±14.0 (4.6-60.0) (Table 1).

Table (2) summarizes the comparison of the hemoglobin level, biochemical parameters and cardiac functions of cases and controls. Mean parathormone of cases was 38.0pg/ml (range 2.3-195pg/ml) versus 34.8±25.5pg/dl (range 3-95.9pg/dl) among the controls and this difference was not statistically significant (*p*=0.6). The results of parathormone revealed that 51 (72.8%) of the studied patients had normal values, 13 (18.6%) had below normal values and 6 (8.6%) patients had high levels exceeding the upper limit of the reference range. The echocardiographic parameters showed no statistically significant differences between cases and control as regards: Fraction shortening (*p*=0.8), ejection fraction (*p*=0.3), MPI (lt) (*p*=0.2) and MPI (rt) (*p*=0.09); while right E velocity, right A velocity, left E velocity and the left A velocity values were significantly higher among cases (*p*=0.01, 0.002, 0.001 and <0.001 respectively).

Table (1): Parameters of iron overload among thalassemia major cases.

Parameter	The studied cases (n=70)
Serum ferritin (ng/ml):	
Mean±SD (Range)	3637.6±2540.9 (263-12128)
Median (IQR[a])	2879.0 (1854.8-4775.5)
LIC[b] mg/dl (n=43):	
Mean±SD (Range)	25.5 ±12.8 (2.3->43.0)
Median (IQR[a])	23.8 (15.5-36.9)
LIC[b] mmol/kg (n=43):	
Mean±SD (Range)	453.4±228.8 (18.8->769.0)
Median (IQR[a])	426 (276.8-661.0)
Cardiac T2 ms (n=43):*	
Mean±SD (Range)	27.4±14.1 (4.6-60.0)
Median (IQR[a])	26.5 (15.2-34.7)

[a]IQR : Inter-quartile range.
[b]LIC : Liver iron concentration.

There was no correlation between PTH and age, frequency of blood transfusion, serum ferritin, cardiac T2*, Calcium, Phosphorus or Alkaline Phosphatase. No correlation was proved between PTH and fractional shortening (FS) (*p*=0.148), ejection fraction (EF) (*p*=0.313), left ventricular MPI (*p*=0.070), right ventricular MPI (*p*=0.187), T E/A velocity (*p*=0.339), M E/A velocity (*p*=0.586). There was a weak correlation between PTH and LIC, LVEF (*r*=0.3, *p*=0.03; *r*=0.3, *p*=0.02 respectively) (Fig. 1).

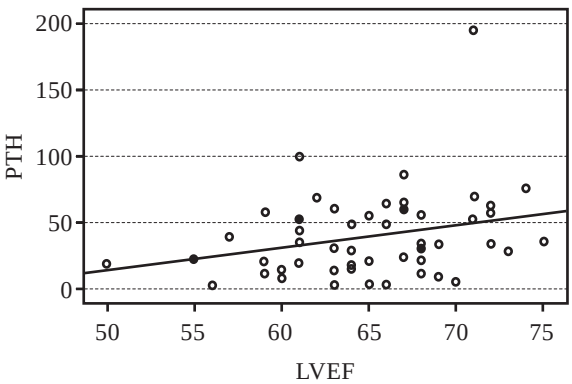

Fig. (1): Correlation between Parathormone and left ventricular ejection fraction in 70 thalassemia major patients (*r*=0.27, *p*=0.047).

Table (2): Comparison of laboratory data and cardiac functions of thalassemia major cases and control group.

Parameter	Cases (n=70)		Control (n=30)		*p* value
	Mean±SD	Range	Mean±SD	Range	
Hemoglobin (g/dl)	7.6±1.6	3.4-12.0	12.4±1.7	8.9-17	<0.001*
iPTH[a] (pg/mL)	38.0±3.5	2.3-195	34.8±25.5	3.0-95.9	0.550
Calcium (mg/dl)	8.6±0.9	6.4-10.1	9±1.1.0	5.3-10.5	0.022*
Phosphorus (mg/dl)	5.6±0.2	3.3-10.5	4.8±0.6	3.4-6.1	<0.001*
Alkaline phosphatase (U/L)	359.4±170.6	79-796	237±90	118-502	<0.001*
Fraction shortening (%)	35.8±5.7	29-69	36.5±5.2	29-48	0.757
Ejection fraction (%)	64±6.4	38-79	65.7±6.3	57-77	0.333
MPI (lt)	0.33±0.04	0.22-0.39	0.32±0.04	0.23-0.4	0.183
MPI (rt)	0.29±0.05	0.19-0.48	0.27±0.06	0.16-0.38	0.090
Right E velocity (m)(m/s)	0.75±0.1	0.51-0.94	0.7±0.1	0.46-0.9	0.012*
Right A velocity (m)(m/s)	0.49±0.06	0.34-0.61	0.44±0.07	0.32-0.55	0.002*
Left E velocity (m)(m/s)	1.03±0.1	0.78-1.29	0.93±0.14	0.63-1.29	>0.001*
Left A velocity (m)(m/s)	0.6±0.06	0.44-0.76	0.55±0.08	0.39-0.77	>0.001*

[a]PTH: Parathormone.
MPI (lt) myocardial performance index of the left ventricle.
MPI (rt) myocardial performance index of the right ventricle.
**p*-value is significant If <0.05.
Right E vel : E velocity across the Tricuspid valve.
Right A vel : A velocity across the Tricuspid valve.
Left E vel : E velocity across the mitral valve.
Left A vel : A velocity across the mitral valve.

On revising the results of cardiac T2* of 47 cases, we found 15 (32%) cases with T2*<20 while 32 (68%) had T2*<20 (Fig. 2).

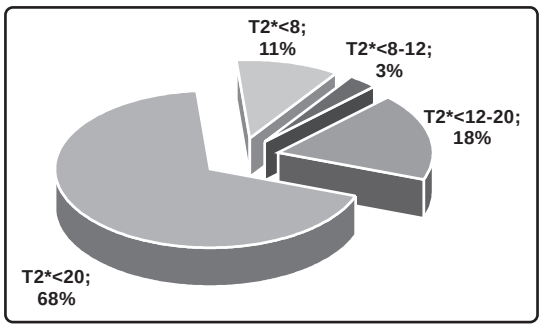

Fig. (2): Distribution of 47 thalassemia major cases according to Cardiac T2* Level.

Table (3) shows a comparison between cases with T2*<20 and cases with T2*<20. Cases with increased cardiac iron (T2*<20) were older, had more frequent transfusions, higher LIC, higher serum ferritin, lower PTH level and lower calcium but all did not reach level of significance (p>0.05).

ROC curve for parathormone in T2 groups:*

Receiver's operating characteristics (ROC) curve was performed to test for the ability of elevated PTH to predict cardiac iron overload among the studied patients with thalassemia major. The area under the ROC curve was 0.6 indicating that overall predictability of PTH is statistically insignificant (p>0.05).

Table (3): Comparison of thalassemia major cases with T2*<20ms and T2*>20ms.

Parameter	T2<20 (n=15)		T2>20 (n=32)		*p* value
	Mean	Std. Deviation	Mean	Std. Deviation	
Age	16.17	2.662	14.79	2.506	0.075
Bl Tr/year	15.67	5.499	12.68	4.839	0.058
Ferritin (ng/ml)	4553.78	3133.694	3600.08	2211.423	0.201
Ca (mg/dl)	8.21	1.018	8.69	0.780	0.054
Ph (mg/dl)	5.976	1.6627	5.549	0.9074	0.22
ALP (units/L)	421.61	187.804	356.16	164.539	0.19
iPTH (pg/ml)	31.35	26.131	43.83	33.062	0.166
LIC (mg/gm)	24.394	14.3699	25.067	10.8402	0.863
LVEF (%)	63.94	5.620	65.46	4.828	0.306

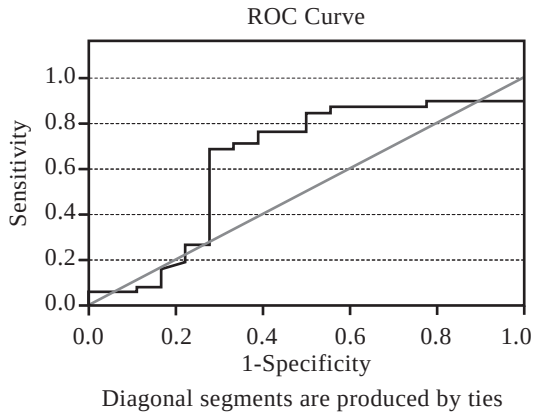

Fig. (3): ROC curve for the ability of elevated PTH to predict cardiac iron overload in patients with thalassemia major (n=47). The area under the ROC curve was 0.6 (*p*-value >0.05).

DISCUSSION

Contrary to expectations, we found low level of PTH in patients having increased myocardial iron deposition. In addition, there was a weak correlation between PTH level and LVEF and no correlations were found between PTH and other echocardiographic parameters and the ROC curve failed to prove the ability of elevated PTH to predict cardiac iron overload among our cases. This oppose the data reported in two recent studies carried out by Wood et al., **[11]** and Dimitriou et al., **[7]** that linked vitamin D deficiency and secondary hyperparathyroidism with cardiac iron uptake.

The pathogenetic mechanism that links PTH and cardiac iron uptake involves the transport of non-transferrin-bound iron (NTBI) in cardiomyocytes through the L-type Calcium channels (LTCCs), as this was initially described by Tsushima et al. **[12]**, as elevated PTH levels have been shown to increase LTCCs activity. Further evidence was obtained from studies showing reduced intracellular myocardial iron accumulation using LTCCs blockers, like amlodipine and verapamil, in mouse models **[6]**.

However, in the literature, not only the elevated PTH levels; but also the level and duration of NTBI exposure are important components for cardiac iron uptake **[13]**. Thus, secondary increases in PTH would not be expected to produce cardiac iron loading in the absence of elevated circulating NTBI, but might independently impair myocardial calcium cycling and cardiac function.

In addition, previous studies reported that the age of onset of parathyroid dysfunction in transfusion-dependant TM patients is usually around the age of 10 years **[14-16]** and abnormal cardiac T2* is rarely found before the age of 10 years, even in patients with high liver iron concentrations **[17]**. This way, the presence of parathyroid hypofunction with subsequent decreased functional reserve might lead to failure to compensate vitamin D deficiency which may induce cardiac iron uptake by mechanisms other than increased PTH. This may explain our findings of cases with increased myocardial iron that were older and had lower PTH and highlighted the fact that cardiac iron deposition is multifactorial and could occur in spite of low PTH.

Our data showed that about one fourth of children and adolescents with TM had abnormal parathormone (PTH) levels with the frequency of low PTH reaching twice that of high PTH. However, mean parathormone of cases was comparable to that of the controls. PTH did not correlate with age, transfusion frequency, serum ferritin, calcium, phosphorus or alkaline phosphatase. We observed weak positive correlation between PTH level and LIC. Our data support the prior evidence that parathyroid dysfunction is one of the common endocrinopathies of ß-thalasemia major due to chronic anemia, hypoxia and iron overload **[18,19]**.

Among our cases, there were no statistically significant differences in fractional shortening (FS %), left ventricular ejection fraction (LVEF %) or MPI between the cases and control group. This is in agreement with the results reported in previous studies **[20,21]**. However, several studies reported a significantly lower LVEF % in thalassemic patients in comparison with healthy age and sex matched individuals **[22-25]** and that MPI of TM cases was significantly increased in comparison to the control group **[24]**. This may be explained by the younger age group included in our study. In addition, supplementary vitamin D at our center is usually prescribed very early and it was reported that vitamin D may improve left ventricular systolic functions **[26]**.

However, the left ventricular diastolic E and E/A indices were significantly higher amongst the cases, indicating restrictive pattern of iron

overload cardiomyopathy and reflecting left ventricular chamber stiffness; this is in agreement with that reported by other studies **[25]**. It is to be mentioned that none of the patients had history of clinically manifesting cardiomyopathy or was receiving any kind of medicine that would affect cardiac functions.

Despite the early introduction of blood transfusion and chelation therapy, the majority of our study populations had post-transfusion hemoglobin below 9g/dl and were moderate to severely iron overloaded as evidenced by serum ferritin, LIC and cardiac T2*. This may be explained by the restrictive transfusion regimen adopted at our center as well as the bad compliance of our cases to chelators. In fact, the financial aspects also share in the problem; due to frequent interrupted availability of these chelators.

In spite of the routine supplementation with calcium and vitamin D, our cases showed biochemical parameters suggestive of hypocalcemia and vitamin D deficiency which may induce parathyroid hyper function **[5]**. This confirms the data reported in the majority of published literature on the high frequency of vitamin D deficiency in TM cases **[3,4,7,11]**.

In fact, our study has some limitations including lack of data about the level of NTBI and vitamin D metabolites among our cases which could impair the strength of our conclusions. But one of the points of strength in this study is the number of cases which was relatively larger than previous reports.

Conclusion: In ß-thalassemia, cardiac iron overload can occur in spite of the low levels of PTH. Subclinical cardiac dysfunction is common in TM patients but not dependant on PTH level. Early and adequate vitamin D and calcium supplementation is mandatory to prevent secondary hyperparathyroidism and to delay the onset of cardiac dysfunctions in pediatric patients with ß-thalassemia major.

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