Pancreatic functions in adolescents with beta thalassemia major could predict cardiac and hepatic iron loading: relation to T2-star (T2*) magnetic resonance imaging

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Accepted 23 January 2016
Published Online First 12 February 2016

ABSTRACT

The aim of this study is to assess the correlation between cardiac and hepatic T2* MRI findings with the endocrine and exocrine pancreatic functions in known patients with β-thalassaemia major (β-TM). A total of 50 adolescent patients with β-TM and 44 healthy controls were investigated via: serum amylase, lipase, triglyceride index, oral glucose tolerance test and T2* MRI, to assess iron content in the heart and liver. Diabetes was found in 20%, and 40% of patients had impaired fasting glucose (IFG). Cardiac T2* was less than 10 ms in 22% indicating heavy load with iron in cardiac tissues. There was a significant decrease in median serum amylase (63.5 vs 87.5 IU/L, p=0.003) and lipase (63 vs 90 IU/L, p=0.017) among patients in comparison with the control group. Patients with β-TM and diabetes had lower serum amylase (32 vs 68 IU/L), lipase (28 vs 79 IU/L), cardiac and hepatic T2* MRI (7 vs 25.5 ms; 3 vs 6 ms, p<0.001 for all) than those without diabetes. Similar results were found among patients with IFG when compared with others (p<0.001 for all). Cardiac and hepatic T2* were inversely correlated to triglyceride index (r=−0.376, p=0.014 and r=−0.475, p=0.001, respectively) and positively correlated to amylase (r=0.791 and r=0.790) and lipase (r=0.784 and r=0.783; p<0.001 for all). The endocrine and exocrine pancreatic functions might become an equivalent predictor to cardiac and hepatic iron overload, especially in countries where MRI is not available or where it is expensive. The early occurrence of these abnormalities warrants more intensive chelation therapy.

Significance of this study

What is already known about this subject?

- Cardiac and hepatic complications are the most important causes of morbidity in transfusion-dependent patients with β-thalassaemia major.
- Cardiac and hepatic MRI T2* is the only presently available non-invasive method with the potential to quantitatively assess iron load.
- MRI is an expensive procedure and is not globally available.
- Chelation therapy is important in order to counteract the toxic effects of iron on the organs of multitransfused patients, particularly the heart and the liver.

What are the new findings?

- Endocrine and exocrine pancreatic functions predict cardiac and hepatic iron overload.
- Pancreatic function parameters are less expensive to assess, more easily available and simpler to use as tools than are cardiac and hepatic MRI T2* machines.
- Patients with β-thalassaemia major and glucose impairments should receive intensive chelation therapy.
- The first cardiac assessment should be performed as early as possible, and it is mandatory whenever poor compliance is suspected or if there is limited access to chelation therapy.

INTRODUCTION

β-thalassaemia major (β-TM) is a significant public health problem in Egypt, where over 1–5 million newborns are expected to be affected with this disorder. There is a high rate of consanguineous marriage in Egypt, which further accumulates deleterious genes in families, reaching 35.3% in our community.[1 2] Regular and frequent red blood cell transfusions have significantly increased the life expectancy of patients with β-TM. The transfusions reduce some of the consequences of anemia, such as growth deficit. However, when no appropriate chelation therapy is available, patients accumulate iron in the heart, liver, spleen, pancreas and endocrine glands, leading to progressive organ dysfunction.[3 4]

Cardiac complications are the most important cause of death in transfusion-dependent patients with β-TM whose iron overload is not adequately chelated.[5] Iron overload-related

To cite: Mokhtar GM, Ibrahim WE, Elbarbary NS, *et al*. *J Investig Med* 2016;**64**:771–781.

Significance of this study

How might these results change the focus of research or clinical practice?

- This study investigated the relationship between pancreatic function and cardiac and hepatic iron burden in adolescents with β thalassaemia major.
- Study of the pancreas and of the correlated glucose metabolism alterations is the new frontier to further pursue the prognosis of thalassaemia major patients. This is particularly true if the analysis is focalised on the paediatric population, and explores the correlation with the heart and liver iron overload.
- The study addresses how to incorporate, in clinical practice, dual pancreatic function measurements as prospective markers of cardiac and hepatic iron overload risk.
- These markers are available inexpensively, and early occurrence of these abnormalities warrants more intensive chelation therapy to prospectively prevent cardiac iron accumulation.

cardiomyopathy is reversible, but the diagnosis is often delayed due to the late appearance of symptoms and echocardiographic abnormalities. A much greater understanding of cardiac damage could be achieved by a comprehensive assessment of the thalassaemic paediatric population, to prevent irreversible organ dysfunction and to further pursue the prognosis in this population.[6] A few studies have attempted to establish the age at which this parameter should first be measured in paediatric patients.[6–8] A recent study reported detectable cardiac damage using a multiparametric cardiovascular MR approach early in major patients with paediatric thalassaemia. Therefore, the first cardiac assessment should be performed as early as feasible to tailor the chelation treatment.[6 9]

Currently, MRI is the only presently available non-invasive method with the potential to quantitatively assess myocardial iron load.[10] L-type Ca++ channels might play a major role in the entrance of iron into cardiomyocytes, as well as in pancreatic β and acinar cells.[11] Earlier studies reported impairment of the endocrine and exocrine function of the pancreas as a common complication in patients with β-TM.[12 13]

Despite extensive research on MRI of hepatic and cardiac iron overload in patients with β-TM, there have been limited data on their relation with pancreatic functions; simpler techniques would be useful to identify patients at risk of tissue iron overload. Today, it is possible to directly quantify the pancreatic iron burden using the pancreatic regional and global T2* MRI technique,[14 15] but no data are available in the current study. The objective of this study was to evaluate cardiac and hepatic iron overload using a non-invasive T2-star MRI (MRI T2*) in young transfusion dependent β-TM patients and to correlate it with glucose disturbances, exocrine pancreatic functions, markers of insulin resistance and iron overload.

SUBJECTS AND METHODS

This cross-sectional study was conducted at Pediatric Hematology Clinic Children's Hospital, Ain Shams University, on transfusion-dependent β-TM data from patients regularly attending the clinic, and they were consecutively collected.

All patients were diagnosed as β-thalassaemia major based on clinical and hematological evaluation. Those having acute systemic infection were excluded from the study, to omit the influence of infection on ferritin, as were those with any systemic disease other than β-TM. All patients were on regular blood transfusion (15 mL packed red blood cells (RBCs)/kg body weight) at 2–4 weeks interval. Patients with β-TM received either mono or combined chelation therapy. Monochelation was in the form of deferoxamine (Desferal, DFO; Novartis Pharma AG, Basel, Switzerland) infused subcutaneously in a dose of 30–40 mg/kg/day (5 days/week), oral deferiprone (Ferriprox; ApoPharma Inc, Toronto, Ontario, Canada) given orally in a dose of 75 mg/kg/day divided on three divided doses for 7 days/week or deferasirox (Exjade, Novartis Pharma AG, Basel, Switzerland) 20 mg/kg/day. For combined chelation therapy, deferoxamine was given for 5 days/week with either daily deferiprone or deferasirox.

In our centre, the recommended starting daily dose of deferasirox is 20 mg/kg body weight. Serum ferritin is monitored monthly, and the dose is adjusted if necessary every 3–6 months based on serum ferritin trends and attainment of clinical goals. According to Yang *et al*,[16] dose adjustment is made in increments of 5 or 10 mg/kg. If the serum ferritin consistently falls below 500 μg/L, temporary interruption of therapy should be considered. In patients not adequately controlled with doses of 30 mg/kg (eg, serum ferritin levels persistently above 2500 μg/L and not showing a decreasing trend over time), doses of up to 40 mg/kg may be considered. Doses above 40 mg/kg are not recommended.[17] Combined oral iron chelators were given to alleviate iron overload in patients who did not comply with deferoxamine or to reduce serum ferritin, and to improve cardiac T2* and quality of life indices. For patients inadequately chelated by a daily dose of deferiprone of 75 mg/kg/day, subcutaneous deferoxamine was combined with deferiprone therapy to improve efficacy of iron chelation.[18]

Forty-four healthy volunteers (20 males and 24 females) with no evident medical disorder and not receiving any medication, served as a control group. This group included children recruited from the same region as the case subjects; most were classmates or acquaintances of the case subjects. They were age-matched and gender-matched healthy individuals with a mean age of 14.2±4.7 years, range 11–19 years.

The study protocol was approved by the Ethical Committee of Ain Shams University, and all the patients, control subjects and parents of the study participants gave their written informed consent after having the study design and tests explained to them.

METHODS

All subjects underwent the following procedures.

Detailed questionnaire

Information was collected on demographic characteristics, disease duration frequency of blood transfusion and

calculation of transfusion index (mL/kg/year). Chelation therapy and compliance to treatment were also reported; good compliance was defined as regularly receiving ≥80% of the calculated dose.[19] In addition, history suggestive of any complications (ie, hepatic, renal, endocrinal, pulmonary or cardiac) and history of operations (ie, splenectomy or cholecystectomy) were taken.

Clinical assessment

Physical examination included noting anthropometric measurements, blood pressure and puberty assessment according to Tanner score,[20] and looking for signs of anemia, hemosiderosis, hepatosplenomegaly and presence of complications (endocrinology, renal, hepatic, cardiac and pulmonary).

Laboratory analysis

Laboratory investigations included complete blood count using a Coulter B66 (Miami, Florida, USA) device, serum ferritin assessed on an Immulite (Diagnostic Products Corporation, 5700 West 96 St, Los Angeles, USA) instrument, liver functions tests, fasting blood glucose (FBG) using a Cobas Integra 800 (Roche Diagnostics, Mannheim, Germany) system, as well as serum triglycerides. The triglycerides glucose (TyG) index was calculated from fasting serum triglyceride and glucose concentration as log (fasting triglycerides (mg/dL)×fasting glucose (mg/dL)/2).[21]

Exocrine pancreatic functions were assessed by measuring serum amylase (ELISA kits) and serum lipase using a colourimetric assay (Roche Diagnostics, Indianapolis, USA). The disturbances of glucose metabolism were assessed by means of an oral glucose tolerance test (OGTT), as described by the WHO.[22] Even if the fasting plasma glucose was normal, all patients performed the OGTT at the time of study at least on two occasions (except those already known to be diabetic and on medical therapy). The OGTT was performed in the morning, as glucose tolerance can exhibit a diurnal rhythm with a significant decrease in the afternoon. The patients were instructed to fast (water was allowed) for 8–12 h prior to the tests. We classified our studied patients according to FBG and OGTT[23] into: (1) patients with normal glucose tolerance having FBG less than 100 mg/dL and a 2 h glucose <140 mg/dL, (2) patients with FBG equal to or above 126 mg/dL and a 2 h glucose ≥200 mg/dL—considered as having diabetes, (3) patients with FBG between 100 mg/dL and 125 mg/dL or a 2 h glucose <140 mg/dL—considered as having impaired fasting glucose (IFG), (4) patients with FBG <126 mg/dL or a 2 h glucose between 140 and 199 mg/dL—considered as having impaired glucose tolerance (IGT).

Radiological examination

All studied patients were subjected to non-invasive standard transthoracic full Doppler echocardiographic study with different modalities, using a Vivid E9 (GE Healthcare, Oslo, Norway) device, performed by an experienced operator. Heart disease was defined by the presence of systolic left ventricle (LV) dysfunction (LV shortening fraction <30% or LV ejection fraction <55%).[24]

ECG was performed using a Siemens–ELEMA AB (Tokyo, Japan) machine, and 24 h Holter monitoring was performed with a three-channel digital Schiller Microvit MT-101 (Schiller AG, Baar, Switzerland) system and analysed by Schiller software (Schiller AG), to detect arrhythmias in the enrolled patients. Cardiac complications were reported in the presence of pericardial effusion, dilated cardiomyopathy, left ventricular dysfunction and arrhythmias, diagnosed only if ECG documented and requiring specific medical treatment. Patients with single supraventricular or ventricular ectopic beats detected by ECG and requiring no medications were not considered as having cardiac complications.

MAGNETIC RESONANCE IMAGING T2* STUDY (T2* MRI IMAGING) OF THE HEART AND LIVER

Technique

All patients underwent MRI examination using a 1.5 T scanner (Philips Intera, Holland) using a 12-element phased-array coil. The prevalence of iron siderosis among our patients was determined using T2* MRI. The liver T2* was determined as a single 10 mm slice through the centre of the liver, which was scanned at 10 simultaneously acquired echo times (TE 1.4–16.6 ms/echo spacing 1.6 ms). The image was acquired using a GRE mFFE-BE sequence (repetition time 100 ms, flip angle 20, matrix 96×128 pixels, field of view 32–36 cm). The signal intensity of the liver parenchyma (region of interest (ROI) was placed over right hepatic lobe in an area totally free from vessels) and the background noise was measured in each of the 10 images using in-house Excel spreadsheet software. Patients were evaluated for liver siderosis using relaxation parameter T2*. Liver iron content (LIC) measurements were assessed according to Hankins *et al*,[25] by acquiring eight consequent T2* values and assessing T2 star decay. Patients with T2* of >11.4 are considered as having no liver iron load, those with T2* between 3.8 and 11.4 ms have mild hepatic iron load, those with T2* between 3.8 and 1.8 ms are considered to have moderate hepatic iron load, and those with T2* <1.8 are considered to be heavily overloaded.

For the measurement of myocardial T2*, a single short axis mid-ventricular slice was performed at eight simultaneously acquired echo times (TE 1.4–13.6 ms/echo spacing 1.6 ms). A gradient echo sequence—GRE black blood—was used (flip angle 35, matrix 128×256 pixels, field of view 32–36 cm). The repetition time was adjusted to the patient's heart rate (99–125 ms). The image was acquired during an 8–12 s breath-hold. The ROI was measured in the left ventricular septum, distant from the cardiac veins, which can be confounded by susceptibility artefacts. The myocardial T2* was calculated employing the same method as that used for the liver.

Mean cardiac iron concentration calculation was performed according to Carpenter *et al*.[26] T2* levels >20 ms indicated absent cardiac iron and very low risk of cardiac disease. Levels 10–20 ms indicated moderate cardiac iron and moderate risk of cardiac disease. Levels <10 ms indicated high levels of cardiac iron and high risk of cardiac disease.[26 27]

Statistical methodology

The data were coded, entered and processed on an IBM-PC compatible computer using the statistical Package for the Social Sciences (SPSS, V.14). Data were expressed as

mean±SD where analysis of variance with post hoc test was used for comparisons or median (IQR), where Kruskal–Wallis and Mann–Whitney tests were used unless specified as number (percentages) using χ^2 test for comparisons. Correlations between variables were assessed using Pearson coefficient of correlation for normally distributed variables and Spearman coefficient of correlation when at least one of the variables in the analysis had a skewed distribution. In order to compare parametric quantitative variables between the two groups, Student t test was applied. The level $p<0.05$ was considered the cut-off value for significance while p value <0.001 was considered highly significant in all analyses.

RESULTS

A total of 66 children with β-TM were recruited; 5 declined to participate in this study and 11 patients were excluded (7 patients did not complete required laboratory investigations while MRIs could not be performed in 4 patients).

Clinical characteristics of the studied population

Data from 50 patients (24 male and 26 female) included in the analysis are shown in table 1. Their ages ranged from 10 to 19 years with a mean of 15.25±4.1 years. A total of 34 (68%) patients were splenectomised. The Mean Transfusion index was 126±45 (mL/kg/year). Twenty-three patients (46%) were on monotherapy of iron chelation while the rest were on combined therapy.

Distribution of exocrine and endocrine functions in the studied population

Serum amylase and lipase levels were significantly decreased among enrolled patients compared to control (figure 1) (63.5 vs 87.5 (U/L), p value=0.003) and (63 vs 90 (U/L) p value=0.017). Of the enrolled patients, 40% had normal glucose tolerance, 40% had IFG while 20% had overt diabetes (6 were known diabetics on insulin therapy and 4 were diagnosed during the study by OGTT, table 1). None of the patients had IGT. In the current study, patients with IFG and those with diabetes showed lower serum amylase and lipase than patients without abnormal glucose metabolism ($p<0.001$ for all, figure 2).

Distribution of cardiac and hepatic imaging in the studied population

Cardiac siderosis was found in 36% of studied patients with cardiac T2* less than 20 ms. Siderosis was detected as early as 12 years of age, where the patient's T2* was 16 ms, while in the oldest patient, aged 19 years, T2* was 2.2 ms. Of the enrolled patients, 22% were heavily loaded with iron in cardiac tissues. Moreover, hepatic siderosis was detected in 60% of studied patients, with liver T2* ranging from 4 to 9 ms.

Patients with IFG had lower cardiac and hepatic T2* than patients without IFG; and they had higher ferritin, triglyceride and TyG index than those with normal glucose metabolism (table 2). Similar results were found among patients with overt diabetes when compared with the other patients (figure 3).

Table 1 Demographic and laboratory data of the studied patients with β-TM

Variable	β-TM (n=50)
Age (years), mean±SD	15.25±4.1
Sex, n (%)	
Male	24 (48)
Female	26 (52)
Weight (kg), mean±SD	40.6±9.2
Height (cm), mean±SD	146.3±103
BMI (kg/m^2), mean±SD	18.8±2.6
Disease duration (years), mean±SD	14.5±2.6
Positive family history, n (%)	40 (80)
Transfusion index (mL/kg/year)	126±45(59–222)
Splenectomised, n (%)	
Yes	34 (68)
No	16 (32)
Chelation therapy, n (%)	
Combined therapy	27 (54)
Mono therapy	23 (46)
Compliance to chelation, n (%)	
Good	28 (56)
Poor	22 (44)
Cardiac complications, n (%)	
Yes	24 (48)
No	26 (52)
Oral glucose tolerance test, n (%)	
Normal	20 (40)
Impaired fasting glucose	20 (40)
Overt diabetes	10 (20)
Triglyceride (mg/dL), median (IQR)	154 (134–211)
Triglyceride index, median (IQR)	8.13 (6.6–10.62)
FBG (mg/dL), median (IQR)	106 (100–143)
2 h BG (mg/dL), median (IQR)	130 (121–195)
ALT (U/L), median (IQR)	44 (34–66)
Albumin (mg/dL), median (IQR)	3.9 (3.5–4.1)
Ferritin (μg/L), median (IQR)	2750 (1319–5516)
Amylase (IU/L), median (IQR)	63.5 (44–84)
Lipase (IU/L), median (IQR)	63 (42–83)
Cardiac T2* (ms), median (IQR)	22 (12–31)
Hepatic T2* (ms), median (IQR)	5.5 (4–9)

ALT, alanine aminotransferase; β-TM, β-thalassaemia major; FBG, fasting blood glucose.

Relationship between clinical and laboratory parameters, and T2* imaging

A significant positive correlation between serum lipase and amylase ($p=0.019$) was found, as well as positive correlation between each of them, and cardiac and hepatic T2* ($p<0.001$, figure 4). Furthermore, an inverse correlation was found between serum amylase and lipase with ferritin, triglyceride and TyG index ($p<0.05$ for all).

A positive correlation between cardiac and hepatic T2* in the enrolled patients ($p=0.031$) was found. There was an inverse correlation between hepatic and cardiac T2* and ferritin ($p=0.01$, $p=0.034$), as well as with transfusion index ($p<0.001$) triglycerides ($p=0.000$) and TyG index ($p=0.014$), which reflects insulin resistance among the studied patients.

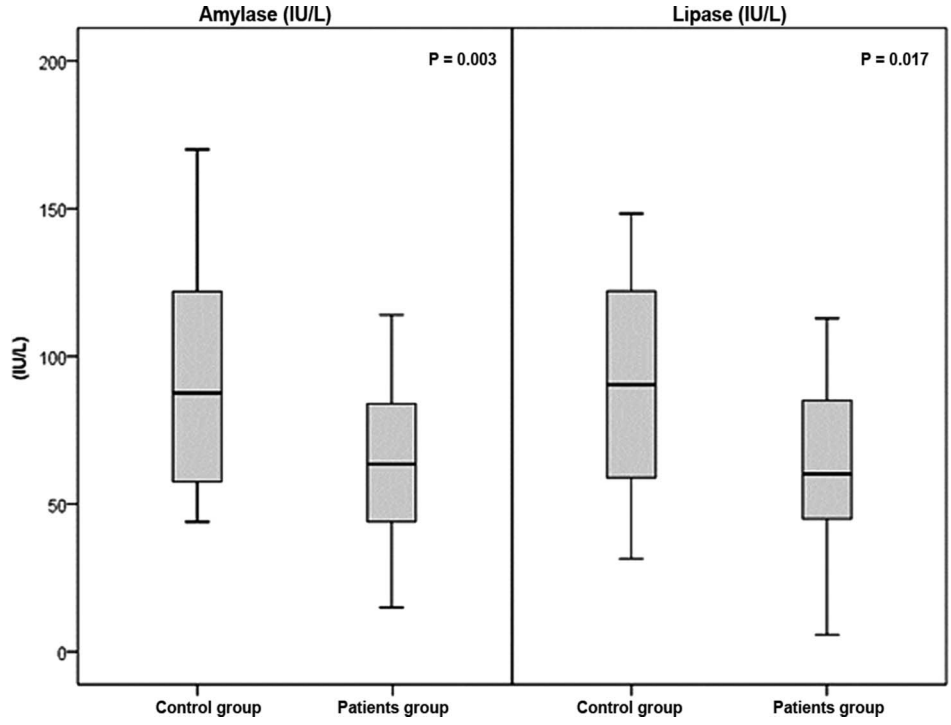

Figure 1 Box and Whisker plot showing comparison between patients and controls, regarding serum amylase and lipase levels. β-TM, β-thalassaemia major.

Laboratory and radiological findings according to disease characteristics in patients with β-TM

Cardiac complications were reported in 48%—those patients had lower serum amylase and lipase than the others (p=0.002 and p=0.003 respectively, figure 5). Moreover, they had lower cardiac T2* (9.75 vs 24 ms p value=0.028) and hepatic T2* (4 vs 6 ms p value=0.009) than other patients.

Splenectomised patients showed lower cardiac T2* compared with non-splenectomised patients (20 ms vs 27 ms with border line significance p=0.05) and were older in age (16.27±4.48 vs 12.8±1.7, p=0.01). However, all

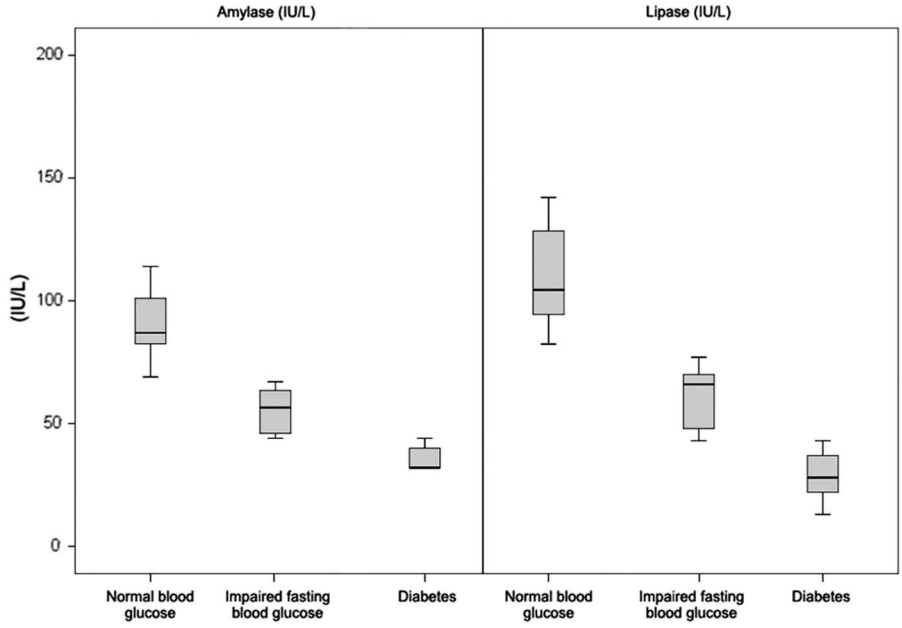

Figure 2 Box and Whisker plot showing comparison between patients with β-TM and normal blood glucose, impaired fasting glucose or diabetes, regarding serum amylase and lipase levels. β-TM, β-thalassaemia major.

Table 2 Comparison between patients with normal blood glucose, impaired fasting blood glucose and diabetes

Variables	Patients with normal blood glucose (n=20)	Patients with impaired fasting blood glucose (n=20)	Patients with diabetes (n=10)	p Value		
				P1	P2	P 3
Age (years), mean±SD	14.46±2.68	15.09±5.06	16.35±3.5	0.625	0.111	0.486
Transfusion index, mean ±SD	126.44±29.33	123.79±36.07	114.79±26.07	0.800	0.192	0.372
Ferritin (μg/L), median (IQR)	1194 (709–1666.5)	4204 (2750–4563)	8798.5 (8765–10,224)	<0.001	<0.001	<0.001
Triglyceride (mg/dL), median (IQR)	128.5 (111 –141)	159 (154–202)	236.5 (233–278)	<0.001	<0.001	<0.001
TYG index, median (IQR)	6.15 (4.56–6.66)	9.15 (8.13–9.9)	13.32 (12.6–15.9)	<0.001	<0.001	<0.001
Amylase(IU/L), median (IQR)	87 (82.5–101)	56.5 (46–63.5)	32 (32–40)	<0.001	<0.001	<0.001
Lipase (IU/L), median (IQR)	103 (93–127)	66 (48–70)	28 (22–37)	<0.001	<0.001	<0.001
Cardiac T2*, median (IQR)	32 (29–41.5)	19 (15–22)	7 (5–8)	<0.001	<0.001	<0.001
Hepatic T2*, median (IQR)	9.5 (7.5–22.5)	5 (4–5.5)	3 (3–4)	<0.001	<0.001	0.005

P1, Patients with normal blood glucose versus impaired fasting glucose.
P2, Patients with normal blood glucose versus those with diabetes.
P3, Patients with impaired fasting glucose versus those with diabetes.

other studied laboratory parameters were non-significant between both groups, including amylase, lipase, ferritin, triglyceride, TyG index and hepatic T2* (p>0.05 for all).

DISCUSSION

To the best of our knowledge, this is the first cardiac and hepatic T2* imaging study in relation to dual pancreatic functions, exocrine and endocrine, as well as markers of insulin resistance, to be conducted in adolescent patients with β-TM. Previous studies tested involvement of the endocrine pancreas alone,[28 29] and only one study tested exocrine functions,[30] with the majority of studies conducted on adult age groups.

The introduction of T2* cardiovascular MR for the estimation of myocardial iron has contributed to improved management of cardiac siderosis and decrease in cardiac-related mortality. Myocardial T2* >20 ms is considered normal and iron accumulation causes a reduction in T2*, with values <10 ms being associated with increased risk of heart failure.[31] In the current study, the diagnosis of cardiac siderosis was found in 36% of patients with cardiac T2* less than 20 ms and 22% with T2* less than 10 ms in those heavily loaded with iron in cardiac tissue.

In the Italian thalassaemia major population, Borgna-Pignatti *et al*[7] examined 35 chronically transfused patients aged 4.2–9.7 years who had undergone MRI; 22

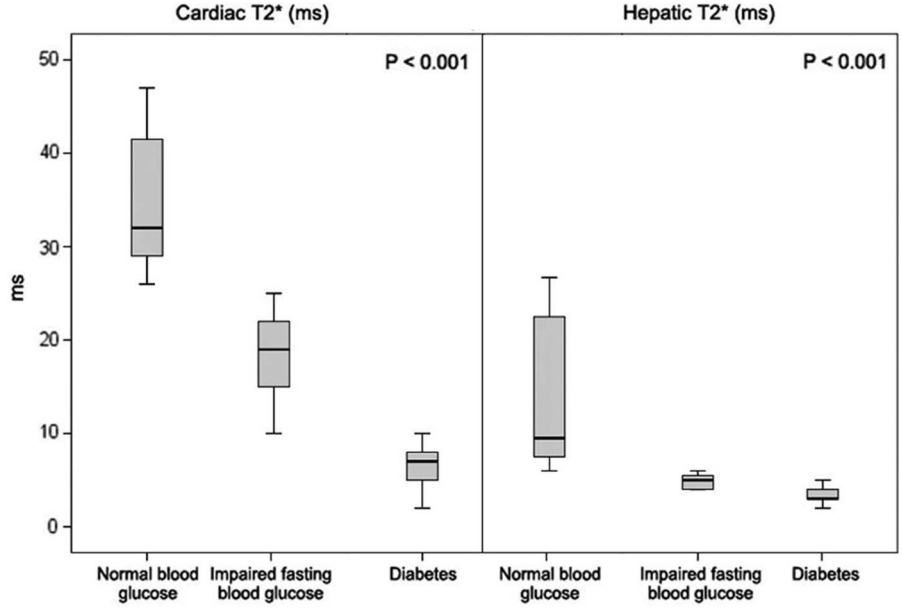

Figure 3 Box and Whisker plot of cardiac T2* and hepatic T2* MRI among patients with β-TM and normal blood glucose, impaired fasting glucose or diabetes. β-TM, β-thalassaemia major.

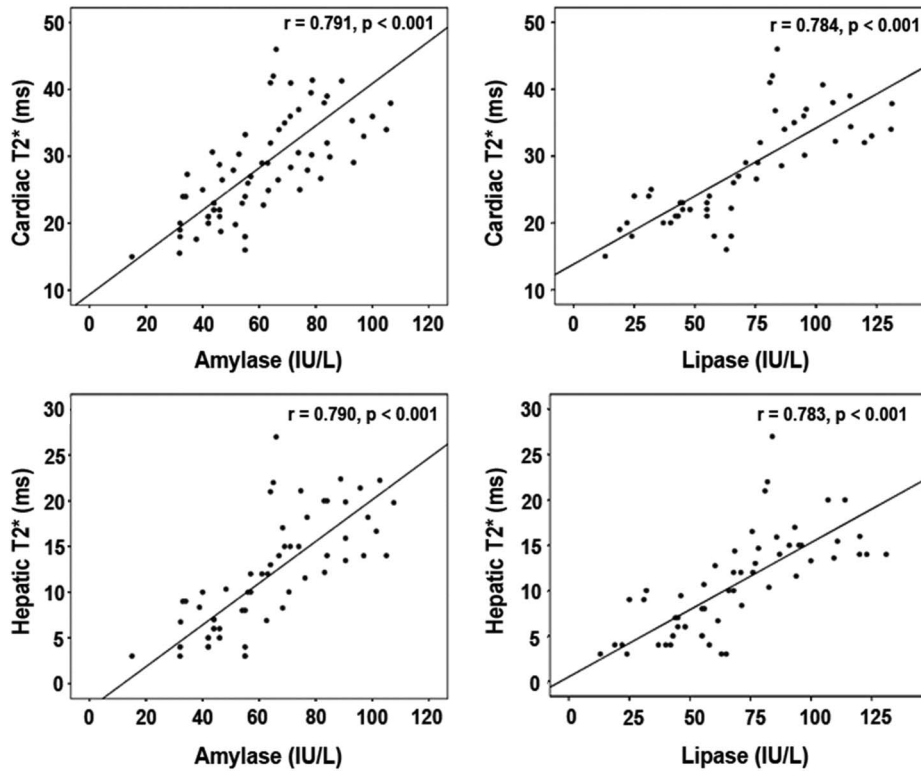

Figure 4 Significant positive correlation between cardiac T2* and hepatic T2* MRI among patients with β-TM in relation to serum amylase and lipase. β-TM, β-thalassaemia major.

patients had heterogeneous myocardial iron overload (MIO) with a T2* global value ≥20 ms while two patients had heterogeneous MIO with a T2* global value <20 ms, and two patients showed homogeneous MIO. Nine patients showed no MIO and none of the patient showed myocardial fibrosis. Among the patients with heart T2* <20 ms, the youngest was 6 years old. The mean liver T2* was 6.7 ±6.5 ms, corresponding to a mean MRI LIC of 8.3

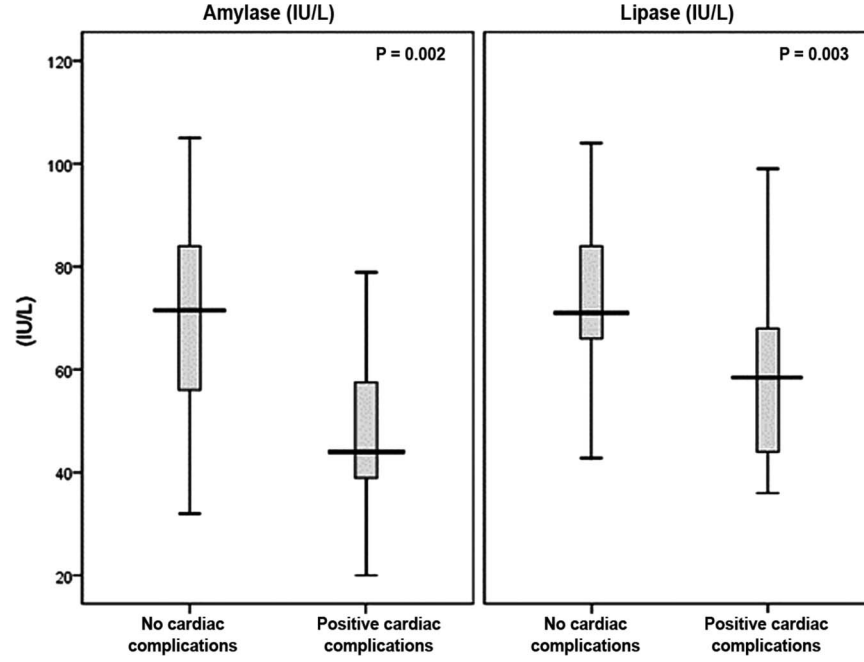

Figure 5 Box and Whisker plot among patients with β-TM and cardiac complications, regarding serum amylase and lipase levels. β-TM, β-thalassaemia major.

±7.1 mg/g dry weight, and 26 patients (74.3%) had hepatic iron overload.

In another recent multicentre study[6] representing the largest report of major patients with paediatric thalassaemia undergoing MRI within MIO using a multiparametric technique, only 37.7% of patients showed no MIO, and the youngest patient with MIO was 7.9 years old. Eighteen patients (16.8%) showed LV dysfunction; of them, 6 (33%) had a global heart T2* value <20 ms. Left ventricular dysfunction was present in the youngest patient involved in the study (a girl of 4.9 years who did not receive any chelation treatment), while 8 patients (7.5%) showed right ventricular dysfunction. Of them, 1 (12.5%) had a global heart T2* value <20 ms, thus suggesting that children can accumulate iron in the heart at an early age. In addition, the vast majority of assessed patients (77.6%) presented with pathological MRI LIC (the youngest 4.2 years old), with moderate to severe liver iron overload in more than half of them (68%).

In a previous work, Mavrogeni *et al*[32] reported that cardiac T2* values ranged between 5 ms and 50 ms, and only the three patients with cardiac iron overload ranging from 5 to 20 ms were among patients with β-TM. Moreover, 48% of our patients had cardiac complication. Iron accumulation occurs in the ventricular myocardium before the atrial myocardium. Also, deposition in the conduction system has been noted and can lead to nodal disease causing bradyarrhythmias, this condition may later progress to a dilated cardiomyopathy with left ventricular systolic dysfunction.[33]

It is worth noting that patients with cardiac complication had lower cardiac T2* than other patients (p=0.028). This is in accordance with Bilge *et al*,[34] who reported that significant reductions of left ventricular ejection fraction and of LV strains in hearts were found with severe iron overload. A previous study[35] stated that cardiac T2* is the best predictor of congestive heart failure (CHF) and of arrhythmias in patients with cardiac siderosis. With T2* <6 ms, approximately 50% of patients develop CHF within 1 year, approximately 90% of patients with CHF have T2* <10 ms whereas about 83% of patients with arrhythmia have cardiac T2* <20 ms. However, the outcome of patients with cardiac siderosis cannot be predicted on the basis of serum ferritin as ferritin is not a suitable predictor of subclinical cardiac disease, and cardiac decompensation can occur with serum ferritin levels <2500 ng/mL.[36] However, Casale *et al*[6] reported that serum ferritin ≥2000ng/mL and liver iron concentration ≥14 mg/g/dw (T2* value <1.8 ms) were found to be significant risk factors for a global heart T2* value <20 ms, and were detected as the best threshold for predicting cardiac iron overload in β-TM children. Our study revealed significantly higher median serum ferritin among patients with low cardiac T2* as well as its negative association with cardiac imaging study. This is in concordance with Fragasso *et al*,[9] who found that serum ferritin was negatively associated with cardiac T2* values. However, other studies[37 38] showed no significant correlation between cardiac T2* and serum ferritin.

The overall prevalence of IFG in our study was 40%, and of diabetes, it was 20%, similar to prior studies where the prevalence of IGT and diabetes in major patients with thalassaemia varied from 8% up to 27%,[3 28 39–42] although our population was made up mostly of adolescents. In a large retrospective cohort of adults patients with β-TM, Pepe *et al*[43] studied the relationship between diabetes mellitus and cardiac complications. Eighty-six (9%) out of 957 patients were affected by DM with a duration of 13.9 ±9.5 years. Of them, (88.4%) were under treatment with insulin, 8.1% with oral antiglycaemic agents and 3.5% did not receive any therapy. It was previously reported[3] that glucose disturbance in β-TM develops after the second decade of life. However, another study[44] reported that the prevalence rises sharply in the second and third decades of life, approaching 70% at 40 years of age. Since iron-mediated diabetes can be partially reversed by intensive chelation,[45] early detection and correction of glucose impairments in young patients may forestall the development of overt diabetes in adulthood. In addition, although established patients with β-TM and diabetes seldom recover normal glucose tolerance,[46] IGT is considered a reversible situation in β-thalassaemia.[12 28]

An increase in fasting serum glucose and triglyceride index accompanied with normoinsulinemia suggests some degree of insulin resistance and relative pancreatic failure, because, normally, the islet cells should produce more insulin to overcome hyperglycemia. It is likely that an elevated level of iron and ferritin causes iron toxicity in the liver and pancreas, as well as insulin dysregulation, due to hepatic and pancreatic dysfunction, which is most likely the cause of impaired glucose metabolism in patients with β-TM,[47] as pancreatic iron deposition is an early event, and many patients initially have normal glucose metrics. Over time, iron-mediated oxidative stress triggers apoptosis, volume loss and fatty replacement, leading to pancreatic dysfunction.[3 48]

Patients showed lower serum amylase and lipase than the control group, with an inverse correlation between the exocrine enzymes with triglyceride index among them. It was suggested[49] that, during the earlier phase of pancreatic damage, pancreatic enzymes leak directly into the circulation, causing increasing enzyme activity in serum until progressive destruction of the acinar tissue takes place with a decline in pancreatic enzyme concentrations. However, the onset of diabetes may follow exocrine pancreatic insufficiency, since endocrine cells are affected to a lesser extent by hemosiderin deposition[28] as patients are continuously loaded with iron due to their transfusion regimen, even if ferritin reaches normal level.[30]

There was a highly significant correlation between serum glucose disturbance and cardiac T2*, as well as with serum amylase, and lipase with cardiac and hepatic T2*. This is in contrast to a previous study,[30] which found a significant correlation between pancreatic lipase and cardiac T2* but not for liver iron concentration. Pancreas and heart iron exhibited a stronger inter-relationship; some have attributed this observation to the presence of L-type calcium channels in these organs. Meloni *et al*[15] showed, for the first time ever, that the association between cardiac and pancreatic hemosiderosis was certain, considering a myocardial segmental analysis and the patterns of iron distribution. In their study, global pancreas T2* values showed a significant positive correlation with global heart T2* values. Of the 137 patients with pancreatic iron overload,

45 (32.8%) had a pathological global heart T2* value. None of the patients with no pancreatic iron overload had MIO. Of the 31 patients with LV dysfunction, none showed a normal pancreatic T2* value. Global pancreas T2* values were significantly higher in patients with normal LV function than in patients with LV dysfunction. Pancreatic iron overload was positively correlated to myocardial iron distribution and LV function. This indicates that pancreas T2* is a powerful predictor for heart iron burden and function. Moreover, Pepe *et al*[43] reported that patients with DM had significantly increased risk for cardiac complications, heart failure, hyperkinetic arrhythmias and myocardial fibrosis than non-diabetic patients with β-TM.

A previous study[44] reported that cardiac T2* was a better predictor of diabetes than pancreas R2*. This is due to the fact that toxicity from pancreatic iron probably represents a combination of iron concentration and duration of iron loading. Since cardiac iron loading is delayed, when compared to pancreatic loading, it implies greater chronicity to the pancreatic iron deposition.[4] These data are worrisome given the improved longevity of patients with β-TM[50] and the potentially synergistic cardiovascular toxicity of diabetes and iron overload. However, a previous study on 131 patients with β-TM[51] described the possibility of predicting cardiac overload almost one decade in advance by studying pancreatic siderosis in them, indicating the strong association between pancreas and cardiac T2*. Furthermore, patients with impaired FBG had a higher cardiac iron overload and triglyceride index than patients with normal FBG. This could be explained by the fact that cardiac T2* correlated strongly with subclinical pancreatic impairment and insulin resistance.[41]

In our study, we observed that patients with β-TM and diabetes had lower serum amylase, serum lipase, cardiac T2* and hepatic T2* than other patients. Another study[30] reported that about 50% of patients with elevated cardiac iron or with decreased serum lipase were affected by diabetes.

There is an inverse correlation between the triglyceride index with cardiac and hepatic T2*, which reflects insulin resistance among studied patients. These data proved that poor chelation and, subsequently, hemosiderosis of the liver and other organs appear to be a major cause for glycaemic abnormalities in patients with β-TM.[3]

Considering the fact that liver biopsy is very invasive and that many patients refuse to be submitted to this procedure, an earlier study[52] showed that grading of liver hemochromatosis by MRI is significantly correlated with grading of hemochromatosis by liver biopsy. In our study, the diagnosis of hepatic siderosis was found in 60% of patients and a significant correlation was found between hepatic T2* and serum ferritin, which was in concordance with previous published studies[3 42 53 54] but contradicted others.[4]

Tangvarasittichai *et al*[55] suggested that liver inflammation due to iron overload and increased oxidative stress secondary to microcytic erythrocyte hemolysis is the main contributor to higher prevalence of diabetes in patients with thalassaemia. This is postulated to be due to iron deposition in the liver, which may interfere with the ability of the insulin to suppress hepatic glucose uptake, and also at the muscle level. This could explain decreased hepatic T2* in patients with diabetes.

The significant correlation found between hepatic and cardiac T2* was in line with Wood *et al*,[56] who reported that baseline hepatic T2* and serum ferritin levels are clinically relevant predictors of cardiac T2*. Another study[31] stated that it is likely that failure to control liver iron over the long-term increases the risk of cardiac iron loading. In contrast to our results, Noetzli *et al*[57] stated that cardiac iron was uncorrelated with liver iron. This can be explained by organ specific mechanisms of iron uptake and release, the heterogeneous distribution of the transferrin receptors and differential iron transport kinetics.[44] Splenectomised patients showed no significant correlation with hepatic and cardiac T2*. However, Brewer *et al*[58] found that surgical splenectomy, which could potentially predispose to greater extrahepatic iron stores by eliminating a large physiological iron depot, was more common in major patients with thalassaemia. Splenectomy was associated with higher cardiac and pancreatic iron burdens, but not hepatic iron or ferritin in patients with β-TM.

In addition, our study shows that our population is affected by pancreatic and cardiac iron overload at an adolescent age, which is earlier when compared with studies in other regions.[51] This may be due to limited access to chelation therapy in our developing country.

There are some limitations of this study that need to be addressed. First, these tests were investigated in a relatively small number of patients, so larger studies will be needed to confirm our findings. Second, it is possible today to quantify pancreatic iron overload using pancreatic regional and global T2* MRI, but we have not performed this technique in the present work.

In conclusion, indirect measurements of pancreatic iron overload by assessment of exocrine and endocrine pancreatic functions provide important feedback, even when cardiac and hepatic T2* results are available. As, it is a notoriously slow and difficult process to remove cardiac iron, if a patient with a normal cardiac T2* demonstrates abnormal exocrine or endocrine pancreatic function, it would be prudent to modify iron chelation to prospectively prevent cardiac iron accumulation rather than waiting for cardiac iron to appear. Therefore, in areas where even cardiac T2* is unavailable, biochemical measurements of exocrine and endocrine pancreatic functions may be used for surrogate assessment of cardiac risk. Cardiac iron loading, unfortunately occurring so early in our young population, warrants more intensive chelation therapy.

Acknowledgements The authors wish to express our sincere appreciation to all the patients and their families following in the Pediatric Hematology/Oncology Clinic, Children's Hospital, Ain Shams University, for their cooperation during the study period.

Competing interests None declared.

Ethics approval The study protocol was approved by the Ethical Committee of Ain Shams University.

Provenance and peer review Not commissioned; externally peer reviewed.

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