Use of echocardiography and glutathione S-transferase to detect heart complications in β-thalassemic patients

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Background and objective
β-Thalassemia major (TM) is an inherited disorder of hemoglobin synthesis and characterized by defective hemoglobin synthesis, resulting in ineffective erythropoiesis, severe anemia, increased erythrocyte turnover, and excessive iron absorption. Accordingly, iron overload develops and may accumulate in the liver, heart, and endocrine organs. Several gene polymorphisms have been studied as protective or predisposing factors for cardiac dysfunction in patients with TM. Moreover, echocardiographic left ventricular (LV) diastolic evaluation is used to detect early myocardial dysfunction secondary to iron overload. This study aimed at determining some diastolic and tissue Doppler echo indices to predict iron load.
Materials and methods
This study included 42 β-thalassemic patients, among whom, 16 proved to have cardiac complications after clinical evaluation. Their age ranged from 3 to 25 years. Participants were subjected to clinical evaluation, molecular analysis to detect glutathione S-transferase M1 (GSTM1) gene polymorphism, and transthoracic color Doppler echocardiography to detect early myocardial dysfunction.
Results
Seven (43.7%) patients had the functional wild-type allele (GSTM1 non-null genotypes), whereas nine (56.2%) patients were homozygous for the GSTM1 null allele. There was a statistically significant increase regarding both right and LV E/A in GSTM1 null genotype when compared with GSTM1 non-null genotype in β-thalassemic patients with cardiac complications. Moreover, LV and right ventricular diastolic function has been significantly affected in participants with GSTM1 null genotype in β-thalassemic patients with cardiac complications, and particularly, LV diastolic function has been significantly impaired in cases experiencing frequent blood transfusion in β-thalassemic patients with cardiac complications.
Conclusion
Follow-up of patients with β-thalassemia and evaluating echocardiographic changes may permit better assessment of patients and early recognition of cardiac affection before disease progression.

Keywords:
β-thalassemia, cardiac complications, echocardiography, glutathione S-Transferase M1

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Introduction

In 1925, Thomas Cooley and Pearl Lee defined β-thalassemia major (TM) as an inherited disorder of hemoglobin synthesis [1].

The defective hemoglobin synthesis in β-thalassemia causes ineffective erythropoiesis, which leads to many symptoms, including severe anemia, increased erythrocyte turnover, and excessive iron absorption, which results in iron overload in the heart, liver, and other organs [2].

These anemic patients need regular blood transfusions, which add to the iron overload. Iron possesses a redox activity that may cause brutal damage and fibrosis to vital organs [3].

Nevertheless, repeated blood transfusions lead to not only iron overload in patients with TM but also increase intestinal iron absorption and peripheral hemolysis. Iron accumulation in the heart leads to cardiac failure, which in most patients causes mortality and morbidity [4,5].

Some gene polymorphisms have been noticed as protective or predisposing to cardiac dysfunction in patients with TM. In this context, Wu and colleagues studied polymorphisms of two endogenous antioxidant enzymes, namely, glutathione S-transferase M1 (GSTM1) and glutathione S-transferase T1 (GSTT1) [6].

The studies revealed that the GSTM1 null (deleted) genotype was linked to decreased signal intensity ratio on MRI, which signifies increased myocardial iron. Other studies revealed that cardiac iron deposition is related to genetic variations of the GSTM1 enzyme [4].

Left ventricular (LV) diastolic evaluation by echocardiography is more sensitive to identify early myocardial dysfunction owing to iron overload compared with systolic evaluation [4,5].

This study aimed at determining some diastolic and tissue Doppler echo indices to predict iron load.

Materials and methods

Materials

This study included 42 β-thalassemic patients, of whom, 16 proved to have cardiac complications after clinical evaluation. Ethics No.: 10-172. Their age ranged from 3 to 25 years. They were included after having their individual written consents or their guardian's approval according to rules of the Medical Research Ethics Committee at the National Research Centre (number: 10-172).

Patients were subjected to detailed registration sheet, which was designed to include personal history, pedigree construction, clinical and genetic history, with special emphasis on family history of hereditary blood disease. The results of all investigations and follow-up were included in the sheet, including anthropometric measurements; height, head circumference, and weight with pubertal assessment.

Cardiac evaluation was performed by clinical assessment and Doppler echocardiographic evaluation of patients and controls.

Molecular analysis methods

Blood samples were collected by a well-trained nurse from each thalassemic patient. Overall, 5 ml of venous blood sample was obtained from each patient into an EDTA tube with smooth shaking. Whole blood samples were used for DNA extraction by salting out method [7,8]. DNA was then stored at −20°C for PCR.

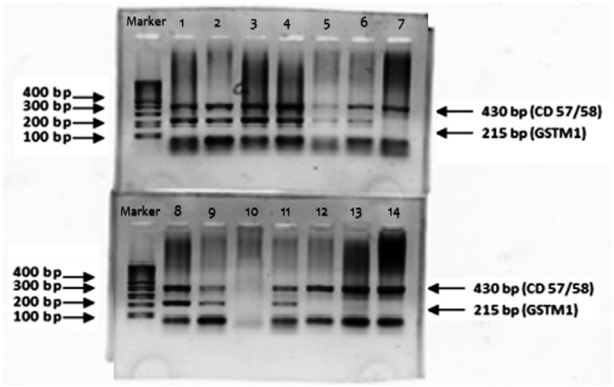
Detection of GSTM1 gene polymorphism was done by PCR technique using the following sequence specific primers: forward: 5′GAA CTC CCT GAA AAG CTA AAG C-3′ and reverse: 5′GTT GGG CTC AAA TAT ACG GTG G-3′. The housekeeping gene Cd 57/58 was simultaneously detected using sequence specific primers as follows: forward: 5′-ATG TGG AGA CAG AGA AGA CTC TTG GGT T-3′ and reverse: 5′-TCA TTC GTCTGT TTC CCA TTC TAA AC-3′. A total volume of 25 µl, including 1 µl DreamTaq DNA polymerase (5 U/µl) (Thermo Fisher Scientific Co., 168 Third Avenue Waltham, MA, USA), 2.5 µl dntps mix (Jena Bioscience GmbH, Jena, Germany), 2.5 µl of forward and reverse primers of both target and housekeeping gene, 2.5 µl 10x DreamTaq buffer (Thermo Fisher Scientific Co.), 5 µl Q-solution 5× (Qiagen, Strasse 1 40724 Hilden Geschäftsführer, Germany), and 3 µl DNA sample in addition to 1 µl nuclease free water, was incubated from the ice directly into the thermocycler (Bio-RAD-PTC-100; 6259 Progressive Avenue, Suite 300, San Diego, CA, USA) at 95°C for 5 min and subjected to 30 cycles of 95°C for 60 s, 60°C for 60 s and 72°C for 60 s, then a final 72°C extension for 5 min. Next, PCR aliquots were electrophoresed using the gel electrophoresis apparatus (Consort EV215-Anachem; 64 Boston Rd, Leicester LE4 1AW, UK) at 120 volts and 80 mA for 20 min on 2% agarose gel (Bioshop, Burlington, Ontario, Canada), stained with ethidium bromide. With the aid of a DNA ladder (100 bp) (Jena Bioscience GmbH), the internal standard fragment of housekeeping gene was identified at 430 bp in length, whereas the amplified gene products of GSTM1 were 215 bp as visualized under UV light using MD25K UV transilluminator (Wealtec, Corp., 1885 Meadowvale Way Sparks, NV, USA).

Transthoracic color Doppler echocardiography for detection of cardiac problems

Transthoracic color Doppler echocardiography was done using a Hewlett-Packard 5500 SONOS (Davis Medical Electronics, Inc., 2441 Cades Way, Building 200 Vista, California, USA) ultrasonic machine phased array sector scanner with 4–8 mHz probes according to age. The patients were examined in the left lateral decubitus position.

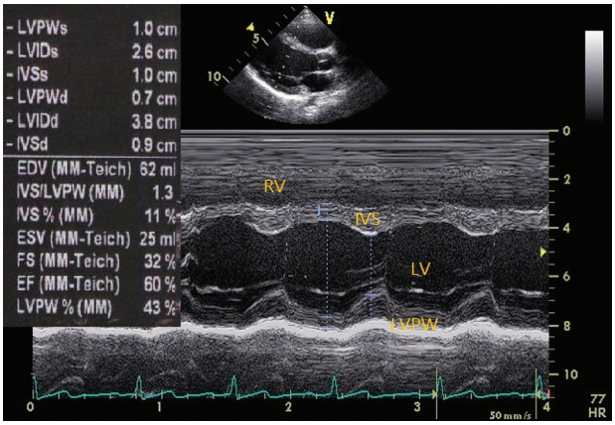
M-mode measurements were performed according to the American Society of Echocardiography in the parasternal long-axis view (Fig. 1). LV end diastolic diameter, LV end systolic diameter, interventricular septum, posterior wall, and calculation of fractional shortening as an indicator of LV systolic function were done according to the American Society of

Figure 1



Two gels for electrophoretic separation of PCR product for GSTM1, exhibiting a 215-bp fragment. Marker is a 100-bp DNA ladder (Jena Bioscience Gmbh, Germany). In the upper gel, lane 1: positive control; lanes 2,3, 4, 5, and 6: positive bands for GSTM1 (215 bp), housekeeping gene (430 bp); and lane 7: negative bands for GSTM1 (215 bp) (GSTM1 null genotype), positive for housekeeping gene (430 bp). In the lower gel, lane 8: positive control; lane 10: negative control; lanes 9 and 11: positive bands for GSTM1 (215 bp), housekeeping gene (430 bp); and lanes 12, 13, and 14: negative bands for GSTM1 (215 bp). (GSTM1 null genotype), positive for housekeeping gene (430 bp). GSTM1, glutathione S-transferase M1.

Figure 2



M-mode echocardiographic measurement. EF, ejection fraction; FS, fractional shortening; IVSd, intraventricular septal width; LVPW, left ventricular posterior wall; LVID, left ventricular internal dimension.

Echocardiography [9]. A fractional shortening of the left ventricle of less than 28% was considered abnormal [10].

Pulsed Doppler right and left ventricles inflow recordings were performed in the apical four-chamber view, by placing the sample volume at the tricuspid and mitral valve tips level, respectively (Fig. 2). E (early) and A (late) peak velocities (m/s) and their ratio were measured as an index of right ventricular (RV) global diastolic function [11].

The ratio of peak early to late diastolic filling velocity (E/A ratio) is the simplest and most commonly used index to assess diastolic filling. Individuals with low E/A (<1) are considered to have impaired early diastolic relaxation, and those with high E/A (>2) have a restrictive filling pattern [12].

RV myocardial performance index (MPI) was calculated by the following formula: the sum of the isovolumetric contraction time (ICT) and isovolumetric relaxation time (IRT) divided by the ejection time (ET) [MPI=(ICT+IRT)/ET] [13].

The normal mean value of RV MPI is 0.28+0.04 [14]. We used RV MPI greater than 0.32 as a cutoff value to indicate global RV dysfunction in this study.

The mean normal value of the MPI or Tei index is 0.39 ±0.05 for the LV. Higher index values correspond to more pathological states with overall cardiac dysfunction [15].

Statistical analysis

Data were expressed as median with range (minimum–maximum). Statistical significance of the difference was analyzed using Statistical Package for the Social Sciences version 18. The nonparametric Mann–Whitney *U*-test was used for comparison of medians. *P* values of less than 0.05 were considered statistically significant.

Analysis of ECHO data was also performed using SPSS 18 (Statistical Package for the Scientific Studies) for Windows. Description of variables was presented as follows:

Description of quantitative variables was in the form of mean, SD, and range with minimum and maximum.

Description of qualitative variables was in the form of numbers and percentage.

Comparisons between quantitative variables were done data were tested for normality using Kolmogorov–Smirnov test of normality. The results of the test showed that the data were normally distributed; the Student's *t*-test was then used for the comparisons of means between groups. χ^2 test was used to detect correlations between qualitative variables. *P* value less than 0.05 was statistically significant.

The rate of blood transfusion was categorized as follows: mild if less than 1/month, moderate if = 1/month and severe if more than 1/month. A χ^2

correlation test was done to test the correlation between rate of blood transfusion and cardiac performance through the LV MPI, LV E/A ratio and the fractional shortening, RV MPI, and RV E/A ratio. Moreover, a χ^2 correlation was done between the gene expression and the left and RV MPI and E/A ratios, and the FS result. Student *t*-test was done to compare groups of patients and their results.

Results

Genetic and basic clinical findings in correlation to GSTM1 genotype are summarized in Table 1. The study included 16 patients descending from 16 pedigrees referred from different Egyptian governorates. Parental consanguinity was positive in 69% of them. They were 6 males and 10 females, and their ages at presentation ranged from 5 and 28 years, with a median age of 12.13 years. Overall, 75% of patients showed mongoloid facies, and hepatosplenomegaly (HSM) was detected in 25% of patients with cardiac complications.

PCR results

Seven (43.7%) patients had the functional wild-type allele (GSTM1 non-null genotypes), whereas nine (56.2%) patients were homozygous for the GSTM1 null allele (GSTM1 null genotype).

Results revealed a statistically significant association between GSTM1 polymorphism non-null genotype in β-thalassemic patients with cardiac complications and HSM ($P<0.05$), whereas, no significant difference was found in correlation to neither sex nor consanguinity or mongoloid features ($P>0.05$).

Moreover, Table 2 presents the hematological profile of patients showing the median and range of hematological parameters as well as age.

Table 1 Comparison between basic clinical data in β-thalassemic patients with cardiac complications using χ^2 correlation test

Parameters	Total (*n*=16)	Gene expression subgroups [*n* (%)]		*P* value
		GSTM1 negative (*n*=9)	GSTM1 positive (*n*=7)	
Sex				
Female	10 (62.5)	5 (50)	5 (50)	0.515
Male	6 (37.5)	4 (66.7)	2 (33.3)	
Consanguinity				
Negative	5 (31)	3 (60)	2 (40)	0.838
Positive	11 (69)	6 (54.5)	5 (45.5)	
Mongoloid facies				
Negative	4 (25)	3 (75)	1 (25)	0.383
Positive	12 (75)	6 (50)	6 (50)	
HSM				
Negative	12 (75)	5 (41.7)	7 (58.3)	0.042*
Positive	4 (25)	4 (100)	0	

GSTM1, glutathione S-transferase M1; HSM, hepatosplenomegaly. *Statistically significant ($P<0.05$). **High Statistical significance ($P<0.01$).

Table 2 Comparison between hematological parameters in β-thalassemic patients with cardiac complications using Mann–Whitney test

Parameters	Total (*n*=16) [median (minimum–maximum)]	Gene expression subgroups [median (minimum–maximum)]		*P* value
		GSTM1 negative (*n*=9)	GSTM1 positive (*n*=7)	
Age (years)	12.13 (6.83–25.00)	14.00 (8.00–25.00)	9.50 (6.83–21.75)	0.314
Rate of blood transfusion (days)	25.00 (10.00–60.00)	20.00 (10.00–30.00)	60.00 (20.00–60.00)	0.005**
Hb (g/dl)	6.65 (4.60–10.20)	6.80 (5.50–7.80)	6.50 (4.60–10.20)	0.874
RBCs ($\times 10^{12}$/l)	3.00 (2.50–3.70)	3.00 (3.00–3.50)	3.00 (2.50–3.70)	0.956
MCV (fl)	67.50 (52.00–83.00)	65.00 (52.00–83.00)	70.00 (61.40–73.50)	0.958
Retics (%)	3.75 (2.50–12.50)	3.30 (2.50–12.50)	4.00 (3.20–9.30)	0.367
A_2 (%)	2.70 (0.30–7.80)	2.70 (0.30–7.80)	2.70 (2.00–4.00)	0.749
F (%)	2.50 (0.00–12.00)	6.90 (0.00–12.00)	2.40 (0.00–9.80)	0.231

GSTM1, glutathione S-transferase M1; Hb, hemoglobin; MCV, mean corpuscular volume; RBC, red blood cell. *Statistically significant ($P<0.05$). **High Statistical significance ($P<0.01$).

Results revealed that the rate of blood transfusion showed a statistically significant increase regarding GSTM1 null genotype (median was 20 days) compared with GSTM1 non-null genotype (median was 60 days) in β-thalassemic patients with cardiac complications. However, no significant difference was encountered comparing the two subgroups regarding other hematological parameters and age.

Echocardiographic data

Table 3 summarizes the Doppler Echocardiography measurements (mean±SD) in patients. Results showed statistically significant increase regarding the LV E/A in GSTM1 null genotype (median was 2.1) when compared with GSTM1 non-null genotype (mean±SD was 1.78) in β-thalassemic patients with cardiac complications.

In the same context, findings revealed a statistically significant increase in RV E/A in GSTM1 null genotype (median was 1.7) when compared with GSTM1 non-null genotype (mean±SD was 1.28) in β-thalassemic patients with cardiac complications. On the contrary, no significant difference between GSTM1 null genotype and GSTM1 non-null genotype in other echocardiographic was revealed.

However, these findings show that LV and RV diastolic function has been significantly affected in participants with GSTM1 null genotype in β-thalassemic patients with cardiac complications.

In β-thalassemic patients with restrictive LV filling (LV E/A >2), the rate of blood transfusion was significantly increased ($P<0.05$) on comparing it with β-thalassemic patients with normal LV E/A=1–2. (Table 4). On the contrary, no significant difference ($P>0.05$) was revealed regarding blood transfusion rate with other parameters.

These results demonstrate that LV diastolic function has been significantly impaired in cases experiencing frequent blood transfusion in β-thalassemic patients with cardiac complications.

Discussion

In this study, basic clinical characteristics of patients showed positive consanguinity in 69% of cases in addition to mongoloid facies in 75% and frequent negative HSM in 75% in β-thalassemic patients with cardiac complications.

Nevertheless, our findings revealed statistically significant increase of HSM in GSTM1 null genotype ($P<0.05$) as compared with GSTM1 non-null genotype in β-thalassemic patients with cardiac complication, yet no significant difference neither in sex nor consanguinity or mongoloid facies ($P>0.05$).

Our results are in concordance with Manzon [16], who documented characteristic changes in facial bones in thalassemic patients including frontal bossing and thickening and roughening of maxilla and zygoma. These changes cause mongoloid appearance as shown by prominent cheekbones and hypertelorism. Our findings came in agreement as well with Qurat-ul-

Table 3 Comparison between echocardiographic measurements in β-thalassemic patients with cardiac complications using Mann–Whitney test

Parameters	Total (*n*=16) [median (minimum–maximum)]	Gene expression subgroups [median (minimum–maximum)]		*P* value
		GSTM1 negative (*n*=9)	GSTM1 positive (*n*=7)	
Ao	2.10 (1.70–3.00)	2.10 (1.80–3.00)	2.00 (1.70–3.00)	0.219
LA	2.80 (1.80–3.90)	2.80 (2.50–3.90)	2.60 (1.80–3.70)	0.285
RV	2.50 (1.60–4.10)	2.65 (2.20–4.10)	2.50 (1.60–3.50)	0.855
PA	1.70 (1.30–2.80)	1.70 (1.60–2.20)	1.75 (1.30–2.80)	0.926
IVS	0.75 (0.60–1.00)	0.80 (0.60–1.00)	0.70 (0.70–0.90)	0.622
PW	0.85 (0.50–2.30)	0.90 (0.55–2.30)	0.80 (0.50–1.10)	0.957
LVED	4.60 (2.70–6.10)	4.70 (4.20–6.10)	4.00 (2.70–5.30)	0.100
LVES	2.70 (1.10–4.10)	2.90 (1.10–4.10)	2.60 (1.80–3.40)	0.339
FS	0.32 (0.26–0.48)	0.32 (0.27–0.45)	0.32 (0.26–0.48)	0.916
Left ventricular MPI	0.40 (0.01–0.85)	0.40 (0.25–0.85)	0.35 (0.01–0.60)	0.368
Left ventricular E/A	2.00 (1.20–3.00)	2.10 (1.70–3.00)	1.78 (1.20–2.10)	0.017*
Right ventricular MPI	0.25 (0.03–0.60)	0.24 (0.15–0.60)	0.26 (0.03–0.40)	0.314
Right ventricular E/A	1.55 (0.38–2.40)	1.70 (0.60–2.40)	1.28 (0.38–2.25)	0.050*

E/A, early to late diastolic filling velocity; FS, fractional shortening; GSTM1, glutathione S-transferase M1; IVS, intraventricular septal width; LA, left arterial; LVED, left ventricular end-diastolic diameter; LVES, left ventricular end-systolic diameter; MPI, myocardial performance index; PA, posterior aortic; PW, posterior wall; RV, right ventricle. *Statistically significant ($P<0.05$). **High statistical significance ($P<0.01$).

Table 4 Comparison between rate of blood transfusion and echocardiographic data in β-thalassemic patients with cardiac complications using χ^2 correlation test

Rate of blood transfusion	Mild <1/month [*n* (%)]	Moderate =1/month [*n* (%)]	Frequent >1/month [*n* (%)]	*P* value
Left ventricular MPI				
≤0.39	3 (37.5)	0	5 (62.5)	0.440
>0.39	4 (50)	1 (12.5)	3 (37.5)	
Left ventricular E/A				
<1	0	0	0	0.002*
1–2	7 (77.8)	1 (11.1)	1 (11.1)	
>2	0	0	7 (100)	
FS				
<28%	1 (50)	0	1 (50)	0.922
>28%	6 (42.9)	1 (7.1)	7 (50)	
Right ventricular MPI				
≤0.32	5 (45.5)	1 (9.1)	5 (45.5)	0.732
>0.32	2 (40)	0	3 (60)	
Right ventricular E/A				
<1	3 (100)	0	0	0.130
1–2	4 (40)	1 (10)	5 (50)	
>2	0	0	3 (100)	

E/A, early to late diastolic filling velocity; FS, fractional shortening; MPI, myocardial performance index. *Statistically significant ($P<0.05$). **High statistical significance ($P<0.01$).

Ain *et al.* [17], who found that total consanguinity rate among parents of β-thalassemic children was 77.39%.

Regarding routine investigations, the present results revealed decrease in hemoglobin, mean corpuscular volume, and red blood cell (RBCs) in β-thalassemic patients compared with the reference ranges. In the same context, reticulocytes were significantly high causing statistically significant increase between the two groups, whereas no significant figures were recorded regarding sex and age.

Findings expected in thalassemic patients as normal Hb synthesis is impaired as formerly affirmed in 2011 by Abdalla. Moreover, we stated significant increase in the rate of blood transfusion in GSTM1 null genotype (median=20.00 days) when compared with GSTM1 non-null genotype (median=0.00 days) in β-thalassemic patients with cardiac complications. On the contrary, no significant difference in other hematological results was revealed between the two groups (GSTM1 null genotype and GSTM1 non-null genotype) in β-thalassemic patients with cardiac complications [18].

This came in agreement with Sharma *et al.* [19] who recommended that necessity of more blood transfusions in participants with GSTT1/M1 gene deletions suggest that the deletion probably has a negative effect on RBCs survival. Our findings are also in agreement with a former Egyptian study conducted by Soliman *et al.* [20] in 2014 who demonstrated that children with β-TM and GST deletion seem to experience enhancement in RBC destruction and hence require RBC transfusion. Accordingly, iron overload occurs as evidenced by high concentration of serum ferritin. Moreover, Chakarov *et al.* [21] suggested that the absence of GST isoenzymes, especially GST-mu due to GSTM1 null genotype, might lead to a major augmentation of iron entry into heart.

Although agreed with some of our findings, Sharma *et al.* [19] in 2010 proved that GSTT1 or GSTM1 gene deletions alone exert no significance on iron overload, unlike our findings that demonstrated a role of GSTM1 gene deletion on cardiac iron overload.

Analysis of ECHO findings of β-thalassemic patients with cardiac complications revealed statistically significant increase in LV E/A in GSTM1 null genotype (median=2.1) when compared with GSTM1 non-null genotype (median=1.78) and RV E/A in GSTM1 null genotype (median=1.7) in comparison to GSTM1 non-null genotype (median=1.28). On the contrary, no significant difference was stated between GSTM1 null genotype and GSTM1 non-null genotype regarding other echocardiographic data.

These findings signify that LV and RV diastolic function was extensively affected in cases with GSTM1 null genotype in β-thalassemic patients with cardiac complications.

This agrees with a study by Chakarov *et al*. [21], which suggested that myocardial damage may increase and hence heart functions may be extensively affected as a result of decreased expression or absence of enzymes related to antioxidant defense (GST enzymes).

Regarding the diastolic indices, increased E/A has previously been discussed as a sign of early impairment of cardiac function in thalassemia patients having normal systolic function. However, it is obvious that the pathophysiology of LV failure of the dilated type is multifactorial, with a considerable involvement of immune-inflammatory and inherited factors. It is worth noting that, myocardial iron deposition does not affect LV relaxation; it directly leads to LV myocardial diastolic dysfunction, as expressed as an echo-Doppler restrictive pattern [22]. Moreover, in concordance with our results, Bay *et al*. [23] stated that heart disease is chiefly expressed by a cardiomyopathy that increasingly leads to heart failure and death.

These statements were previously explained by Origa [24]. They stated that when GSTM1 is deleted in the presence of iron accumulation, the entry of iron into the heart notably increases. This might be owing to the difference in permeability among the divalent cations. However, his hypothesis is supported by the finding that L-type Ca^{2+} channels are high-capacity pathways of ferrous iron uptake into cardio-myocytes under iron overload situations.

Phulukdaree *et al*. [25] added proof that, the GSTM1 null genotype is linked with coronary artery disease in young South Africans of Indian origin. Although findings demonstrated a considerable association between null polymorphism of GSTM1 and CHD risk, no remarkable association between variation in GST genotypes and CHD risk was noticed when the integrated studies were stratified by control source [26]. In fact, a single deletion of only one gene GSTT1 or GSTM1 had no important effect on cardiac overload [6].In our study, the rate of blood transfusion was appreciably enhanced ($P<0.05$) in β-thalassemic patients with restrictive LV filling (LV E/A >2) in comparison with β-thalassemic patients with normal LV E/A=1–2. On the contrary, no considerable change was noted ($P>0.05$) in the rate of blood transfusion on LV MPI, RV E/A, and RV MPI.

These results indicate that LV diastolic function was considerably impaired in cases with frequent blood transfusion in β-thalassemic patients with cardiac complications. This agrees with a study conducted by Origa *et al*. [24], who noted that the heart is the target lethal organ for iron accumulation in TM and that the major cause of death in transfusion-dependent patients is heart failure secondary to iron overload.

Noori *et al*. [27] agreed with our results, where they discovered that, although there was a disturbance in the left and right diastolic functions in participants with TM, only some of the echocardiographic parameters of the left side considerably changed. Proper understanding of these differences could allow better assessment of patients with TM with a view to an early recognition of cardiac involvement and early treatment initiation before disease progression.

Bosi *et al*. [28], in contrary to our findings, noted no change in LV compliance in the early stage of the disease.

However, in 2011, Montazare Lotfe Elahi *et al*. [29] noticed early cardiac dysfunction in β-thalassemic patients with chronic iron overload. A similar statement was reported by Saha *et al*. [30], who stated that restrictive diastolic LV filling might theoretically signify an early indication of iron-induced cardiomyopathy, which may progress to heart failure in thalassemic patients.

Conclusion

In conclusion, thorough follow-up of patients with β-thalassemia and proper understanding of echocardiographic changes could allow better assessment of patients with a view to an early recognition of cardiac involvement and early treatment initiation before disease progression.

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Conflicts of interest

There are no conflicts of interest.

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