Clinical Investigations

Early Detection of Left Ventricular Dysfunction with Strain Imaging in Thalassemia Patients

Address for correspondence:
Beste Ozben, MD
Yildiz Caddesi Konak
Apartmani No: 43/24
34353 Besiktas/Istanbul, Turkey
bestes@doctor.com,
besteozben@yahoo.com

Ahmet Kaya Bilge, MD; Engin Altinkaya, MD; Beste Ozben, MD; Figen Pekun, MD; Kamil Adalet, MD; Selim Yavuz, MD
Istanbul University, Istanbul Faculty of Medicine, Department of Cardiology (Bilge, Adalet), Istanbul, Turkey; Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine (Altinkaya), Istanbul, Turkey; Marmara University, Faculty of Medicine, Department of Cardiology (Ozben), Istanbul, Turkey; Okmeydani Teaching and Research Hospital, Department of Pediatrics (Pekun), Istanbul, Turkey; Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Hematology (Yavuz), Istanbul, Turkey

ABSTRACT

Background: Iron-mediated cardiomyopathy is the main cause of death in thalassemia patients. Early detection of cardiac abnormalities is important as aggressive chelation therapy may improve prognosis in these patients. The aim of this study is to evaluate left ventricular (LV) functions by tissue velocity imaging (TVI) and strain imaging (SI) in thalassemia patients without overt heart failure.

Methods: At total of 32 patients with β-thalassemia major (mean age: 24.2 ± 8.0 y, 22 male) and 25 healthy controls (mean age: 22.8 ± 2.1 y, 20 male) were included. Conventional echocardiography, TVI, and SI were performed on all subjects. Tissue velocity imaging and SI measures included peak systolic, early and late diastolic myocardial velocities, peak systolic strain and strain rate, and early and late diastolic strain rate at the basal segments of the LV lateral and septal walls.

Results: There were no significant differences in LV ejection fraction and fractional shortening between the groups. However, systolic myocardial velocity of the lateral wall and systolic strain and strain rate of the septal and lateral walls were significantly lower in thalassemia patients. There was a significant negative correlation between LV mass index and systolic myocardial velocity of the lateral wall ($r = -0.29$, $P = .045$) and septal systolic strain ($r = -0.45$, $P < .001$).

Conclusion: Thalassemia patients have regional systolic dysfunction in the LV lateral and septal walls, even if they do not have overt heart failure. Strain imaging is helpful in early detection of LV systolic dysfunction in thalassemia patients.

Introduction

The occurrence of thalassemia is from the mutation of genes encoding hemoglobin α and β chains. Thalassemia patients have extra vasal hemolysis and ineffective erythropoiesis resulting in severe anemia. Thus they require regular blood transfusions, which results in iron overload. Patients receive between 0.3 and 0.5 mg/kg/d of iron through transfusions.[1] Thalassemia patients absorb more iron than normal individuals. Iron overload results in iron deposition in a variety of parenchymal tissues including the heart, liver, gonads, and pancreas in these patients.

Iron is an essential component of hemoglobin, myoglobin, and enzymes necessary for normal cell growth and proliferation. It serves as a biological catalyst for critical redox reactions necessary for energy production. Iron is stored in cells in the form of ferritin, hemosiderin, and free iron. In excess concentrations, free iron in the cells is highly toxic and stimulates formation of free radicals, which results in peroxidative damage of membrane lipids and proteins provoking cellular injury.[1]

In the heart, excess free iron leads to impaired function of the mitochondrial respiratory chain, which is clinically manifested by the reduction of cardiac muscular contractility, progressive systolic dysfunction, and development of heart failure.[2] Additionally, increased intracellular ferrous iron inhibits the ryanodine sensitive calcium channels of the sarcoplasmic reticulum, which modulates calcium release, resulting in further reduction of cardiac function, conduction problems, and arrhythmia development.[3]

Iron-mediated cardiomyopathy is the main cause of death in thalassemia patients.[4,5] Anemia causes enlargement of the ventricular chambers, increased cardiac output, and reduced total vascular resistance. Cardiac iron overload due to long-term transfusion causes further chamber dilation, decreased contractility, and arrhythmia. Aggressive

Received: November 16, 2008
Accepted: January 18, 2009

chelation therapy may prevent, delay, or even reverse myocardial dysfunction.

Many thalassemia patients remain asymptomatic until decompensation occurs. However, once overt heart failure is present, only 50% of patients survive.[6] Therefore, early recognition of thalassemia patients at risk of heart failure is needed. The conventional echocardiographic parameters such as left ventricular ejection fraction (LVEF) or fractional shortening (LVFS) are not sensitive enough to detect subclinical cardiac dysfunction. Global ventricular function and exercise capacity may remain normal until late in the disease process.[6]

Recent tissue Doppler imaging modalities including tissue velocity imaging (TVI) and strain imaging (SI) have proven to be very sensitive for the assessment of myocardial dysfunction.[7] The aim of this study is to evaluate left ventricular (LV) functions by TVI and SI in thalassemia patients without overt heart failure and explore the value of these imaging modalities in the early detection of myocardial dysfunction.

Methods

Study Population

The investigation conforms to the principles outlined in the Declaration of Helsinki. The study was approved by the local ethics committee. All participants gave informed consent.

A total of 32 patients with β-thalassemia major (mean age: 24.2 ± 8.0 y, 22 male) and 25 healthy controls (mean age: 22.8 ± 2.1 y, 20 male) were included. Thalassemia patients were selected among cases followed by the Hematology Department of Istanbul Faculty of Medicine and Okmeydani Teaching and Research Hospital. The diagnosis of thalassemia was based on hemogram, blood smear, hemoglobin electrophoresis, and clinical evaluation. All patients needed 10 to 20 units of blood transfusion per year and were under chelation therapy with an oral iron chelator (deferiprone, 75 mg/kg/d) and a parenteral iron chelator (deferoxamine, 40 mg/kg 2 days a week). Serum ferritin levels were noted as ng/mL. None of the patients had significant congenital, rheumatic, or ischemic heart disease or overt heart failure. None of them were using cardiovascular medication including a β-blocker, calcium channel blocker, angiotensin-converting enzyme inhibitor, or diuretic.

Echocardiographic Examination

All patients and controls underwent echocardiographic examination by General Electric Vivid 7 Expert (GE Vingmed Ultrasound, Horten, Norway) echocardiography device. Echocardiographic examinations were evaluated by a single blinded cardiologist. In addition to conventional echocardiographic parameters, TVI and SI parameters were also evaluated. All patients were in sinus rhythm at the time of examination. Measurements were performed on 3 consecutive heart beats and an average of the 3 measurements was calculated.

Left atrial diameter (LA), LV end-diastolic diameters (LVED), LV end-systolic diameters (LVES), diastolic interventricular septal thickness (IVSd), systolic interventricular septal thickness (IVSs), diastolic posterior wall thickness (PWd), systolic posterior wall thickness (PWs), LVFS, and LVEF (calculated by the Teichholz method[8]) were measured. LV mass (LVM) was calculated as $1.04 \times [(\text{LVED} + \text{IVSd} + \text{PWd})^3 - (\text{LVED})^3] \times 0.8 + 0.6$ while LVM index (LVMi) was calculated as LVM/body surface area. Conventional Doppler echocardiography was performed to obtain early and late transmitral diastolic velocities (E and A velocities) and their ratio (E/A) deceleration time of the early transmitral diastolic flow (DT).

Color coded tissue Doppler data were acquired and stored as cine-loops. Both TVI and SI measures were obtained from off-line analysis of stored loops by using custom software (Echopac, GE Systems, Oslo, Norway). Pulsed TVI analysis of the mitral annulus was performed in the apical 4-chamber view. A 5 millimeter sample volume was placed at the septal and lateral sites of the annulus. Settings were adjusted for a frame rate between 120 and 180 Hz, and a cineloop of 3 to 5 consecutive heart beats was recorded. Tissue velocity imaging measures included peak systolic myocardial velocity, early and late diastolic myocardial velocities, and their ratio at the basal segments of the LV lateral wall and septal wall. Care was taken to obtain an ultrasound beam parallel to the direction of mitral annulus motion. Strain imaging measures included peak systolic strain and strain rate, peak early and late diastolic strain rate at the basal segments of the LV lateral wall and septal wall as SI imaging is angle-dependent, and longitudinal velocities are the highest in the basal segments of the LV and diminish towards the apex. For all strain parameter measurements, the sample volume, oval and 6×12 mm in size, was placed in the basal inner half of the LV myocardium at the septum and the lateral wall to keep the angle between the Doppler beam and the endocardium smaller than 30-degrees. The narrowest possible image sector angle was used to achieve the maximum color Doppler frame rate. The frame rates ranged from 154 to 184 fps. Strain rate was expressed as 1/ s. The strain data were expressed as negative % values and measured from systolic strain curves. All TVI and SI measurements were calculated from 3 consecutive cycles and an average of 3 measurements was recorded. The intraobserver variability for strain was $3.7\% \pm 4.2\%$.

Statistical Analysis

All statistical analyses were performed by statistical software (SPSS 11.0 for Windows, Chicago, IL). Continuous variables were expressed as mean ± standard deviation. Student *t* test was used for comparison of parametric values while the Mann-Whitney test was used for comparison of

Published online in Wiley InterScience. (www.interscience.wiley.com)
DOI:10.1002/clc.20584

nonparametric data. Correlation analysis was performed by Spearman's correlation test. Linear regression analysis was modeled to determine the independent determinants of LVMi and LA, SI and TVI parameters were included into the model. A P value $<.05$ was considered statistically significant.

Results

There were no significant differences in age and gender distribution between the groups ($P = .41$ and $P = .34$, respectively). Body weight and height of the patients and controls were also similar (56.9 ± 14.2 kg vs 62.3 ± 4.4 kg, $P = .07$ and 162.2 ± 13.4 cm vs 167.1 ± 4.8 cm, $P = .09$, respectively).

Systolic and diastolic blood pressures of thalassemia patients were significantly lower than those of controls (112.0 ± 6.0 mm Hg vs 118.0 ± 5.4 mm Hg, $P < .001$ and 68.7 ± 6.5 mm Hg vs 73.0 ± 6.2 mm Hg, $P < .01$, respectively). Heart rate of thalassemia patients were significantly higher than that of controls (94 ± 9/min vs 76 ± 11/min, $P < .001$).

Mean serum ferritin level was 3096 ± 2000 ng/mL (range, 744–8700 ng/mL).

Conventional Echocardiographic Parameters

The mean values of conventional echocardiographic parameters are presented in Table 1. LA, LVED, LVM, and LVMi of thalassemia patients were significantly higher than those of controls. LVEF and LVFS did not significantly differ between groups. Both E and A velocities were significantly higher while DT is significantly lower in patients compared to controls.

TVI and SI Parameters

Tissue velocity imaging and SI measures of the thalassemia patients and controls are presented in Table 2 and 3. The peak systolic myocardial velocity of the LV lateral wall and the systolic strain and strain rate values at the basal segments of the septal and lateral walls were significantly lower in thalassemia patients compared to controls. The diastolic myocardial velocities and diastolic strain rate measures of the septal and lateral wall were not significantly different.

Correlation Analysis between LVMi and LV Function

There was a significant positive correlation between LVMi and LA ($r = 0.69$, $P < .001$) and mitral E velocity ($r = 0.50$, $P = .002$) with a negative correlation between LVMi and DT ($r = -0.29$, $P = .030$). However, there was no significant correlation between LVMi and E/A ratio ($r = 0.10$, $P = .69$), lateral early/late diastolic myocardial velocity ratio ($r = 0.15$, $P = .31$), lateral early or late diastolic strain rate ($r = 0.18$, $P = .26$ and $r = 0.10$, $P = .61$, respectively).

Table 1. Conventional Echocardiographic Parameters of the Thalassemia Patients and Controls

	Thalassemia Patients	Controls	*P* Value
LA (mm)	37.0 ± 5.4	31.5 ± 3.1	<.001
LVED (mm)	52.4 ± 7.2	48.4 ± 3.3	.013
LVES (mm)	31.0 ± 4.3	29.6 ± 3.3	NS
IVSd (mm)	10.0 ± 1.8	9.8 ± 0.8	NS
IVSs (mm)	14.6 ± 3.6	14.3 ± 2.5	NS
PWd (mm)	8.9 ± 2.1	8.6 ± 1.5	NS
PWs (mm)	14.2 ± 2.5	14.0 ± 1.4	NS
LVFS (%)	39.0 ± 6.4	38.5 ± 5.1	NS
LVEF (%)	68.5 ± 7.8	68.0 ± 6.5	NS
LVM (g)	228.0 ± 94.0	174.0 ± 25.0	.008
LVMi (g/m^2)	139.0 ± 42.0	92.0 ± 12.0	<.001
E (m/s)	1.08 ± 0.19	0.89 ± 0.11	<.001
A (m/s)	0.70 ± 0.24	0.60 ± 0.09	.042
E/A	1.7 ± 0.5	1.5 ± 0.4	NS
DT (s)	165 ± 34	183 ± 22	.028

A = late transmitral diastolic velocity; DT = deceleration time of the early transmitral diastolic flow; E = early transmitral diastolic velocity; IVSd = diastolic interventricular septal thickness; IVSs = systolic interventricular septal thickness; LA = left atrial diameter; LVED = left ventricular end-diastolic diameter; LVEF = left ventricular ejection fraction; LVES = left ventricular end-systolic diameters; LVFS = left ventricular fractional shortening; LVM = left ventricular mass; LVMi = left ventricular mass index; NS = not significant; PWd = diastolic posterior wall thickness; PWs = systolic posterior wall thickness.

There was no significant relation between LVMi and LVEF ($r = 0.17$, $P = .24$) or LVFS ($r = 0.19$, $P = .17$). However, there was a significant negative correlation between LVMi and septal peak systolic strain values ($r = -0.45$, $P < .001$) and peak systolic myocardial velocity of the LV lateral wall in thalassemia patients ($r = -0.29$, $P = .045$).

Linear regression analysis (adjusted R2: 0.565, $P < .001$) revealed that LA (standardized β: 0.534, $P < .001$) and septal peak systolic strain values (standardized β: 0.534, $P < .001$) were independently associated with LVMi. Ferritin levels did not correlate with any echocardiographic parameters including LVMi.

Discussion

Cardiac iron deposition due to long-term transfusion causes formation of free radicals and impairment in the function of the mitochondrial respiratory chain and sarcoplasmic

Published online in Wiley InterScience. (www.interscience.wiley.com)
DOI:10.1002/clc.20584

Table 2. Tissue Velocity Imaging Measures of Thalassemia Patients and Controls

	Thalassemia Patients	Controls	*P* Value
Septal systolic myocardial velocity (cm/s)	8.8 ± 1.3	8.9 ± 1.1	NS
Septal early diastolic myocardial velocity (cm/s)	12.1 ± 2.0	13.0 ± 2.5	NS
Septal late diastolic myocardial velocity (cm/s)	8.0 ± 2.2	8.2 ± 1.4	NS
Septal early/late diastolic myocardial velocity	1.6 ± 0.5	1.7 ± 0.5	NS
Lateral systolic myocardial velocity (cm/s)	9.5 ± 2.6	11.1 ± 2.1	.017
Lateral early diastolic myocardial velocity (cm/s)	16.7 ± 3.5	15.2 ± 2.6	NS
Lateral late diastolic myocardial velocity (cm/s)	8.3 ± 2.5	7.5 ± 1.7	NS
Lateral early/late diastolic myocardial velocity	2.2 ± 0.7	2.2 ± 0.5	NS

Abbreviations: NS = not significant.

Table 3. Strain Imaging Measures of Thalassemia Patients and Controls

	Thalassemia Patients	Controls	*P* Value
Septal systolic strain rate (1/ s)	0.9 ± 0.2	1.3 ± 0.2	<.001
Septal early diastolic strain rate (1/ s)	2.0 ± 0.7	2.1 ± 0.6	NS
Septal late diastolic strain rate (1/ s)	1.2 ± 0.6	1.2 ± 0.5	NS
Lateral systolic strain rate (1/ s)	0.9 ± 0.3	1.1 ± 0.2	.007
Lateral early diastolic strain rate (1/ s)	1.4 ± 0.7	1.5 ± 0.8	NS
Lateral late diastolic strain rate (1/ s)	1.2 ± 0.6	0.9 ± 0.8	NS
Septal systolic strain (%)	−19.0 ± 4.3	−24.2 ± 4.5	<.001
Lateral systolic strain (%)	−12.1 ± 5.1	−15.5 ± 4.1	.008

Abbreviations: NS = not significant. The strain data were expressed as negative % values.

reticulum, resulting in heart failure and arrhythmia.[2,3] Aggressive chelation therapy may improve prognosis and should not be delayed until development of overt heart failure.[6] Modell et al[9] reported a marked improvement in survival and reduction in deaths due to cardiac iron overload in β-thalassemia major by early identification of myocardial siderosis by cardiac magnetic resonance imaging and appropriate intensification of iron chelation treatment. Therefore, early recognition of cardiac abnormalities is essential in these patients, but not easy as global ventricular function and exercise capacity may remain normal until late in the disease process.[6]

The novel contribution of our study was the demonstration of superiority of SI over the conventional echocardiographic parameters, LVEF and LVFS, in the detection of regional myocardial function. Thalassemia patients in our study had normal LVEF and LVFS and did not have overt heart failure. However, they had lower septal and lateral systolic strain and strain rate values and lower systolic myocardial velocity of the LV lateral wall, indicating presence of regional systolic dysfunction in the septal and lateral LV walls. These wall motion abnormalities may represent an early sign of cardiac disease despite preserved global ventricular function.[4] We suggested that SI parameters might be more sensitive than conventional echocardiographic parameters. Septal systolic myocardial velocity of thalassemia patients was also lower than that of controls, but the difference was not significant. This might be explained by either the small sample size or superiority of SI over TVI in detection of early regional systolic dysfunction.

Magnetic resonance imaging[6] and acoustic densitometry using ultrasonic backscatter techniques[10] were reported to be effective in early detection of myocardial dysfunction in asymptomatic thalassemia patients. There are only 2 studies about the use of TVI and SI in the early detection of regional myocardial dysfunction in thalassemia patients.[11,12] Similar to our study, these 2 studies also demonstrated abnormal TVI and SI parameters indicating early myocardial involvement in thalassemia patients although they did not have overt clinical cardiac dysfunction.

The limitation of conventional echocardiographic parameters and the superiority of TVI and SI in the detection of subclinical cardiac dysfunction has also been demonstrated in other systemic diseases.[7,13–15] Tissue velocity imaging and SI were shown to be effective for detecting right and left ventricular myocardial involvement in patients with systemic sclerosis,[16] amyloidosis,[17] Duchenne muscular dystrophy,[18] and Fabry disease.[19] All these studies indicated that impairment of longitudinal fiber motion is a sensitive marker of early myocardial dysfunction.

There is no single perfect echocardiographic method for assessment of LV diastolic dysfunction. Transmitral diastolic velocities may be affected by age, volume load, heart rate, and anemia.[20] In our study, the transmitral Doppler parameters revealed increased E and A velocities

Published online in Wiley InterScience. (www.interscience.wiley.com)
DOI:10.1002/clc.20584

reflecting an increase in the preload state due to chronic anemia. This is in concordance with previous reports.[21,22] Similar to our study, Hahalis et al[23] reported that LV filling characteristics indicated increased preload without abnormal alteration. Although we found increased E and A velocities in thalassemia patients, the diastolic strain rate measures were not significantly different indicating absence of overt global LV diastolic dysfunction early in the disease process. Kremastinos et al[24] also showed that global LV diastolic function might be well preserved until the final stages of disease.

Henry et al[25] investigated echocardiographic abnormalities in patients at risk for myocardial iron deposition and found that LA, LVED, and LVMi were increased. Similarly in our study, LA, LVED, LVM, and LVMi were significantly higher in thalassemia patients. The increase in LV dimensions was attributed to the increased cardiac output caused by the chronic anemia in these patients.[26,27] We demonstrated a significant negative correlation between LVMi and septal peak systolic strain values and peak systolic myocardial velocity of the LV lateral wall. As increased LVMi is a parameter of cardiac involvement in the thalassemia patient, our demonstration of a significant correlation between LVMi and systolic strain and myocardial velocity is important. We did not find any significant correlations between LVMi, LVEF, and LVFS. This also suggests that systolic strain and systolic myocardial velocity are superior to LVEF and LVFS in the estimation of myocardial function.

The major limitation of our study is the small sample size and we believe that randomized and blinded studies in larger populations will help define the eventual role of SI and TVI in the determination of LV functions in thalassemia patients.

Conclusion

Thalassemia patients have early regional systolic dysfunction in the septal and LV lateral walls even if they do not have overt heart failure. Conventional echocardiographic parameters, LVEF and LVFS, do not provide adequate information about ventricular function. Strain imaging is helpful in early detection and quantitative assessment of LV longitudinal systolic functions in thalassemia patients and may provide additional data for management of thalassemia patients suspected of iron-mediated cardiomyopathy.

References

1. Aessopos A, Berdoukas V, Tsironi M. The heart in transfusion dependent homozygous thalassaemia today—prediction, prevention and management. *Eur J Haematol*. 2008;80:93–106.
2. Glickstein H, El RB, Link G, et al. Action of chelators in iron-loaded cardiac cells: accessibility to intracellular labile iron and functional consequences. *Blood*. 2006;108:3195–3203.
3. Kim E, Giri SN, Pessah IN. Iron (II) is a modulator of ryanodine-sensitive calcium channels of cardiac muscle sarcoplasmic reticulum. *Toxicol Appl Pharmacol*. 1995;130:57–66.
4. Kremastinos DT, Tiniakos G, Theodorakis GN, Katritsis DG, Toutouzas PK. Myocarditis in β-thalassemia major. A cause of heart failure. *Circulation*. 1995;91:66–71.
5. Peng CT, Chang JS, Wu KH, Tsai CH, Lin HS. Mechanisms of and obstacles to iron cardiomyopathy in thalassemia. *Front Biosci*. 2008;13:5975–5987.
6. Vogel M, Anderson LJ, Holden S, Deanfield JE, Pennell DJ, Walker JM. Tissue Doppler echocardiography in patients with thalassaemia detects early myocardial dysfunction related to myocardial iron overload. *Eur Heart J*. 2003;24:113–119.
7. Weidemann F, Strotmann JM. Use of tissue Doppler imaging to identify and manage systemic diseases. *Clin Res Cardiol*. 2008;97:65–73.
8. Teichholz LE, Kreulen T, Herman MV, Gorlin R. Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence of absence of asynergy. *Am J Cardiol*. 1976;37:7–11.
9. Modell B, Khan M, Darlison M, Westwood MA, Ingram D, Pennell DJ: Improved survival of thalassaemia major in the UK and relation to T2* cardiovascular magnetic resonance. *J Cardiovasc Magn Reson*. 2008;10:42.
10. Di Paola R, Di Guardo G, Lo Giudice P, Caruso V, Magnano C, Mangiameli S. Preclinical thalassemic cardiopathy: a study by acoustic densitometry. *Ital Heart J*. 2003;4:413–418.
11. Hamdy AM. Use of strain and tissue velocity imaging for early detection of regional myocardial dysfunction in patients with beta thalassemia. *Eur J Echocardiogr*. 2007;8:102–109.
12. Magrì D, Sciomer S, Fedele F, et al. Early impairment of myocardial function in young patients with β-thalassemia major. *Eur J Haematol*. 2008;80:515–522.
13. Nikitin NP, Witte KK. Application of tissue Doppler imaging in cardiology. *Cardiology*. 2004;101:170–184.
14. Yip G, Abraham T, Belohlavek M, Khandheria BK. Clinical applications of strain rate imaging. *J Am Soc Echocardiogr*. 2003;16:1334–1342.
15. Weidemann F, Kowalski M, D'hooge J, Bijnens B, Sutherland GR. Doppler myocardial imaging. A new tool to assess regional inhomogeneity in cardiac function. *Basic Res Cardiol*. 2001;96:595–605.
16. D'Andrea A, Stisi S, Bellissimo S, et al. Early impairment of myocardial function in systemic sclerosis: non-invasive assessment by Doppler myocardial and strain rate imaging. *Eur J Echocardiogr*. 2005;6:407–418.
17. Koyama J, Ray-Sequin PA, Falk RH. Longitudinal myocardial function assessed by tissue velocity, strain, and strain rate tissue Doppler echocardiography in patients with AL (primary) cardiac amyloidosis. *Circulation*. 2003;107:2446–2452.
18. Mertens L, Ganame J, Claus P, et al. Early regional myocardial dysfunction in young patients with Duchenne muscular dystrophy. *J Am Soc Echocardiogr*. 2008;21:1049–1054.
19. Fiuza M, Avó LB, Oliveira EI, Gonçalves S, Lopes MG. Detection of preclinical left ventricular dysfunction in Fabry disease: the contribution of tissue Doppler. *Rev Port Cardiol*. 2006;25:613–637.
20. Harrison MR, Clifton GD, Pennell AT, DeMaria AN. Effect of heart rate on left ventricular diastolic transmitral flow velocity patterns assessed by Doppler echocardiography in normal subjects. *Am J Cardiol*. 1991;67:622–627.
21. Iarussi D, Di Salvo G, Pergola V, et al. Pulsed Doppler tissue imaging and myocardial function in thalassemia major. *Heart Vessels*. 2003;18:1–6.
22. Vaccari M, Crepaz R, Fortini M, et al. Left ventricular remodeling, systolic function, and diastolic function in young adults with β-thalassemia intermedia: a Doppler echocardiography study. *Chest*. 2002;121:506–512.
23. Hahalis G, Manolis AS, Gerasimidou I, et al. Right ventricular diastolic function in β-thalassemia major: echocardiographic and clinical correlates. *Am Heart J*. 2001;141:428–434.

24. Kremastinos DT, Rentoukas E, Mavrogeni S, Kyriakides ZS, Politis C, Toutouzas P. Left ventricular filling pattern in β-thalassaemia major—a Doppler echocardiographic study. *Eur Heart J*. 1993;14:351–357.
25. Henry WL, Nienhuis AW, Wiener M, Miller DR, Canale VC, Piomelli S. Echocardiographic abnormalities in patients with transfusion-dependent anemia and secondary myocardial iron deposition. *Am J Med*. 1978;64:547–555.
26. Bosi G, Crepaz R, Gamberini MR, et al. Left ventricular remodelling, and systolic and diastolic function in young adults with β thalassaemia major: a Doppler echocardiographic assessment and correlation with haematological data. *Heart*. 2003;89:762–766.
27. Kremastinos DT, Tsiapras DP, Tsetsos GA, Rentoukas EI, Vretou HP, Toutouzas PK. Left ventricular diastolic Doppler characteristics in β-thalassemia major. *Circulation*. 1993;88:1127–1135.

Published online in Wiley InterScience. (www.interscience.wiley.com)
DOI:10.1002/clc.20584