Use of strain, strain rate, tissue velocity imaging, and endothelial function for early detection of cardiovascular involvement in patients with beta-thalassemia

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ABSTRACT

Background : Global ventricular function often remains normal in patients with beta-thalassemia major (β-TM) until late. Tissue Doppler and strain imaging may be useful to assess regional myocardial function abnormalities in these patients.

Methods : Systolic (Sm), early diastolic (Em), and late diastolic (Am) (Em/Am) myocardial velocities at basal lateral and septal left ventricular (LV) segments, strain (S), and strain rate (SR) in basal and mid LV, right ventricular (RV) and septum were measured in 30 patients (β-TM, 12.4 ± 5.2 years, serum ferritin 2603.1 μg/L) and twenty controls (12.5 ± 5.2 years). Flow-mediated dilatation (FMD) vasodilatation as a measure of endothelial function was also assessed.

Results : Patients had significantly higher LV mass index (169.45 ± 61.14 vs. 104.66 ± 24.42; P = 0.009) while global LV Sm and diastolic function was similar to controls. Patients had significantly lower lateral Em velocity, Em (10.12 ± 1.16 vs. 17.9 ± 2.11; P = 0.002), Em/Am ratio (0.811 ± 0.192 vs. 2.06 ± 0.62; $P \le$ 0.001) at the basal lateral LV, lower strain values at the basal lateral LV (19.5 ± 4.17 vs. 24.196 ± 1.81; P = 0.002), mid lateral LV (19.07 ± 3.98 vs. 25.56 ± 2.62; P = 0.042), basal septum (17.04 ± 3.44 vs. 25.43 ± 2.53; $P \le$ 0.001), and mid septum (20.49 ± 5.34 vs. 24.45 ± 2.20; P = 0.001) as compared to controls. SR at the basal and mid segment of the lateral LV wall and at the basal and mid septum was also significantly lower in patients. SR in basal and mid RV although lower was not significantly different from controls. Patients also had significantly lower FMD (7.57 ± 3.16 vs. 18.08 ± 1.9, P = 0.018) implying endothelial dysfunction.

Conclusions : Tissue Doppler, strain and SR imaging are useful to quantify regional myocardial function in asymptomatic β-TM patients with preserved global Sm and diastolic function.

Keywords : Echocardiography, strain, strain rate, thalassemia, tissue Doppler

INTRODUCTION

Cardiac involvement is common in beta-thalassemia major (β-TM) due to tissue iron overload and myocardial parenchymal damage with progressive systolic (Sm) and diastolic dysfunction of the left ventricular and/or right ventricular (LV/RV).[1,2] Although cardiac failure

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due to iron overload is the most frequent cause of death in patients with β-TM, cardiac involvement is often multifactorial secondary to iron deposition, myocarditis, immunogenic mechanisms, and endothelial dysfunction.[3-5] Conventional echocardiographic (ECG) parameters are often inadequate to detect subtle abnormalities of cardiac dysfunction in patients with β-TM since global ventricular function remains normal until late stages of the disease. Tissue velocity imaging (TVI), ventricular strain imaging (SI), and strain rate imaging (SRI) are superior to routine ECG techniques for analysis of regional and longitudinal myocardial function.[6-8] Previous studies of tissue Doppler and SI in patients with β-TM have yielded wide-ranging results.[9-13] The aims of the present prospective study were to assess conventional, TVI, SI, and SRI ECG parameters in patients with β-TM and compare them with matched controls. In addition, assessment of endothelial function was done by measuring vascular endothelial-dependent (flow mediated dilatation [FMD]) and endothelial-independent (nitrate mediated dilatation [NMD]) function of the brachial artery.

METHODS

Patients with β-TM, under routine follow-up of the Genetics Department of our institute, were included in the study following the method of the consecutive sampling. Patients with β-TM were included in the study after obtaining informed consent, and the study conformed to the institutional ethical guidelines. Age- and gender-matched healthy subjects were recruited as the control group. The diagnosis of thalassemia was based on hemogram, blood smear, hemoglobin electrophoresis, and clinical evaluation. Patients with congenital or rheumatic valvular heart disease, present or past history of heart failure, arrhythmias or those on cardiac medications (including β-blocker, calcium channel blocker, angiotensin-converting enzyme inhibitor, or diuretics) were excluded from this study. All cases underwent a detailed clinical examination to exclude any overt sign of heart failure and routine laboratory investigations, namely, hemoglobin, fasting glucose, total cholesterol, and serum ferritin levels were estimated in all. The mean serum ferritin level was derived by averaging the previous 6–8 values obtained over the past 1 year every 2 monthly while in controls additional blood samples were drawn for same.

Echocardiographic evaluation

Detailed ECG was performed using a GE Vivid 7 ECHO machine (GE Healthcare, Waukesha, WI, USA) by an operator who was blinded to the clinical data. Conventional echo-Doppler parameters including LV end-diastolic dimensions and LV end-diastolic volumes, LV end-systolic dimensions and LV end-systolic volumes,

interventricular septal thickness (IVST), LV posterior wall thickness (LVPWT), LV fractional shortening (LVFS), LV stroke volume (LVSV), and LV ejection fraction (LVEF) were measurements according to the American Society of Echocardiography guidelines.[14] The LV dimensions were obtained from M-mode parasternal long-axis views while LV volumes were obtained from the apical four- and two-chamber views using standard transducer positions. Using the modified Simpson's rule, ejection fraction (EF) was automatically calculated as the difference between end-diastolic volume and end-systolic volume normalized to end-diastolic volume. The corrected LV mass (g) was calculated as 0.8 (1.04 ([LVEDD + LVPWT + IVST]^3 – [LV internal diameter in diastole]^3)) +0.6 and indexed LV mass index (LVM) as LVM/body surface area.[15] The LV end-systolic (meridional) stress was calculated as 0.334 P × end-systolic diameter (ESD)/h (1 + h/ESD), where *P* is LV peak pressure, h is the end-systolic posterior wall thickness, and ESD is the end-systolic internal dimension.[16] LV work (in mm Hg × L/min) was calculated as LVSV times heart rate times arterial blood pressure (BP) at end systole (Pes), which was calculated as follows: Pes = 2/3 systolic BP + 1/3 diastolic BP measured by sphygmomanometer.[17,18]

Diastolic Doppler parameters were recorded including early and late transmitral diastolic velocities (Evel and Avel) and their ratio (E/A) and early deceleration time (EDT) of the transmitral diastolic flow. Tei index or myocardial performance index (MPI), an ECG Doppler load independent index of combined Sm and diastolic function, was calculated as isovolumic relaxation time plus isovolumic contraction time divided by ejection time.[19] Measurements were performed over three heartbeats, and an average of the three measurements was taken.

Tissue velocity imaging measures

TVI analysis of the mitral annulus was performed in the apical four-chamber window. A 5 mm sample volume was placed at the desired area of interest and Sm velocities at the basal segments of the lateral LV wall (LV-Sm), septal wall (septal-Sm), early and late diastolic myocardial (Am) velocities (Em, Am), and their ratio (Em/Am) of the same basal segments of lateral LV wall (i.e., LV-Em, LV-Am, and LV-Em/Am for the basal lateral LV segment) and septal wall (septal-Em, septal-Am and septal-Em/Am for the basal septal segment) were also recorded.

Strain imaging and strain rate imaging measures

The apical four-chamber view was used to record the cardiac cycle in tissue Doppler imaging mode at a frame rate of more than 100/s following which 2 mm volume samples were placed at four segments of the LV and two segments of the RV in apical four chamber view for strain analysis. Strain and SRI measures included longitudinal systolic strain and strain rate (SR) at the

basal and mid segment of the lateral LV wall (S-LV-Basal, S-LV-Mid and SR-LV-Basal, SR-LV-Mid), basal and mid septum (S-septal-basal, S-septal-mid and SR-septal-basal, SR-septal-mid), and basal and mid-RV (S-RV-Basal, S-RV-Mid and SR-RV-Basal, SR-RV-Mid). The TVI, strain, and SRI measures were obtained from the offline analysis of the stored loops. Strain values were expressed as the negative percentage values, and the SR was expressed as negative 1/s values.

All above measurements were made from three consecutive beats at end expiration, and results were averaged to get the final value. End-diastole was defined by the peak of the R, and the end systole was defined by the end of the T-wave on the ECG.

The intraobserver variability for strain parameters was observed to be 3.1% ± 2.2% while the interobserver variability was 5.7% ± 3.1%.

Vascular studies

The arterial endothelial function of the brachial artery was assessed noninvasively by ultrasound examination of the vasodilation response to endothelium-dependent and -independent stimuli, as previously reported.[20] The right brachial artery, proximal to the antecubital fossa, was imaged longitudinally using the linear-array transducer. Flow-mediated endothelium-dependent (FMD) vasodilatation was assessed by measuring the brachial artery diameter at baseline and during reactive hyperemia. Reactive hyperemia was induced by deflating a cuff previously inflated to 300 mm Hg for 4.5 min in the forearm. Arterial flow velocity was measured at baseline and during reactive hyperemia using pulsed-wave Doppler. After 10–15 min, the endothelium-independent response (NMD) was assessed by the change in artery diameter at 3–4 min after a 5 mg dose of sublingual glyceryl trinitrate. The parameters were measured for three consecutive cardiac cycles, and the average was taken.

Carotid intimal medial thickness

For the measurement of carotid intimal medial thickness (CIMT), the subject was made to lie supine with the neck turned contralaterally to the side being examined. The measurement was taken in the 1 cm segment of the common carotid artery (CCA) proximal to the carotid bulb which was defined as the point of the deviation of the far wall from the parallel plane of the distal CCA. The CIMT was measured by the caliper as the distance between the inner echogenic line representing the intima blood interface and the outer echogenic line representing the adventitia-media junction. Measurements were repeated three times unfreezing the image on each occasion and relocating the position of the maximal intima – media thickness. The mean value of the each set of the readings represented

the mean CIMT that was taken for the final analysis. The intraobserver variability for CIMT was 5.8% ± 3.7% while the interobserver variability was 7.6% ± 4.2%.

Statistical analysis

The data are expressed as the mean ± standard deviation statistical analysis was performed using the Statistical Package for Social Sciences, version 17, for Windows (SPSS, Chicago, Illinois, USA). The independent *t*-test and Chi-square test were used for comparison of the continuous and categorical variables between groups, respectively. To study correlations, Pearson's and Spearman's coefficients were used for continuous and categorical variables, respectively. $P < 0.05$ was considered statistically significant.

RESULTS

The study included 30 β-TM patients (23 males, mean age 12.4 ± 5.2 years) and twenty healthy subjects (13 males, mean age 12.5 ± 5.2 years). All patients were on long-term blood transfusions since the age of 6 months to 1 year, and on an average, each patient required ~10–18 transfusions per year. Chelation therapy was prescribed as decided by the Genetics Department, according to individual serum ferritin values and included either deferiprone, 75 mg/kg/d or deferoxamine, 40 mg/kg twice weekly or both. Thirteen patients (43.33%) had been splenectomized.

The baseline characteristics are summarized in Table 1 and were comparable amongst the two groups. None of the patients had hypertension, diabetes, or dyslipidemia. The mean hemoglobin was lower and serum ferritin levels significantly higher in patients than in the controls. All patients had serum ferritin level >1200 μg/L. All included patients were asymptomatic and in New York Heart Association (NYHA) functional Class I.

Conventional ECG parameters including LV dimensions, volumes, EF, fractional shortening (FS), E/A velocity, and MPI were not significantly different among the patients and controls [Table 2]. The LVEF (62.15 ± 7.96 vs. 59.94 ± 6.02, $P = 0.27$) was also similar in the two groups, and none of the patients had global LVEF <50%. Patients with β-TM had higher LV mass and lower deceleration time of the mitral inflow velocity (EDT) as compared to controls [Table 2].

Systolic and diastolic tissue velocity imaging parameters [Table 3]

The Sm and Em/Am velocities at the basal segments of the septal LV wall (septal-Sm, septal-Em, septal-Am) and their ratio septal-Em/Am although slightly lower in patients, were not statistically different among the two groups. In contrast, velocities at the basal segments of the lateral LV wall revealed significant differences in patients and

Table 1: Demographic and hematologic characteristics of the patient and the control group

Parameter	Patients	Controls	*P*
Sex (male:female)	23:7	13:7	
Age (years)	12.4±5.2	12.5±5.2	0.702
	11 (5-24)	12 (6-24)	0.992
Height (cm)	136.5±12.5	139.8±13.2	0.91
	138.5 (108-155)	138.6 (113.4-166.0)	0.532
Weight (kg)	30.58±9.36	28.08±9.90	0.377
	28.5 (15-49)	29.38 (17.2-43.06)	0.620
Body surface area (m^2)	1.079±0.202	1.00±0.291	0.33
	1.05 (0.72-1.44)	0.985 (0.51-1.65)	0.251
Heart rate	73±11	73±12	0.809
	75 (50-94)	72 (60-96)	0.843
Systolic BP	116±10	121±10	0.093
	116 (92-138)	123 (94-136)	0.047
Diastolic BP	57±11	62±11	0.155
	58 (50-90)	66 (56-80)	0.047
Mean serum ferritin (µg/L)	2603.1±203	176.4±51.83	<0.001
	2606.3 (2019-3154)	186.7 (79.04-284)	<0.001
Hemoglobin (g/dl)	13.5±1.9	14.9±0.94	0.002
	13.6 (9.0-17.3)	14.7 (13.5-16.9)	0.007
Blood sugar (mg/dl)	93.82±12.02	95.04±9.6	0.693
	97.2 (62.8-114)	94.8 (80-115)	0.789

BP: Blood pressure

controls. Although the Sm velocity at the basal portion of lateral LV wall (LV-Sm) was similar to that in controls, the early diastolic myocardial velocity (LV-Em) and the ratio of Em/Am velocity at the basal segment of lateral LV (LV-Em/Am) were significantly lower in patients.

Strain parameters [Table 4]

Values of LV strain at the basal and mid segment of the lateral LV wall (S-LV-Basal and S-LV-Mid) as well as at the basal and mid septum (S-septal-basal and S-septal-mid) were significantly lower in the patients as compared to controls. Although the strain parameters of the basal RV were also depressed in patients with β-TM (S-RV-Basal), the strain at mid RV (S-RV-Mid) was not different in comparison to controls.

Strain rate imaging [Table 5]

SRI values at the basal and mid segment of the lateral LV wall (SR-LV-Basal, SR-LV-Mid) and at the basal and mid septum (SR-septal-basal, SR-septal-mid) were all significantly lower in patients. The corresponding values of SRI at the RV basal and mid-RV segments though lesser in patients, the trend was not statistically significant.

Vascular studies

The CIMT was similar between the patients and healthy controls (0.366 ± 0.134 vs. 0.357 ± 0.112, $P = 0.3$). On the other hand, vascular endothelium-dependant FMD was significantly impaired compared with controls (7.57 ± 3.16 vs. 18.08 ± 1.9, $P = 0.018$) while endothelium-independent nitrate-mediated dilatation (NMD) was not significantly different amongst the two groups (16.6 ± 2.1 vs. 17.4 ± 3.2, $P = 0.56$). The percentage of patients with FMD <5.5% in patients versus controls was 33% (10/30) versus 0% (0/20, $P = 0.004$).

Correlation analysis for serum ferritin, flow mediated dilatation, and left ventricular mass$_i$

Serum ferritin levels had a significant positive correlation with LV mass$_i$ ($r = 0.536$, $P \leq 0.001$) and LV Am (0.663, $P \leq 0.001$) while a negative correlation was noted with DT ($r = -0.313$, $P = 0.027$), LV-Sm ($r = -0.651$, $P \leq 0.001$), LV-Em/Am ($r = -0.83$, $P \leq 0.001$), S-LV-Basal ($r = -0.566$, $P \leq 0.001$), S-LV-Mid ($r = -0.692$, $P \leq 0.001$), S-septal-basal ($r = -0.795$, $P \leq 0.001$), SR-septal-basal ($r = -0.648$, $P \leq 0.001$), SR-septal-mid ($r = -0.43$, $P = 0.002$), and SR-RV-Basal ($r = -0.601$, $P \leq 0.001$). A strong negative correlation was also seen between ferritin levels and FMD ($r = -0.895$, $P \leq 0.001$) (all $P < 0.004$).

FMD had a significant positive correlation with LV-Sm ($r = 0.545$, $P \leq 0.001$), LV Em ($r = 0.778$, $P \leq 0.001$), LV Em/Am ($r = 0.755$, $P \leq 0.001$), S-LV-Basal ($r = 0.522$, $P \leq 0.001$), S-LV-Mid ($r = 0.562$, $P \leq 0.001$), S-septal-basal ($r = 0.66$, $P \leq 0.001$), S-RV-Basal ($r = 0.51$, $P \leq 0.001$), SR-septal-basal ($r = 0.544$, $P \leq 0.001$), SR-RV Basal ($r = 0.607$, $P < 0.001$) while it had a negative correlation with serum ferritin ($r = -0.895$, $P \leq 0.001$) and LV Am ($r = -0.635$, $P \leq 0.001$) (all $P < 0.004$)

LV Mass$_i$ showed a significant positive correlation with septal Em ($r = 0.397$, $P < 0.03$), SR-septal-mid ($r = 0.37$, $P = 0.004$), and SR-RV-Mid ($r = 0.46$, $P = 0.001$).

Predictors of flow mediated dilatation [Table 6]

On univariate analysis, significant predictors of FMD included serum ferritin (odds ratio [OR] -0.004 [confidence interval (CI) -0.005–-0.004, $P \leq 0.001$]), LV mass$_i$ (OR -0.44 [CI -0.070–-0.017, $P = 0.002$]), LV-Sm (OR 2.125 [CI 1.175–3.074, $P \leq 0.001$]), LV-Em (OR 1.083 [CI 0.829–1.337, $P \leq 0.001$]),

Table 2: Conventional (systolic and diastolic) echocardiographic parameters of patients and controls

Parameter	Patients	Controls	*P*
Left atrial diameter (cm)	28.6±6.35	25.6±5.37	0.346
	29.0 (17-44)	26 (18-36)	0.193
AO diameter (cm)	21.86±3.45	23.35±3.26	0.799
	22 (15-28)	24 (16-28)	0.077
LVEDD (cm)	40.26±4.33	41.28±4.72	0.944
	41.5 (32-47)	40.5 (33-52)	0.811
LVESD (cm)	25.36±3.045	23.37±3.5	0.844
	24 (21-32)	23.00 (18.00-30.00)	0.016
IVST (cm)	8.3±1.20	7.15±1.04	0.321
	8.0 (6-10)	7.00 (5.0-9.0)	0.002
Posterior wall thickness (cm)	7.8±1.18	7.55±1.19	0.974
	8.0 (5-9)	8.0 (5.0-10.0)	0.316
LVEDV (ml)	66.16±22.84	67.37±20.078	0.365
	62.00 (35-16)	71.00 (19-115)	0.476
LVESV (ml)	25.4±10.46	30.0±15.18	0.349
	24 (10-45)	30.05 (5-53.00)	0.205
LVSV (ml)	40.76±13.95	33.75±12.29	0.445
	38 (20-71)	36.5 (8.0-54.00)	0.317
LVEF (%)	62.15±7.96	59.94±6.02	0.27
	61.1 (50-83)	58.56 (47.99-73.31)	0.342
LVFS (%)	0.367±0.06	0.358±0.07	0.261
	0.368 (0.18-0.48)	0.349 (0.21-0.52)	0.488
LV mass (g)	177.05±52.51	120.01±30.49	0.01
	169.0 (101.38-285.35)		<0.001
LV mass$_i$ (g/m^2)	169.45±61.14	104.66±24.42	0.009
	155.40 (82.69-342.80)	112.84 (61.95-146.36)	<0.001
Evel (m/s)	1.153±0.226	1.09±0.223	0.407
	1.12 (0.68-1.58)	1.16 (0.47-1.45)	0.451
Avel (m/s)	0.6866±0.176	0.695±0.228	0.152
	0.69 (0.29-1.5)	0.677 (0.24-0.99)	0.651
Evel/Avel ratio	1.76±0.474	1.714±0.451	0.722
	1.174 (0.87-3.69)	1.69 (0.82-2.47)	0.949
EDT (s)	165±34	181±20	0.012
	167.8 (93.87-221.8)	184 (138-222)	0.043
MPI (Tei Index)	0.349±0.031	0.352±0.04	0.845
	0.344 (0.30-0.40)	0.350 (0.28-0.43)	0.905
LVESS (dyn/cm^2)	98.74±21.11	94.6±7.9	0.33
	99.87 (77.1-114.5)	95.3 (86.8-103.4)	0.45
LV work (mm Hg×L/min)	292644±115811	1299328±152499	0.86
	294524 (178713-410581)	1301121 (146828-451827)	0.76

LVEDD: Left ventricular end-diastolic diameter, LVESD: Left ventricular end-systolic diameter, IVST: Interventricular septal thickness, LVEDV: Left ventricular end-diastolic volume, LVESV: Left ventricular end-systolic volume, LVSV: Left ventricular stroke volume, LVEF: Left ventricular ejection fraction, LVFS: Left ventricular fractional shortening, LV mass: Left ventricular mass, LV massi: Left ventricular mass index to body surface area, Evel: Transmitral early flow velocity, Avel: Transmitral late flow velocity, EDT: Early deceleration time, MPI: Myocardial Performance Index, LVESS: Left ventricular end-systolic stress, AO: Aortic, LV: Left ventricular

Table 3: Left ventricular tissue velocity imaging (cm/s) parameters of the patient and control groups

Parameter	Patients	Controls	*P*
Septal Sm velocity	10.84±1.09	11.08±0.956	0.409
	10.83 (8.68-12.99)	10.88 (8.89-12.38)	0.452
Septal-Em velocity	11.86±2.73	12.25±2	0.564
	11.40 (7.18-17.66)	12.36 (7.94-15.84)	0.327
Septal-Am velocity	8.38±1.96	9.18±2.21	0.202
	8.235 (4.61-12.23)	9.14 (4.9-13.85)	0.143
Septal Em/Am velocity ratio	1.47±0.421	1.81±2.18	0.499
	1.46 (0.82-2.26)	1.86 (1.2-3.49)	0.285
Lateral LV-Sm velocity	8.76±1.10	10.85±1.13	0.248
	9.00 (7-12)	10.00 (9.00-13.00)	<0.01
Lateral LV-Em velocity	10.12±1.16	17.9±2.11	0.002
	10.1 (8.2-12.7)	17.75 (15.10-21.60)	<0.001
Lateral LV-Am velocity	12.85±1.84	7.18±1.84	0.903
	13.20 (8.9-16.2)		<0.01
Lateral LV-Em/Am velocity ratio	0.811±0.192	2.06±0.62	<0.001
	0.755 (0.56-1.27)	1.86 (1.2-3.4)	<0.001

Sm: Systolic myocardial, Em: Early diastolic myocardial, Am: Late diastolic myocardial, LV: Left ventricular

Table 4: Systolic strain (in %) parameters of the patient and control groups

Parameter	Patients	Controls	*P*
Basal lateral wall (strain-LV-basal)	−19.5±4.17	−24.196±1.81	0.002
	−18.5 (−12.8-−29.0)	−23.95 (−21.28-−28.39)	<0.001
Mid-lateral wall (strain-LV-mid)	−19.07±3.98	−25.56±2.62	0.042
	−18.00 (−12.6-−27.5)	−26.03 (−19.47-−31.47)	<0.001
Basal septal wall (strain-septal-basal)	−17.04±3.44	−25.43±2.53	<0.001
	−15.45 (−12.00-−23.20)	−25.38 (−18.80-−29.96)	<0.001
Mid-septal wall (strain-septal-mid)	−20.49±5.34	−24.45±2.2	0.001
	−18.9 (13.5-−34.00)	−24.05 (−20.79-−28.86)	0.001
RV basal wall (strain-RV-basal)	−18.46±5.78	−23.01±3.89	0.05
	−17.00 (−11.00-−31.00)	−22.99 (−17.05-−29.70)	0.002
RV mid wall (strain-RV-mid)	−24.24±7.35	−23.97±7.27	0.89
	−24.45 (−12.20-−47.90)	−25.53 (−11.86-−34.13)	0.843

LV: Left ventricular, RV: Right ventricular

Table 5: Systolic strain rate imaging (in 1/s) parameters of the patient and control groups

Parameter	Patients	Controls	*P*
Basal lateral wall (SR-LV-basal)	−0.8273±0.538	−0.887±0.38	0.005
	−0.720 (−0.07-−1.68)	−0.805 (−0.31-−1.85)	0.774
Mid-lateral wall (SR-LV-mid)	−0.671±0.230	−1.07±0.417	0.029
	−0.685 (−0.34-−1.3)	−0.885 (−0.44-−1.6)	0.009
Basal septal wall (SR-septal-basal)	−0.674±0.2606	−1.28±0.481	0.001
	−0.665 (−0.20-−1.20)	−1.31 (−0.42-−2.07)	<0.001
Mid-septal wall (SR-septal-mid)	−0.676±0.294	−1.02±0.48	0.05
	−0.700 (−0.04-−1.30)	−1.08 (−0.02-−1.82)	0.002
RV basal wall (SR-RV-basal)	−1.182±0.995	−2.417±0.433	0.145
	−0.960 (−0.09-−5.68)	−2.45 (−1.62-−3.2)	<0.001
RV mid wall (SR-RV-Mid)	−1.114±0.918	−1.16±1.00	0.871
	−0.900 (−0.34-−4.94)	−1.00 (−0.62-−2.97)	0.635

SR: Strain rate, LV: Left ventricular, RV: Right ventricular

Table 6: Univariate predictors of flow mediated dilatation

Parameter	*P*	Coefficient	Lower limit CI	Upper limit CI
Ferritin	<0.001	−0.895	−0.005	−0.004
IVS	0.008	−0.373	−2.987	−0.48
AO	0.36	0.297	0.034	0.988
SV	0.011	−0.357	−0.271	−0.037
LV mass$_i$ (g/m^2)	0.002	−0.435	−0.07	−0.017
LV-Sm	<0.001	0.545	1.175	3.074
LV-Em	<0.001	0.778	0.829	1.337
LV-Am	<0.001	−0.635	−1.864	−0.892
LV-Em/Am	<0.001	0.755	4.378	7.332
Septal-Am	0.027	0.312	0.103	1.664
SR-septal-basal	<0.001	0.544	3.832	10.048
SR-RV-basal	<0.001	0.607	2.158	4.804
Stain-LV-basal	<0.001	0.522	0.392	1.098
Stain-LV-mid	<0.001	0.562	0.401	0.999
Stain-RV-basal	<0.001	0.51	0.276	0.806

Sm: Systolic myocardial, Em: Early diastolic myocardial, Am: Late diastolic myocardial, IVS: Interventricular septal, AO: Aortic, CI: Confidence interval, SV: Stroke volume, LV massi: Left ventricular mass index to body surface area

LV-Am (OR − 1.378 [CI − 1.864-−0.892, *P* ≤ 0.001]), LV-Em/Am (OR 5.855 [CI 4.378-7.332, *P* ≤ 0.001]), septal-Am (OR 0.884 [CI − 0.103-−1.664, *P* ≤ 0.001]), S-LV-Basal (OR 0.745 (CI 0.392-1.098, *P* ≤ 0.001]), S-LV-Mid (OR 0.700 [CI 0.401-0.999, *P* ≤ 0.001]), S-RV-Basal (OR 0.541 [CI 0.276-0.806, *P* ≤ 0.001]), SR-septal-basal (OR 6.94 [CI 3.83-10.05, *P* ≤ 0.001]), and SR-RV-Basal (OR 3.481 [CI 2.158-4.804, *P* ≤ 0.001]) (all *P* < 0.004).

On multivariate linear regression analysis, only serum ferritin (adjusted OR = −0.004, *P* ≤ 0.001) and SR-septal-mid (adjusted OR = 4.506, *P* = 0.003) were observed to be significant independent predictors of FMD (both *P* < 0.004).

DISCUSSION

In this study of thirty children with β-TM all of whom were in NYHA Class I, ECG analysis revealed that patients had significantly higher LV mass as compared to controls, although resting heart rate and Sm/diastolic BP were similar to controls. Increased LV mass in patients with β-TM is postulated to be due to chronic myocardial parenchymal iron overload. The observed absolute and indexed LV mass (177.05 ± 52.51 g and 169.45 ± 61.14 g/m^2 respectively) in our study is consistent with previous reports of increased LV mass in patients with β-TM (absolute/indexed LV mass 80.92 ± 39.49/86.14 ± 25.47, respectively,[10] 228.0 ± 94.0/139.0 ± 42.0,[12] 113.8 ± 38.0/82.4 ± 17.5 gm/m^2).[15]

Patients had significantly higher LV mass and although conventional parameters of Sm and diastolic function like LV dimensions, volumes, EF, FS, transmitral E and A velocity and their ratio were not altered significantly in patients, E/E' ratio at the lateral annulus was significantly higher as compared to controls representing

subtle impairment of diastolic function. This was also accompanied by slight shortening of EDT in patients with β-TM. Previous reports have also documented that global LV diastolic function might be well preserved till late in the course of the disease.[21]

Tissue velocity imaging analysis

We found that β-TM patients had significantly lower Em velocity as well as lower Em/Am velocity ratio at the lateral LV as compared to controls. The septal velocity profile although slightly lower than controls was not significantly different. Comparison of TVI parameters in patients with β-TM revealed that Sm lateral wall velocity (LV-Sm, 8.76 ± 1.10) was significantly lower than Sm septal velocity (septal Sm, 10.84 ± 1.09), lateral Am velocity (LV-Am, 12.85 ± 1.84) was higher than septal Am velocity (septal-Am, 8.38 ± 1.96) and ratio Em/Am was lower in the lateral LV (0.811 ± 0.192) as compared to the septal LV (1.47 ± 0.421). Hence, despite an overall normal LVEF, there was a differential abnormality of TVI in the lateral versus the septal LV regions in patients with β-TM.

Strain and strain rate imaging

We found that LV strain at the basal and mid segments of the lateral LV wall as well as at the basal and mid septum were significantly lower in β-TM patients. Strain rate was also impaired in patients with β-TM. The SRI values at the basal and mid segment of the lateral LV wall and at the basal and mid septum were significantly lower in patients.

In some previous studies of SI, myocardial velocities and strain parameters were measured only in the basal segments of the LV, RV, and septum since initially the longitudinal velocities progressively decrease from base to apex in diseased states.[11,12] Other studies have assessed TVI and strain values in both basal and mid segments of LV, RV, and septum (as performed by us) to obtain a more detailed analysis of regional ventricular function.[10,13] While some studies have also previously reported lower SRI at the basal lateral and septal LV, others reported higher SRI at the basal lateral LV wall in patients with β-TM.[12,10]

Regional impairment as detected by TDI and SI reflects the often patchy and nonhomogeneous deposition of iron within cardiac myocytes rather than the interstitium.[22] Our observations further suggest that SI is not only more superior to conventional ECG parameters such as LVEF and LVFS, but also better than tissue Doppler parameters (TVI) in the detection of regional myocardial function. Although none of our patients had abnormal LVEF/FS or overt heart failure, analysis of TVI detected velocity parameters only at the lateral LV wall, while septal parameters were not different from controls. In contrast, SI and SRI detected lower Sm strain and strain rate both at the septal as well as the lateral across both the basal and mid segments.

Right ventricular strain analysis

We found that basal RV strain was also depressed in patients while strain at mid-RV was not different in comparison to controls. Although SRI at the RV basal and mid-RV segments was lesser in patients, the trend was not statistically significant. Hence, there may be a differential regional involvement of the two ventricles in patients with β-TM with more pronounced changes in lateral and septal LV as compared to the RV.

Although serum ferritin levels correlated with LV mass$_i$ and strain parameters, this does not automatically establish a cause-effect relationship. Correlation analysis of LV mass and strain parameters revealed further mechanistic insights. It is possible that higher serum ferritin levels in patients with β-TM, translate into higher LV mass and eccentric hypertrophy. This in turn causes abnormal tissue deformation with abnormalities in strain and SRI parameters.

Vascular analysis

Although we did not observe any differences in CIMT in patients versus controls, FMD was significantly more impaired in patients with β-TM. No differences in NMD were noted in the two groups. Altered vascular stiffness in patients with β-TM may be due to increase oxidative stress, endothelial dysfunction with disruption and fragmentation of vascular elastic lamellae, vascular calcification, and cell apoptosis.[23-25] While CIMT and arterial stiffness both indicate vascular function, endothelial dysfunction, and arterial stiffening may be affected earlier on in the course of vascular involvement in β-TM. Hence, CIMT measurement may be normal and therefore not reflect early vascular changes which may be better detected by FMD assessment.

Timely initiation and up-titration (if and when needed) of chelation therapy before the myocardial iron deposition leads to irreversible damage is important in these patients. Effective chelation of the labile iron molecules can help establish a negative iron balance and may help in improving survival if started early enough. Parameters such as TVI, strain, and strain rate are sensitive makers of early impairment of myocardial function, despite overall normal LV-Sm function. Although it is postulated that these abnormalities are secondary to myocardial iron deposition, this has not been validated in the current study. Early detection of subclinical regional myocardial dysfunction by deformation analysis including strain and strain rate may help in individualized tailoring of chelation therapy in patients with β-TM and should be the focus of future studies.

In developing countries like ours where the burden of thalassemia is large, and ECG is an inexpensive and

readily available modality, our study adds to the existent literature confirming its use to detect subtle myocardial dysfunction in patients with β-TM.

Limitations

A small number of patients (n = 30) represents an obvious limitation, and more studies with larger number of patients are needed to help further define the role of these newer ECG parameters in assessment of LV function in patients with β-TM. A single point ECG analysis is also an important limitation; serial longitudinal studies with measurement of parameters in follow-up are necessary to clarify if these abnormalities are reversible. Since there was a significant difference in LV mass among the two groups, LA volumes may have been significantly different in patients and controls. The fact that only LA diameters have been obtained represents a limitation of the study. The fact that T2* MRI imaging for *in vivo* assessment of myocardial iron concentration was not performed also represents an important limitation since how far do changes in ECG deformation parameters correlate with myocardial iron content is uncertain. Although we have measured serum ferritin levels, it is important to realize that this is not the gold standard to reflect the extent of cardiac iron overload and with increasing myocardial siderosis the correlation of ferritin with cardiac T2* values becomes even more unreliable. Strain and strain rate parameters are sensitive to loading conditions and whether the abnormalities in β-TM are secondary to altered loading conditions or increased myocardial iron stores needs to be further characterized.

CONCLUSIONS

Since global cardiac Sm and diastolic function often remain normal until late stages in patients with β-TM, novel imaging techniques are necessary to detect early myocardial involvement. Our study demonstrates the ability of SI and SRI to quantify regional myocardial function in asymptomatic β-TM patients with preserved global Sm and diastolic function.

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Conflicts of interest

There are no conflicts of interest.

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