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Ventricular Diastolic Dysfunction in Sickle Cell Anemia Is Common But Not Associated With Myocardial Iron Deposition

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Abstract

Background—Cardiac failure from myocardial iron deposition is a severe complication in patients with transfusion-related iron overload. Progressive heart damage from iron overload can cause left ventricular systolic and diastolic dysfunction in patients with hematologic disorders. Since non-transfused patients with sickle cell anemia (SCA) have a high incidence of diastolic dysfunction, we investigated the relationships among transfusional iron burden, myocardial iron deposition, and diastolic ventricular dysfunction by T2*-MRI and tissue Doppler echocardiography in iron-overloaded children with SCA.

Procedure—Children (≥7 years) with SCA and iron overload (serum ferritin >1000 ng/ml or ≥18 lifetime transfusions) were eligible. Serum ferritin and hepatic iron content (HIC) were measured and participants underwent nonsedated T2*-MRI of the heart, echocardiogram, electrocardiogram, and multi-uptake gated acquisition (MUGA) scan. Age-matched normative echocardiographic data were used for comparison.

Results—Among 30 children with SCA (median age, 13 years) and iron overload, mean (±SD) HIC and serum ferritin were 10.8 mg Fe/g (±5.9 mg Fe/g) and 3089 ng/mL (±2167 ng/mL), respectively. Mean T2*-MRI was 33 msec (±7 msec, range 22-49). Echocardiography showed a high prevalence of diastolic dysfunction (77% and 45% abnormally low mean mitral annular velocity and mean tricuspid annular velocity, respectively); however, echocardiogram and MUGA scan findings were not significantly associated with HIC or T2*-MRI.

Conclusions—Diastolic dysfunction is not associated with transfusional iron burden or myocardial iron deposition among children with SCA. Diastolic dysfunction likely results from disease pathophysiology and severity rather than iron overload.

Keywords

T2*MRI; echocardiography; multi-uptake gated acquisition; diastolic dysfunction; systolic dysfunction; sickle cell disease

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INTRODUCTION

Cardiac failure is a major complication for patients with hematological disorders who have increased body iron burden because of repeated blood transfusions. Myocardial iron deposition leads to progressive heart dysfunction and remains the leading cause of death in patients with beta thalassemia major (TM).[1;2] Myocardial hemosiderosis can also occur in other hematological diseases treated with repeated blood transfusions such as Diamond–Blackfan anemia, myelodysplasia, and, in some cases, sickle cell anemia (SCA).[3]

The severity of cardiac dysfunction depends on the amount of iron deposited in the myocardium[4] and the overall body iron burden; hepatic iron content (HIC) persistently greater than 15 mg Fe/g of dry weight liver is associated with cardiac morbidity.[5] Left ventricular systolic dysfunction (decreased ejection fraction) is a late finding of heart disease from iron accumulation, since cardiac iron deposition is associated with the duration of blood transfusions.[3] In contrast, left ventricular diastolic dysfunction occurs earlier in the development of overall cardiac dysfunction (heart failure), and its presence is an independent risk factor for mortality among adults with SCA.[6]

Diastolic dysfunction, measured by tissue Doppler imaging (TDI) echocardiography, has been documented in patients with hematological diseases; left ventricular diastolic dysfunction occurs in 9.7–18% of patients with SCA without iron overload.[6;7] Patients with TM having high ferritin values also have restrictive left ventricular filling abnormalities, suggesting ventricular diastolic impairment.[8] Small-scale studies have suggested a relationship between iron overload and diastolic dysfunction in SCA. Children with SCA receiving blood transfusions have a higher left ventricular myocardial performance index (LVMPI – a measure of combined left ventricular systolic and diastolic function) than control patients with SCA not receiving transfusion.[9;10] In these reports, systolic function was normal and the high LVMPI was thought to be secondary to diastolic left ventricular dysfunction, however exclusive measures of diastolic function were not performed.

No previous studies on SCA have investigated the relationships between myocardial iron deposition measured by magnetic resonance imaging (MRI), transfusional iron burden, and diastolic ventricular dysfunction. We investigated the relationships between the degree of myocardial iron accumulation and ventricular diastolic function by comparing heart T2*-MRI measurements with echocardiogram tissue Doppler findings in iron-overloaded children with SCA (≥7 years old) who received medical care at St. Jude Children's Research Hospital (St. Jude).

DESIGN AND METHODS

Patient Selection and Data Collection

This prospective study was approved by the St. Jude Institutional Review Board. Study participants or their legal guardians provided signed informed consent before any study-related activity; all children signed an informed assent. Children with SCA receiving medical care at St. Jude were eligible to participate in the study if they were 7 years or older and had a diagnosis of iron overload. Patients were defined as having iron overload if they had serum ferritin values of 1000 ng/mL or more within 3 months of enrollment or had received 18 or more lifetime erythrocyte transfusions.[11;12] Patients who had any contraindication for MRI testing (e.g., presence of ferromagnetic material in the body), could not tolerate MRI without sedation, or were pregnant were not enrolled in the study. In addition, patients with history of prior heart surgery were not included in the present analysis.

Medical records of patients were reviewed for transfusion history prior to study enrollment, use of cardiac medication at the time of enrollment, and symptoms of heart failure. Study participants underwent a nonsedated T2*-MRI exam of the heart followed by echocardiogram, complete blood count, reticulocyte count, chemistries, electrocardiogram (EKG), multi-uptake gated acquisition (MUGA) nuclear scan, and one single serum ferritin measurement, all within 30 days. The hemoglobin (Hb) concentration value closest to the echocardiogram exam (usually the pre-transfusion Hb) was ascertained. To calibrate the T2*-MRI technique for assessing liver iron content, participants also underwent a percutaneous liver biopsy with HIC quantitation, and these findings have already been reported.[13]

Echocardiogram and MUGA Scan

All studies were performed using GE Vivid 7 ultrasound equipment with 3.5 or 2.5 MHz transducers. Standard transthoracic 2D images were obtained. M Mode analysis was done from a parasternal long axis projection of the left ventricle (LV). Color flow Doppler, spectral Doppler, and tissue Doppler measurements were obtained. LV dimension, systolic function, and LV mass measurements were made using the M Mode data. Measurements were made with the internal equipment electronic calipers on the digital image, or for videotape, the image was calibrated by using reference lines on the video screen image. Measurement of systolic function included estimation of the LV shortening fraction (LVSF). The left ventricular dimensions, systolic function, and LV mass were estimated from the M Mode according to guidelines of the American Society of Echocardiography.[14] In order to compare our results to published data in patients with SCA, the LVMPI (or Tei) was obtained. LVMPI was calculated from the mitral valve inflow and the LV outflow obtained close in time. The mitral closing to opening time (*a*) was measured at the end of the interval from the end to the onset of the mitral inflow velocity profile. The LV ejection time (*b*) was measured from the onset to the end of the LV outflow velocity profile. LVMPI was calculated by the formula $(a - b)/b$.[15] Mean values were obtained by averaging the 3 best-quality signals from a sequence. Tissue Doppler was done from the apical projection with the presets from the equipment. For each velocity, an average of 3 beats was used. The peak mitral annular velocity (e') and early filling of the left ventricle (e) were measured from digital image videotape by using the echo machine calibration tool and electronic calipers. The e/e' ratio was used as a measure of global left ventricular diastolic function. The peak tricuspid annular velocity was used as a measure of right ventricular diastolic function. Decreasing e' (and therefore increasing e/e' ratio) values reflect the decreasing relaxation ability (increasing stiffness) of the left ventricle, whereas decreasing peak tricuspid annular velocities reflect increasing right ventricular stiffness.

Cardiac gated nuclear medicine blood pool studies (MUGA scan) were performed on patients in a resting state. An *in vivo* method of labeling red blood cells was employed. Patients were intravenously administered 3 mg of sodium pertechnetate 30 min before injecting ^{99m}technetium at a dose of 15mCi times their body surface area (maximum dose 20mCi). Patients were placed in a supine position on the gamma camera (Siemens Duet, Chicago, IL) and 45° left lateral oblique (LAO) and 70° left lateral projection images were obtained over 5 min at a frame rate of 16 per cardiac cycle. The left ventricular ejection fraction (LVEF) was calculated on the basis of LAO projection images. Cine clips were evaluated by a nuclear medicine physician or a radiologist to assess regional wall motion abnormalities.

MRI

Study participants underwent a nonsedated single breath-hold examination of the heart by a single 1.5T MRI scanner (Siemens Magnetom Symphony, Siemens, Malvern, PA) within 30

days of the echocardiogram, EKG, and MUGA scan. A short axis mid-ventricular 8-mm-thick slice positioned halfway between the base and apex of the left ventricle was acquired at 12 separate short echo times (TEs) in a single breath-hold (TEs ranging from 2 to 21.1 msec with equal echo spacings). To ensure best possible cardiac motion compensation, EKG gating was performed while scanning.

Images were transferred to a computer workstation for postprocessing region of interest (ROI) T2* measurement. Quantitative T2* maps were calculated offline, using custom written MATLAB software, and the signal intensity drop over the image series was fitted on a pixel-by-pixel basis to a monoexponential decay, using the least-squares fit method by Levenberg-Marquardt.[16;17] ROIs were drawn either on the source images or on T2* maps, in the left ventricular septum, distant from lungs and cardiac veins. Images were considered inadequate if a sufficient amount of pixels in the ROI could not be fitted, either due to iron accumulation (lack of MR signal) or the presence of cardiac motion artifacts (bright signal across the image). The total MR table bedtime required for the T2*-MRI examination of the heart was approximately 20 min.

Statistical Analyses

To avoid intrapatient association and thereby a confounded interpretation of results, each patient participated in the study only once. LVEF values (measured by the MUGA scan) below 60% and LVSF values (measured by echocardiogram) below 28% were considered abnormal. Echocardiogram measurements were categorized as normal or abnormal, using normative age-specific published data.[18] The association of echocardiogram and MUGA variables (both as continuous and categorical variables) with T2*-MRI measurements was investigated using Fisher's Exact test, the Wilcoxon-Mann-Whitney test, or Spearman's rank-order correlation with exact *p*-values estimated by Monte Carlo simulation. If the correlation coefficient was significantly different from zero, *p*-values were reported; *p*-values were considered significant if < 0.05.

RESULTS

Patient Characteristics

Thirty-two patients with SCA underwent both heart T2*-MRI and cardiac tests (MUGA scan, EKG, and echocardiography), however two patients were excluded from the analysis: one due to prior heart surgery, and one due to artifacts in the chest that precluded MRI interpretation. Of the 30 patients included in the analysis, 15 were male and 15 were female, and their SCA genotypes were Hb SS (28) and Hb $S\beta^0$-thalassemia (2); their median age was 13 years (range, 8–18 years). Reasons for patients to receive chronic transfusions were secondary stroke prevention (10), primary stroke prevention (12), and prophylaxis against recurrence of vasoocclusive events such as acute chest syndrome (8). None of these 30 patients had a history of heart disease or used any heart medication. Mean (± SD) Hb concentration was 9.9 g/dL (± 1.5 g/dL), and was obtained within 7 days in all but one patient who had it done within two weeks of cardiac testing. The mean (± SD) HIC and serum ferritin values were 10.8 mg Fe/g of dry weight liver (±5.9 mg Fe/g) and 3089 ng/mL (±2167 ng/mL), respectively. These 30 patients had received erythrocyte transfusions for a median of 45 months (range, 7–162 months) before study enrollment. One patient came to our program in her teenage years and her records reported only 7 erythrocyte transfusions; however, since her serum ferritin was more than 1000 ng/mL, the total number of erythrocyte transfusions she received may have been underestimated. Fourteen (47%) of these patients had history of chelation therapy at the time of study enrollment, and their median duration of chelation therapy was 7.5 months (range 1 – 65 months).

Echocardiogram, EKG, and MUGA Findings

The mean left ventricular shortening fraction was normal (above 28%) in all patients (40.8% ± 4.1, range 33.3 – 48.6%). The mean MUGA LVEF values were also normal (above 60%) in our pediatric patients (71.2% ±4.8%, range, 62%–80%). Comparisons with age-matched normative echocardiographic data identified a high prevalence of both right and left ventricular diastolic dysfunction (Table I). Twenty-three (77%) patients had abnormally low mean mitral annular velocity (*e′*), and 13 (45%) patients had abnormally low mean tricuspid annular velocity, corresponding to left and right ventricular diastolic functions, respectively. In addition, all patients had *e/e'* values higher than the normal reference for age. LVMPI (a combined measure of systolic and diastolic functions) was abnormally high in 17% of our patients, consistent with prior reports of values higher than normal in children with SCA and iron overload.[9;10] Five (17%) patients had LV myocardial mass index values higher than expected (Table I), but they were not associated with peak tricuspid annular velocity or *e′*. There was no significant association between any echocardiographic measurement and HIC. In addition, there were no significant associations between laboratory parameters such as Hb concentration, lactate dehydrogenase (LDH), reticulocyte count, and aspartate aminotransferase (AST), and measures of right or left diastolic dysfunction (peak tricuspid annular velocity and *e′*).

Six (20%) patients had a prolonged heart rate–corrected QC (QTc) interval (defined as QTc interval > 440 msec). The median QTc interval was 430 msec (range, 400–460 msec). Of the patients, 6 (20%) had left ventricular hypertrophy, 3 (10%) had flat T waves in frontal plane and lateral leads, 1 (3%) had right ventricular hypertrophy, and 1 (3%) had incomplete right bundle branch block. QTc interval was not significantly associated with *e/e′* (correlation coefficient = –0.16; *p*-value = 0.47). The presence of LV hypertrophy was not significantly associated with peak tricuspid annular velocity or *e′*.

T2*-MRI Findings

The mean heart T2*-MRI value for the 30 patients was 33 msec (±7 msec; range, 22-49 msec). Comparison of T2*-MRI values of the myocardium with echocardiographic findings showed that heart T2* was not associated with LVMPI, LVSF, LVMI, peak tricuspid annular velocity, *e′*, or the *e*/*e′* ratio. There were no significant associations between T2*-MRI values and echocardiogram measurements, even when echocardiogram normative data were used to categorize echocardiographic findings as normal or abnormal. T2*-MRI findings were not associated with MUGA results. T2* values of the liver and heart were not significantly associated. The heart T2*-MRI values of four patients transfused for greater than 10 years (range, 11 – 13.5 years) ranged from 24.7 to 38.1 msec; their HIC ranged from 13.5 to 19.7 mg/g, and 3 of them received chelation for a median of 60 months.

DISCUSSION

Myocardial iron deposition develops as a consequence of repeated erythrocyte transfusions and is associated with increased morbidity and mortality in TM patients with iron overload.[4;19;20] Measuring and monitoring cardiac iron should be part of the clinical standard of care for iron-overloaded patients, since proper treatment can minimize iron toxicity and also reduce mortality.[21;22] MRI relaxation techniques (e.g.; T2*) provide a noninvasive means of quantifying iron deposition in the heart, and therefore potentially allow early treatment and prevention of heart dysfunction. Echocardiogram TDI is a relatively new technique that provides effective quantification of the motion within a myocardial segment, including quantification of systolic and diastolic ventricular functions, while minimizing measurement artifacts. TDI allows accurate quantification of global and especially regional LV function.[23;24] TDI is done by obtaining an apical four-chamber image of the heart and sampling

the movement of the myocardium at the site where the atrio-ventricular valves anchor into the myocardium. This technique is angle-dependent and is also influenced by the heart rate and patient's age. TDI velocities have proven useful in pediatric patients to evaluate several different conditions, including cardiomyopathy, pericardial disease, cardiac effects of hypertension, and in TM patients with iron overload.[25]

Cardiac systolic dysfunction (decreased LVSF) can be predicted by increased myocardial iron measured by T2*-MRI in patients with TM.[3;4;26] Although heart T2*-MRI calibration is not feasible due to the risks associated with myocardial biopsy, a T2*-MRI value less than 20 msec has been associated with decreased LVSF in patients with TM.[4] Systolic dysfunction is usually severe and progresses rapidly to heart failure. Diastolic dysfunction, in contrast, is an early marker of heart dysfunction that can precede the onset of systolic dysfunction. Left ventricular diastolic dysfunction may be the result of myocardial ischemia, fibrosis, iron deposition, and ventricular hypertrophy.[8;27-29] Although there was a high prevalence of diastolic dysfunction in our pediatric SCA population, T2*-MRI did not accurately identify it. Myocardial iron has not been consistently shown to predict left ventricular diastolic dysfunction. In a study wherein the diastolic function of patients with TM was measured by MRI, there was only a weak association between increased cardiac iron and left ventricular diastolic dysfunction. [30] No association was observed, however, between T2*-MRI and the indices of left ventricular diastolic dysfunction such as e'/e or the Tei index measured by tissue Doppler in patients with TM.[26] It is unclear why cardiac iron deposition disproportionally affects systolic in comparison with diastolic ventricular function. It is possible that the relaxation process of the heart is less sensitive to iron-related damage, but this hypothesis has not yet been investigated.

TDI e' velocities reflect the early relaxation of the ventricle, which creates rapid filling partly via a suction effect.

Since systolic function is preserved in most patients with SCA, the mechanical untwisting or recoiling of the ventricle attributed to systole alone cannot be implicated as the causative factor of diastolic dysfunction. Rather, the active process of relaxation may be impaired in diastolic dysfunction. Diastolic dysfunction is caused by factors intrinsic or extrinsic to the myocardium.[31] Iron accumulation is one of the intrinsic myocardial causes, and iron appears to preferentially deposit in the myocytes rather than the interstitium.[32] In our study population of patients with SCA, however, correlations of diastolic dysfunction with myocardial iron loading were not significant. This implies that the impairment of ventricular relaxation in these patients may not be due solely to myocyte injury, but could involve the extracellular matrix that surrounds the cardiac muscle cells or involve abnormal activation of neurohormones in the heart. Among neurohormonal activators, subendocardial nitric oxide (NO) release is crucial during ventricular relaxation and filling periods. NO exerts significant effects on the relaxation phase of the cardiac cycle, such as enhancement of myocardial relaxation and increase in diastolic distensibility and modulation of myocardial oxygen consumption.[33-35] The ongoing intravascular hemolysis that occurs in SCA rapidly inactivates NO via consumption by cell-free hemoglobin and also by a reaction between ferrous iron and ferric iron that forms nitrosylhemoglobin, which consumes NO.[36] Hemolysis also releases arginase, which causes depletion of arginine, the substrate for NO production. The increased inactivation and decreased production of NO may be involved, therefore, in the development of diastolic dysfunction in patients with SCA. We were unable to demonstrate a significant association between markers of hemolysis (LDH, AST, reticulocyte count) in our patient population, however the chronic use of blood may have reduced the degree of hemolysis and therefore prevented the identification of a significant association. Possible extramyocardial causes of diastolic dysfunction include

increased hemodynamic load from anemia, which leads to myocardium hypertrophy and increased stiffness.[37]

We found no association between systolic function indices (echocardiogram LVSF and MUGA LVEF) and T2*-MRI. This is likely due to the fact that none of our pediatric subjects had evidence of systolic dysfunction and none had abnormally low T2*-MRI values (T2* <20 msec). Increased cardiac iron concentration is not a common finding in iron-overloaded patients with SCA. Patients with SCA do not accumulate iron in the heart or develop heart disease to the same degree as do patients with TM,[3;38;39]. The most likely reason, however, for the absence of significant amounts of cardiac iron in our cohort is the relative short duration of transfusions.

Our study was limited by a relatively small sample size and comparisons of diastolic function in patients with SCA to normative data from the general population. Although no race-matched echocardiographic data was used for comparison, the normative data included a sample from a general pediatric population from Houston, Texas,[18] that did not make any distinction with respect to patients' racial or ethnic background. It is possible, however, that these normative cardiac parameters do not apply to patients with SCA; therefore, prospective large-scale studies on age- and race-matched patients are necessary to determine the role of diastolic dysfunction in overall heart function and development of iron overload. Although diastolic dysfunction has been associated with pulmonary hypertension,[6;40] we cannot address the relationship between diastolic function and pulmonary hypertension, since we did not quantify tricuspid regurgitation (a surrogate marker for pulmonary hypertension) in our patients.

Our data suggest that in young, iron-overloaded patients with SCA, diastolic dysfunction is common and likely linked to cardiac dysfunction from the severity of the disease itself, rather than solely from iron loading. Since our patients were young and not exposed to blood transfusions for long periods of time (only 4 patients had been transfused for more than 10 years), we could not assess the long-term role of ongoing iron accumulation in the heart and diastolic dysfunction in overall heart function. The concentration of iron in our patients' hearts was not very high, likely a reflection of the limited duration of transfusion and is probably the main reason why no statistically significant associations were found with cardiac function tests measures. We recommend that patients with SCA who receive blood transfusions should be followed prospectively with measurements of cardiac iron concentration, as well as diastolic functions such as isovolumetric relaxation time and mitral *e*-wave deceleration time. Longitudinal assessment by echocardiography and MRI will shed light on the importance and role of diastolic function and iron accumulation in the hearts of patients with SCA, and the role of therapies that specifically target cardiac hemosiderosis and diastolic dysfunction.

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Table I

Echocardiography findings in the iron-overloaded patients with sickle cell anemia (SCA) and normal reference values

	Age Range (years)								
	6 -9			10 - 13			14 - 18		
	Reference Value	*Patients with SCA*	*N(%) abnormal*	*Reference Value*	*Patients with SCA*	*N(%) abnormal*	*Reference Value*	*Patients with SCA*	*N(%) abnormal*
Mean mitral annular velocity – *e′* (cm/sec)	17.2±3.7 (16.2-18.3)	17.0±3.3 (15.0-23.7)	5/6 (83%)	19.6±3.4 (18.7-20.5)	16.4±3.5 (11.3-20.7)	10/13 (77%)	20.6± 3.8 (19.7-21.4)	17.6±4.2 (11.0-25.7)	8/11 (73%)
***e/e′* ratio**	5.8± 1.9 (5.3-6.4)	7.8±0.9 (6.3-8.8)	100%	4.9±1.3 (4.6-5.2)	8.7±3.3 (5.0-14.6)	100%	4.7±1.3 (4.4-5.0)	7.7±2.7 (4.8-13.6)	100%
Mean tricuspid annular velocity (cm/sec)	16.5±3.0 (15.7-17.4)	17.4±3.0 (13.3-22.2)	2/6 (33%)	16.5±3.1 (15.7-17.4)	17.1±3.0 (13.0-22.0)	6/12 (50%)	16.7±2.8 (16.0-17.3)	15.9±2.8 (9.3-18.7)	5/11 (45%)
Left ventricular myocardial performance index	0.32±0.06	0.21±0.12 (0.07-0.36)	1/5 (20%)	0.34±0.06	0.25±0.11 (0.11-0.52)	1/13 (8%)	0.34±0.08	0.30±0.17 (0.10-0.72)	3/11 (27%)
Left ventricular mass index (g/m2)	82.3±28.3	101.5±25.8 (79.0-148.0)	5/6 (83%)	110.1±32.9	80.8±13.5 (62.0-103.0)	0	158.4±48.5	90.8±18.9 (61.0-133.0)	0

Notes: Results presented as mean ± 1 STD (95% confidence interval), unless noted otherwise. *e/e′* ratio, mean tricuspid annular velocity, left ventricular myocardial performance, and left ventricular mass index were not obtained in one patient. *e′* and mean tricuspid annular velocity were considered abnormal if below the reference range for age. *e/e′* ratio, left ventricular performance index, and left ventricular mass index were considered abnormal if above the reference range for age. Reference values extracted from published norms in general pediatrics.[18]