Published in final edited form as:
Cancer. 2013 October 1; 119(19): 3555–3562. doi:10.1002/cncr.28256.

Impact of hemochromatosis gene mutations on cardiac status in doxorubicin-treated survivors of childhood high-risk leukemia

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Abstract

Background—Doxorubicin is associated with progressive cardiac dysfunction, possibly by forming doxorubicin-iron complexes leading to free-radical injury. We determined the frequency of hemochromatosis (HFE) gene mutations associated with hereditary hemochromatosis and their relationship with doxorubicin-associated cardiotoxicity in survivors of childhood high-risk acute lymphoblastic leukemia.

Methods—Peripheral blood was tested for two common HFE allelic variants: C282Y and H63D. Serum cardiac troponin-T (cTnT) and N-terminal pro-brain natriuretic peptide (NT-proBNP), biomarkers of cardiac injury and cardiomyopathy, respectively, were assayed during therapy. Left ventricular (LV) structure and function were assessed with echocardiography.

Results—184 patients had DNA results for at least one variant, and 167 had both: 24% carried H63D and 10% carried C282Y. Heterozygous C282Y genotype was associated with multiple elevations in cTnT concentrations (p=0.039), but not NT-proBNP. At a median of 2.2 years (1.0–

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Conflicts of Interest Disclosure: No conflicts of interest or disclosures are noted.

3.6) after diagnosis, mean [SE] *Z*-scores for LV fractional shortening (−0.71 [0.25], p=0.008), mass (−0.84 [0.17], p<0.001), and end-systolic (−4.36 [0.26], p<0.001) and end-diastolic posterior wall thickness (−0.68 [0.25], p=0.01) were abnormal in children with either allele (n=32). Non-carriers (n=63) also had below-normal LV mass (−0.45 [0.15], p=0.006) and end-systolic posterior wall thickness (−4.06 [0.17], p<0.001). Later follow-up showed similar results.

Conclusions—Doxorubicin-associated myocardial injury was associated with C282Y HFE carriers. Although LV mass and wall thickness were abnormally low overall, they were even lower in HFE carriers, who also had reduced LV function. Screening newly-diagnosed cancer patients for HFE mutations may identify those at risk for doxorubicin-induced cardiotoxicity.

Keywords

Cardiotoxicity; Doxorubicin; Hemochromatosis; Leukemia; Pediatrics

Introduction

Children with acute lymphoblastic leukemia (ALL) have long-term, event-free survival rates >80%.[1] Late effects often include anthracycline-associated cardiovascular abnormalities.

Among more than 300,000 U.S. childhood cancer survivors,[2] over 50% have been treated with anthracyclines.[3] Anthracyclines have been associated with progressive cardiotoxicity.[4] Thus, understanding the causes, mechanisms, and magnitude of doxorubicin-related cardiotoxicity is important. Reducing cardiotoxicity could improve long-term survivor outcomes.

Doxorubicin-associated cardiotoxicity risk is greater in girls, Down syndrome, and those treated at younger ages, with higher doxorubicin dose-rates and cumulative doses, as well as with longer follow-up since receiving doxorubicin.[4,5,6] However, some patients appear to be more vulnerable than others, independent of these risk factors. Identifying patients at the highest risk of late cardiotoxicity is a priority because they should be the focus of alternative chemotherapies[7] and novel preventive treatments to maintain oncologic efficacy while reducing toxicity and late effects.

Doxorubicin-induced cardiotoxicity is caused, in part, by myocardial doxorubicin-iron complexes generating doxorubicin semiquinone free radicals, which after reacting with oxygen lead to lipid peroxidation and DNA damage.[8,9] The cardioprotective properties of dexrazoxane, a compound that chelates iron thereby reducing this free radical formation, given before doxorubicin doses supports this hypothesis.[10,11]

We hypothesized that conditions leading to higher iron tissue concentrations may favor the development of doxorubicin cardiotoxicity. *In vitro* and animal studies suggest that tissue iron-loading potentiates anthracycline cardiotoxicity, and increases biomarker concentrations of cellular damage.[8,12]

Hereditary hemochromatosis (HH) is a genetic iron metabolism disorder resulting in iron overload-associated tissue injury. The most common HH disease gene is HFE; most affected individuals carry at least one copy of a founder mutation, C282Y. Homozygosity for the C282Y mutation is present in 52–100% of HH patients.[13] Adults with non-cancer-associated idiopathic dilated cardiomyopathy and early pathologic LV remodeling[14] have higher C282Y homozygosity than healthy controls.[15]

Postmortem testing of an anthracycline-naïve 14-year-old with high-risk ALL, iron overload, hepatic failure, and cardiac dysfunction revealed a homozygous C282Y mutation,

confirming previously undiagnosed HH.[16] Other alleles, such as H63D, are less often associated with clinical disease,[13] but may be more commonly found in non-cancer-associated idiopathic dilated cardiomyopathy than in healthy controls.[17]

Given the importance of iron,[8,12] and potential genetic involvement[18,19] in anthracycline-induced cardiac injury, we determined HFE disease-associated allelic frequency in at-risk patients for doxorubicin cardiotoxicity. Knowing the HFE disease allele frequency in doxorubicin-treated children with ALL may help clarify the relationship between their genetic predisposition to cardiotoxicity and cardiovascular status. If HFE gene mutations predict late cardiotoxicity, then patients with HFE mutations could be identified at diagnosis and be given cardioprotective agents to minimize their cardiac risk.

Methods

Participants

Between 2005 and 2007, patients with high-risk ALL treated on Dana-Farber Cancer Institute (DFCI) ALL Consortium Protocol protocols from 1991 onward and who had no prior relapse were enrolled in this study. Informed consent was obtained from parents if the patient was ≤18 years old and from patients >18 years. Total planned cumulative doxorubicin dosage for high-risk patients was 300–360 mg/m^2.

HFE genotyping

5-mL of peripheral blood was drawn from each patient with 2.5-mL shipped to the DFCI central testing laboratory for genetic analysis.

Cell Purification—Mononuclear cell fractions were obtained using standard methods. Blood was diluted with an equal volume of serum-free medium and mixed gently. The sample was layered on top of Ficoll (10-mL of Ficoll and 25-mL of diluted blood/50-mL conical bottom Falcon tube) and then centrifuged for 30 minutes at 1500 rpm at room temperature. The mononuclear cell layer from the Ficoll:plasma interface was placed into a new 50-mL tube and brought to 5-mL with RPMI medium. The pellet was washed twice, re-suspended and counted in an automated cell counter. Cell concentrations were adjusted to about 10^6/mL. The pellet was frozen in 1-mL aliquots at −80°C for several hours and transferred to liquid nitrogen for long-term storage. About 1.5×10^6 cells were kept unfrozen to generate DNA.

DNA Isolation—Isolated DNA was prepared using NucleoSpin DNA isolation kits (BD Biosciences Clontech) in all samples at the time of mononuclear cell fraction isolation. Sample volume was adjusted to 200-μL (1.5×10^6 cells), and 25–50-μL proteinase K was added. After adding 200–400-μL of Buffer B3, the mixture was incubated at 70°C for 60 minutes. The sample was vortexed after 210–420-μL of 96–100% ethanol was added, placed in the NucleoSpin column, and centrifuged at 11,000 × g for 1-minute at room temperature. The flow-through was discarded; 500-μL of buffer BW was added to the spin column and centrifuged at 11,000 × g for 1-minute at room temperature. Buffer B5 (600-uL) was added to the spin column and centrifuged at 11,000 × g at room temperature. The flow-through was discarded and the column was re-centrifuged at 11,000 × g at room temperature to remove B5 completely. The column was placed in a 1.5-mL tube, eluted with 50–100-μL BE buffer (warmed to 70°C) by incubation for 10 minutes, and then centrifuged. Contaminating protein and DNA were measured by UV spectroscopy. DNA was stored at −80°C.

Detection—The two most common HFE alleles associated with HH, the cysteine-to-tyrosine substitution at amino acid position 282 (C282Y) and the histidine-to-asparatic acid

substitution at amino acid position 63 (H63D), were detected by directly-sequencing genomic DNA with a commercial, clinically-validated kit (Biotage Inc.; PyroMark HFE Cat #40-0053) run on a Pyrosequencer instrument (PSQ HS 96, Biotage Inc.) or by Sequenome and Taqman genotyping assays performed at the Harvard Partners Center for Genetics and Genomics.

Genetic counseling

Before enrolling, patients and families were invited to genetic counseling regarding study implications. Results were disclosed to patients with homozygous HFE deficiency because this may precede clinically-important iron overload, and re-testing in a CLIA-certified laboratory was advised.

Sample collection for biomarkers

The DFCI protocol 91-01 study[20] was conducted before serum cardiac biomarkers were collected. Patients from this cohort were excluded from the cardiac biomarker analysis, but were included in all other analyses.

At DFCI protocol 95-01 and 00-01 study enrollment, serum samples were collected at diagnosis, daily for 1-week after the first induction doxorubicin dose, before each subsequent doxorubicin dose, and for 7-days after the final doxorubicin dose.[20] Serum was stored at −70°C until analysis. Hemolyzed samples were excluded. Cardiac troponin-T (cTnT) concentrations were determined centrally using the Elecsys Troponin-T STAT Immunoassay (Roche Diagnostics Corporation, Indianapolis, IN; sensitivity, 0.01 ng/mL). Concentrations of N-terminal pro-brain natriuretic peptide (NT-proBNP) were measured using an immunoassay (Elecsys immunoanalyzer, Roche Diagnostics, Indianapolis, IN; sensitivity, 5 pg/mL). Concentrations of cTnT greater than 0.01 ng/mL and NT-proBNP concentrations greater than 150 pg/mL in infants <1 year or greater than 100 pg/mL in children aged ≥1 year old at sample collection indicated myocardial injury and cardiomyopathy, respectively.

Cardiac assessment

Echocardiograms were obtained at diagnosis, after doxorubicin therapy, and every 2 years thereafter at local treatment sites and centrally re-measured at a single facility by study staff blinded to treatment status.[21] Children were eligible for cardiac follow-up throughout their first continuous complete remission. Left ventricular status was assessed with LV end-systolic and end-diastolic dimensions; LV mass; LV end-systolic and end-diastolic posterior wall thicknesses; LV thickness-to-dimension ratio; and LV fractional shortening, an index of LV systolic performance influenced by heart rate, LV preload, LV afterload, and LV contractility.

We standardized echocardiographic measurements with *Z*-scores,[22] which are the number of SDs the measurement is above or below the mean value of a normative population, to adjust for age, body-surface area, and growth-related changes. We calculated *Z*-scores from the difference between LV outcome values in patients and known values in healthy children, divided by the SD of a distribution of values in healthy children.

We calculated the predicted value for each outcome in healthy children with a regression model, using data from 285 healthy children measured in a single center and in the same manner as the study patients.[23,24] The *Z*-scores for LV mass, LV end-systolic and end-diastolic posterior wall thicknesses, and LV dimensions were adjusted for body-surface area, and *Z*-scores for LV fractional shortening were adjusted for age at echocardiography.

Statistical analysis

Means of normally-distributed outcome variables (e.g., echocardiographic measurements) were compared with t-tests; means of non-normally-distributed variables (e.g., age), with Wilcoxon rank-sum tests. Differences in proportions of dichotomous values (e.g., mutation carrier) between groups were compared with Fisher's exact tests.

For associations with serum cardiac biomarkers, each HFE variant was dichotomized into carriers or non-carriers, and wild-type only. For associations with echocardiographic *Z*-scores, the two loci were combined as a result of a small sample. Carriers were hetero- or homozygous for either H63D or C282Y, and non-carriers were wild-type only for both. Unadjusted and adjusted logistic regressions of multiple abnormalities in NT-proBNP and cTnT concentrations were performed to determine the association of HFE carrier status for both H63D and C282Y combined and separately. The models were adjusted for treatment including doxorubicin with and without dexrazoxane. All analyses were done using SAS (version 9.2).

Results

For the 184 high-risk ALL patients, the median time from registration on the original ALL therapy protocol to enrollment on the HFE testing protocol was 7.0 years (range, 1.1–17.1). Of these, 68 (37%) had received doxorubicin alone and 116 (63%) had received dexrazoxane and doxorubicin (Table 1).

Genetic detection was successful for 172 patients for H63D and 179 patients for C282Y. Two patients (1%) were homozygous and 39 (23%) were heterozygous for H63D. One (<1%) was homozygous and 17 (9%) were heterozygous for C282Y. Of the 167 patients with results for both tests, 113 (68%) were homozygous wild-type at both loci.

The percentage of patients (31%) with two or more abnormal cTnT concentrations during doxorubicin therapy was higher in C282Y carriers than in non-carriers (6%) (Table 2). Multiple elevations in cTnT concentrations were associated with carriers of the C282Y allele (OR: 7.23 [95% CI, 1.78–29.4]; p=0.006) in univariate analyses. This association remained significant after adjusting for dexrazoxane treatment (adjusted OR: 9.21 [1.11–76.5; p=0.039]). No associations were found with NT-proBNP. Neither H63D heterozygosity nor homozygosity was associated with either cardiac biomarker (data not shown).

At a median of 2.2 years (range 1.0–3.99) after diagnosis, mean *Z*-scores for LV mass and end-systolic posterior wall thickness were significantly worse than normal for all children (carriers: mean [SE] *Z*-score, −0.84 [0.17], p<0.001 and −4.36 [0.26], p<0.001, respectively; non-carriers: −0.45 [0.15], p=0.006 and −4.06 [0.17], p<0.001, respectively). However, carriers alone also had abnormally low mean [SE] *Z*-scores for LV fractional shortening (−0.71 [0.25], p=0.008) and end-diastolic posterior wall thickness (−0.68 [0.25], p=0.01) (Table 3). Although power to detect such differences was less in later follow-up due to the number of echocardiograms available, similar results were obtained and no differences in *Z*-scores between carriers and non-carriers were detected. The median follow-up for the 114 patients with a post-baseline assessment was 6.1 years (1.0–16.1).

Discussion

In this study of survivors of childhood high-risk ALL, heterozygosity for C282Y was associated with multiple elevations of cTnT concentrations after controlling for dexrazoxane treatment. Furthermore, compared to a normal population, patients with the C282Y and/or

H63D allelic variants had significantly lower LV function, mass, and wall thickness 2 years after diagnosis.

The HFE prevalence in our study is similar to the US population, where the estimated prevalence for C282Y-H63D heterozyogosity is between 1.5% and 2.5%.[25] For the C282Y mutation, the prevalence is 7–9% for heterozygotes and 0.12–0.5% for homozygotes.[25] For the H63D mutation, the prevalence is 20–23% for the heterozygotes and 1.5–2.4% for the homozygotes.[25]

In vitro and animal studies suggest that iron-loaded tissues enhance anthracycline cardiotoxicity, which increases concentrations of markers of cellular damage.[12,26] HFE gene mutations predispose rodents to doxorubicin-induced cardiotoxicity.[26] Mice with a targeted mutation of the HFE gene (homozygotic $HFE^{-/-}$ mice) were iron-overloaded in multiple organs, including the heart, compared to wild-type controls.[26] In addition, when treated with doxorubicin, $HFE^{-/-}$ mice had concentrations of serum markers for acute cardiac injury, mitochondrial damage, myofibril degeneration, and mortality that were significantly higher than those in wild-type mice. In HFE heterozygotic mice ($HFE^{+/-}$), chronic doxorubicin administration caused mitochondrial degeneration and increased mortality rates, although not to the same extent as in $HFE^{-/-}$ animals.[26] This result suggests that homozygous or heterozygous mutations of HFE or perhaps even of other genes linked to increased iron stores and HH may increase susceptibility to doxorubicin cardiotoxicity.

The magnitude of the *Z*-score differences in this study, while small relative to those that guide the daily clinical decisions by cardiologists, are consistent with findings in doxorubicin-treated pediatric cancer survivors,[4–6] who years later have increased rates of CHF and cardiac mortality compared with controls.[27] Although there were no significant differences between carriers and non-carriers in our study, the *Z*-scores are characteristic of anthracycline-associated cardiomyopathy in that acute evidence of injury is often followed by normalization of ventricular size and function, presumably by ventricular remodeling (cellular hypertrophy compensating for cell loss). Nonetheless, late evidence of abnormal ventricular structure with reduced LV wall thickness is a frequent outcome.[4] Furthermore, the risk of anthracycline-related CHF may be modified by the presence of HFE variants, as shown in anthracycline-treated survivors of hematopoietic cell transplantation.[28]

Cascales et al. retrospectively evaluated cardiac iron, cardiac events, and HFE genotypes (C282Y and H63D) in 97 consecutive necropsies of cadavers with solid and hematological cancers, 48 of which had been anthracycline-treated and 49 who received no chemotherapy (n=25) or non-anthracycline chemotherapy (n=24).[29] Cases treated with cumulative anthracycline doses >200 mg/m^2 had higher heart iron concentrations (490 versus 240 μg/g dry weight; p=0.01), independently of liver iron load or transfusion history, than did controls. Mutations in HFE were associated with higher heart tissue iron deposits, but not with global cardiac events or CHF. Multivariate linear regression showed that both HFE genotypes and anthracyclines contributed to cardiac iron concentrations (R^2=0.284), a result suggesting cardiac iron accumulation modulation by both anthracyclines and HFE, supporting the importance of HFE status as a predictor of anthracycline cardiotoxicity.[29]

Myocardial injury (elevated cTnT) during doxorubicin-therapy in patients with C282Y alleles puts them at greater risk for subsequent reduced LV wall thickness and mass due to loss of cardiomyocytes during therapy.

Carriers had significantly reduced LV function 2 years post-ALL diagnosis compared to healthy children, a finding only marginally observed when ALL non-carriers were compared with healthy children. This suggests that the remaining cardiomyocytes are adversely affected by the doxorubicin-HFE carrier combination, going beyond cardiomyocyte demise

during therapy (elevated cTnT concentrations) resulting in late reduced LV wall thickness and mass being the sole type of late doxorubicin cardiotoxicity. Unhealthy residual myocardium in doxorubicin-treated long-term ALL survivors with HFE alleles is supported by a reduced LV thickness-to-dimension ratio, a trend consistent with early pathologic LV remodeling in this population. Such an association has been described in non-cancer HFE carriers with idiopathic dilated cardiomyopathy.[13]

Patients with C282Y homozygosity more frequently have genetic variations in antioxidant enzymes and mitochondrial DNA, which may increase susceptibility to myocardial iron-induced oxidative stress and dysfunction, respectively.[30,31]

Dexrazoxane iron chelation therapy may prevent the enhanced doxorubicin-mediated damage in iron-overloaded heart.[32] Children with high-risk ALL treated with dexrazoxane before doxorubicin have fewer and milder subclinical biomarker and echocardiographic signs of cardiotoxicity.[32–38]

Identifying all high-risk patients for anthracycline-induced cardiotoxicity is difficult since factors known to increase this risk do not identify many patients who subsequently develop cardiomyopathy.[6,8] Although generalized population screening for HFE mutations is not recommended, case-finding by employing HFE screening in high-risk groups to detect affected individuals might be beneficial.[39] Screening for HFE mutations common in children with newly-diagnosed high-risk ALL to be treated with anthracyclines might inform treatment decisions regarding chemotherapy, cardioprotectant therapy, transfusions, and magnetic resonance imaging to determine early myocardial iron overload.[13,40]

Acknowledgments

We thank all of the patients and families involved in this study, as well as the data managers and study personnel from the 8 participating centers for this study in the DFCI Consortium.

Funding/Support: This study was supported in part by grants from the National Institutes of Health (HL072705, HL078522, HL053392, CA127642, CA068484, HD052104, AI50274, HD052102, HL087708, HL079233, HL004537, HL087000, HL007188, HL094100, HL095127, HD80002), the Children's Cardiomyopathy Foundation, the Women's Cancer Association, the Lance Armstrong Foundation, the STOP Children's Cancer Foundation, the Scott Howard Fund, and the Michael Garil Fund.

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Table 1

Patient characteristics

	n (%)
Number of patients evaluable	184
Time from original protocol registration to 05-159, years, median (range)	7.0 (1.1–17.1)
Sex	
Male	101 (55)
Female	83 (45)
Age at diagnosis, median (range)	6.3 (<1–17.9)
<10 years	109 (59)
≥10 years	75 (41)
Age at time of enrollment on DFCI Protocol 05-159, median (range)	15.2 (3.1–31.4)
<10 years	44 (24)
≥10 years	140 (76)
Immunophenotype at diagnosis	
T-cell	34 (18)
B-cell	150 (82)
White blood cell count at diagnosis (k/μl), median (range)	20.1 (1.3–740.4)
Serum iron concentration (μg/dL)†	75.0 (15.0–235.0)
Serum ferritin concentration (ng/mL)†	67.2 (7.21–1998.0)
Treatment	
Doxorubicin	68 (37)
Dexrazoxane before doxorubicin	116 (63)
Cumulative doxorubicin dose (mg/m^2), median (range)	300 (204–420)
Time from registration on original protocol to post-baseline echocardiogram, years, median (range)	6.1 (1.0–16.1)
No. Echocardiograms	
T1 (1–3.99 yrs from Registration)	95 (52)
T2 (4–6.99 yrs from Registration)	53 (29)
T3 (7+ yrs from Registration)	47 (26)

†Data from only 97 patients available. T=time.

Table 2

Allelic variants by patient characteristics

	Combined H63D/C282Y (n=167)			H63D (n=172)			C282Y (n=179)		
Characteristics	**Carrier (n=54)**	**Non-Carrier (n=113)**	**p value**	**Carrier (n=41)**	**Non-carrier (n=131)**	**p value**	**Carrier (n=18)**	**Non-carrier (n=161)**	**p value**
Age at Diagnosis, years median (range)	6.8 (<1–17.9)	6.1 (<1–17.1)	0.51	9.8 (1.5–17.6)	6.1 (<1–17.9)	0.22	3.9 (<1–17.6)	7.0 (<1–17.6)	0.44
Age at Enrollment, years median (range)	15.2 (4.6–31.4)	15.2 (3.1–27.2)	0.50	15.8 (6.4–31.4)	15.2 (3.1–29.2)	0.27	14.3 (4.6–29.2)	15.3 (3.1–31.4)	0.61
Sex, Male, n (%)	33 (61)	58 (51)	0.25	23 (56)	71 (54)	0.86	13 (72)	85 (53)	0.14
Treatment, n (%)									
Doxorubicin only	20 (37)	41 (36)	0.99	14 (34)	49 (37)	0.85	9 (50)	57 (35)	0.30
Dexrazoxane Before Doxorubicin	34 (63)	72 (64)		27 (66)	92 (63)		9 (50)	104 (65)	
Cumulative Doxorubicin Dose (mg/m^2), median (range)	300 (204–382)	300 (240–366)	0.54	300 (204–382)	300 (240–420)	0.79	300 (288–360)	300 (204–382)	0.82
Number of Patients Evaluable	38	83		29	95		13	114	
Multiple Abnormal cTnT Measurements During Doxorubicin Treatment	4 (11)	7 (8)	0.74	1 (3)	10 (11)	0.46	4 (31)	7 (6)	**0.015**
Other *	34 (89)	76 (92)		28 (97)	85 (89)		9 (69)	107 (94)	
Number of Patients Evaluable	38	82		29	94		13	113	
Multiple Abnormal NT-proBNP Measurements During Doxorubicin Treatment	33 (87)	72 (88)	0.99	25 (86)	82 (87)	0.99	11 (85)	100 (88)	0.65
Other *	5 (13)	10 (12)		4 (14)	12 (13)		2 (15)	13 (12)	

* Other: patients with no abnormal cTnT/NT-proBNP biomarker measurements or < 2 abnormal measurements

Table 3

Left Ventricular Structure and Function *Z*-scores from echocardiograms

Left Ventricular Measurement	Combined H63D/C282Y				
	HFE Carrier Mean (SE)	Carrier vs. Normal p value	HFE non-carrier Mean (SE)	Non-carrier vs. Normal p value	Carrier vs. Non-carrier p value
T1 (1–3.99 years)					
End-Systolic Dimension	0.28 (0.17)	0.11	0.02 (0.21)	0.90	0.41
End-Diastolic Dimension	−0.09 (0.16)	0.55	−0.27 (0.19)	0.16	0.54
Fractional Shortening	−0.71 (0.25)	**0.008**	−0.60 (0.30)	0.053	0.81
Thickness-to-Dimension Ratio	−0.50 (0.33)	0.12	0.29 (0.31)	0.32	0.09
Mass	−0.84 (0.17)	<**0.001**	−0.45 (0.15)	**0.006**	0.14
End-Systolic Posterior Wall Thickness	−4.36 (0.26)	<**0.001**	−4.06 (0.17)	<**0.001**	0.33
End-Diastolic Posterior Wall Thickness	−0.68 (0.25)	**0.011**	−0.06 (0.22)	0.77	0.08
T2 (4–6.99 years)					
End-Systolic Dimension	0.20 (0.34)	0.56	0.62 (0.23)	**0.012**	0.29
End-Diastolic Dimension	−0.43 (0.39)	0.28	0.25 (0.17)	0.15	0.08
Fractional Shortening	−0.93 (0.27)	**0.003**	−1.05 (0.53)	0.054	0.86
Thickness-to-Dimension Ratio	0.07 (0.31)	0.82	−0.63 (0.28)	**0.031**	0.11
Mass	−0.96 (0.32)	**0.009**	−0.79 (0.21)	<**0.001**	0.64
End-Systolic Posterior Wall Thickness	−4.63 (0.34)	<**0.001**	−4.13 (0.21)	<**0.001**	0.20
End-Diastolic Posterior Wall Thickness	−0.29 (0.39)	0.46	−0.61 (0.27)	**0.032**	0.49
T3 (7-7+ years)					
End-Systolic Dimension	−0.08 (0.27)	0.77	0.55 (0.27)	0.051	0.18
End-Diastolic Dimension	−0.20 (0.38)	0.62	0.24 (0.21)	0.26	0.29
Fractional Shortening	−0.27 (0.45)	0.56	−0.59 (0.27)	**0.033**	0.51
Thickness-to-Dimension Ratio	−1.13 (0.52)	**0.045**	−0.94 (0.19)	<**0.001**	0.66
Mass	−1.15 (0.59)	0.092	−1.04 (0.32)	**0.004**	0.87
End-Systolic Posterior Wall Thickness	−4.16 (0.86)	**0.005**	−4.20 (0.26)	<**0.001**	0.95
End-Diastolic Posterior Wall Thickness	−0.77 (0.56)	0.20	−1.08 (0.22)	<**0.001**	0.54