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HIV Infected Children in Malawi have Decreased Performance on the 6 Minute Walk Test with Preserved Cardiac Mechanics Regardless of Antiretroviral Treatment Status

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Abstract

Introduction—We aimed to (1) determine if cardiac disease can be detected in HIV-infected children by strain imaging, and (2) to evaluate differences in exercise performance between HIV-infected on and not yet on antiretroviral therapy (ART) and in HIV-uninfected children by 6-minute walk tests (6MWT).

Methods—This cross-sectional study evaluated cardiac function by echocardiogram and exercise performance by 6MWT in HIV-infected and uninfected children ages 4 to 18 years in Lilongwe, Malawi. Analyses compared HIV-uninfected, HIV-infected not yet on ART, and HIV-infected on ART. Comparisons used Chi-square, t-test, ANOVA, and multiple linear regression.

Results—No differences were found in ejection fraction (EF), shortening fraction (SF), or strain in 73 children not yet on ART, 149 on ART, and 77 HIV-uninfected controls. As viral load (VL) increased, children had worse circumferential strain. In addition, children receiving ART had better circumferential strain than those not yet on ART. Increased CD4 percentage was associated with better longitudinal strain and farther 6MWT distance. As longitudinal strain worsened, the 6MWT distance decreased. HIV-infected children not yet on ART walked a mean of 25.8m less than HIV-uninfected children, and HIV-infected children on ART walked 25.9m less ($p=0.015$ comparing 3 groups).

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Conclusions—HIV-uninfected children performed better on the 6MWT than HIV-infected children. Lower VL, being on ART, and higher CD4% were associated with better strain measures. Better longitudinal strain was associated with a farther 6MWT distance. Overall, EF, SF and strain measures between groups were similar, so cardiac strain did not detect cardiac dysfunction in this young population.

Keywords

speckle-tracking; strain imaging; echocardiography; pediatric HIV; cardiac function

INTRODUCTION

Before the availability of antiretroviral therapy (ART), children with HIV often suffered from severe cardiac sequelae, including pericarditis, myocarditis, cardiomyopathy, pulmonary arterial hypertension, valvular disease, aortic dilation, and arrhythmias(1–4). In children, one third of HIV-related deaths in the pre-ART era could be attributed to cardiac causes (2, 3). Even as ART has decreased the burden of cardiac disease in children infected with HIV in both resource rich and limited settings(1), concern has shifted to cardiac complications that present later in life(5). HIV-infected children are often exposed to ART while the cardiovascular system is still developing and sub-clinical cardiac dysfunction detected in childhood could develop into symptomatic cardiomyopathy in adulthood (6). Asymptomatic HIV infection in adults often manifests mild functional and morphologic cardiac abnormalities (5, 7). In addition, lipid and metabolic derangements have been commonly found in the HIV infected population, both with and without ART (8). Adolescents perinatally infected with HIV have been shown to have an increased atherosclerotic cardiovascular disease risk (9).

Multiple studies have demonstrated the accuracy and reliability of strain and strain rate in the assessment of myocardial function (12–18). Global strain has been validated in the pediatric population using tagged magnetic resonance imaging as the gold standard (19).

Strain analysis uses speckle tracking to track the displacement and velocities of acoustic markers of individual segments of the myocardium as they move. Strain measures the regional fractional shortening of the myocardium, and strain rate is the integration of strain over time. Strain can be measured in the circumferential, longitudinal, and radial directions. We chose to only measure longitudinal and circumferential strain and strain rate because radial strain has been shown to be less reproducible and more error-prone (20).

Strain and strain rate echocardiographic imaging may provide a means for earlier detection of cardiac dysfunction than traditional measures of cardiac function such as ejection fraction (EF) and shortening fraction (SF) (10) in this population. Abnormal strain or strain rates may indicate that patients are at an increased risk of cardiac events and require more vigilant cardiac follow-up.

The six-minute walk test (6MWT) evaluates how far the person being tested can walk in six minutes. The ability to walk for a set time is a quick, easy and inexpensive way to assess

physical function. The 6MWT has been validated as a reliable test for assessing exercise tolerance and endurance (11).

Malawi is a small land-locked country in Southeast Africa with a population of over 16 million people, with over 1 million people infected with HIV, and an adult HIV prevalence rate of 10.3% (21). The government has made HIV a priority, and access to free antiretroviral medications among adults and children is improving.

We conducted a cross-sectional study to evaluate differences in traditional cardiac functional measures, strain, and strain rate as determined by echocardiography and in exercise performance as determined by a 6MWT between HIV-infected children on ART, HIV-infected children not yet on ART and HIV-uninfected children in Malawi. For the age group that we studied, Malawi national guidelines recommended starting ART in children who were WHO stage 3 or 4, or who had a CD4 count of less than 350 cells/mm³. We also explored if there was a relationship between HIV-specific factors such as HIV RNA (hereby referred to as viral load, or VL) and CD4 count and EF, SF, strain, strain rate and 6MWT.

MATERIALS AND METHODS

HIV-infected children ages 4 to 18 years were recruited among patients attending routine outpatient visits at the Baylor Children's Clinical Center of Excellence (COE) in Lilongwe, Malawi between November 2011 and May 2012. Recruitment took place each clinic day except Wednesday and Friday (when the PI had other clinical duties). Target sample size was 250 HIV positive and 100 controls. Our sample recruited provided 90% power to detect a standard effect size of 0.49 in the one-way ANOVA analyses.

Inclusion criteria included ages 4 through 18, and perinatally acquired HIV. Participants were recruited from those coming for regularly scheduled (not sick) visits. Children with known or suspected heart problems were not specifically excluded.

The Baylor COE is an outpatient pediatric HIV clinic in Lilongwe, Malawi that serves as a pediatric HIV referral center for the entire country and provides care for over 3200 infants, children, and adolescents with HIV infection or exposure. Potential study participants' clinical files were taken from the file queue of children waiting to be seen for outpatient visits after the previous study patient was finished. If the age of the patient met study criteria, the patient was approached, together with their guardians, for study participation. Not all eligible children each day were approached because of the time it took to complete the echo and walk test.

HIV-uninfected control participants were recruited from the community, children of clinic staff, or HIV-uninfected siblings of participants. An HIV test was performed if there was not already one documented. A repeat test was done if the participant was over 12 years old and one had not been performed within the past 30 days.

Each echocardiogram was performed by the study primary investigator (PI) (a pediatric cardiologist) on a CX50 echocardiography machine (Philips Medical Systems, Bothell, WA, USA) with a 5 MHz phased array transducer. Echos were performed without sedation.

Frame rates on all studies were performed within a range of 60–80 frames/sec. Studies were saved under a study number on the echo machine. Clinical factors, such as current medical problems and ART history were obtained through interviews and reviewing the medical record. A physical exam was then performed. The physical exam included vital signs (heart rate, blood pressure, respiratory rate, pulse oximetry, height and weight), as well as a general exam, which included a cardiac exam, respiratory exam, and abdominal exam. The patient's nadir CD4 count, current CD4 count and viral load were abstracted from the medical records of children with HIV infection. Labs were drawn if they had not been obtained for clinical purposes within the previous six months.

6MWTs were performed on children ages 5 and above. A 25-meter course was set up behind the clinic. The course was marked with cones at either end, and children were asked to walk between the cones for six minutes. They were told to stop after 6 minutes, and the distance walked was measured. The output measure was how far each child walked in six minutes. A "better" result was a longer distance walked. The walk test was performed following the echocardiogram. Study data was collected and managed using REDCap electronic data capture tools hosted at Children's National Medical Center, Washington, DC (22).

Laboratory measurements performed on the HIV-infected study participants included CD4 T-cell count and percentage, and viral load by reverse transcription polymerase chain reaction (using an Abbott 2000 platform). Labs were drawn at the time of the visit if they had not been obtained in the previous 6 months. Laboratory tests were performed at the University of North Carolina Project laboratory in Lilongwe, Malawi.

The PI read each echocardiogram off-line. The echocardiograms were saved under study numbers and batched so the PI who performed the echo did not know the HIV status of the echocardiogram being read offline. General echocardiographic parameters were measured and recorded for each participant and control. Traditional measures of left ventricular (LV) systolic function such as ejection fraction (EF) and shortening fraction (SF), as well as indicators of diastolic function such as mitral inflow E/A ratio and tissue Doppler-derived E/E' ratio were evaluated. LV EF was calculated using the single-plane modified Simpson's rule in the four-chamber view. LV mass was calculated from M-mode tracings using the method of the American Society of Echocardiography in the short-axis view (23). Tricuspid regurgitation velocity as an indicator of pulmonary artery pressure was also measured. Tissue Doppler velocities were measured from the septal annulus from a single representative beat. All measurements were performed by a pediatric cardiologist according to American Society of Echocardiography standards (23).

Global circumferential and longitudinal speckle-tracking derived strain and strain rate were measured and recorded off-line using Philips Qlab software. Each study was analyzed from the parasternal short-axis and apical four-chamber views. Each tracing was performed during end systole as the endpoint, using end diastole as a reference. The short-axis view was analyzed at the level of the papillary muscles. Six segments were used to calculate global strain from the short-axis and apical four-chamber views. Short-axis measurements included circumferential strain (%) and circumferential strain rate (1/sec). Longitudinal

strain (%) and longitudinal strain rate (1/sec) were recorded from the apical four-chamber view.

Statistics

Summary statistics were stratified by the following groups: HIV-uninfected, HIV-infected not yet on ART, and HIV-infected on ART. Comparisons between groups were performed using Chi-square test, t-test, and ANOVA as appropriate. Linear regression was performed to evaluate the difference in outcomes (strain measures, traditional echo function measures, and 6 minute walk distance) between groups adjusting for age, sex, and body mass index (BMI). When group was significant overall all pairwise comparisons between the three groups were performed adjusting for multiple comparisons using Tukey's method. Linear regression was also used to evaluate the association between the outcomes and log transformed viral load, CD4 count, CD4 %, nadir CD4 count, and history of tuberculosis among the HIV-infected groups adjusting for age, sex, BMI, and ART use. All analyses assumed independent observations.

Ethics

This study was approved by four IRB's: the Malawi National Health Sciences Research Committee (Lilongwe, Malawi), Children's National Medical Center (Washington, D.C.), Baylor College of Medicine (Houston, TX), and University of North Carolina (Chapel Hill, NC).

RESULTS

Of 226 eligible HIV-infected participants that were asked to participate, 222 children were enrolled. Three chose not to participate because of time constraints, and one was excluded because HIV was likely not perinatally acquired. Of the 77 control participants, 21 were HIV-uninfected siblings of study participants. Four of those children were exposed to single dose nevirapine in utero, and one had a mother who was on ART (lamivudine/stavudine/nevirapine) during pregnancy.

HIV-uninfected children were slightly older and had higher BMI than the HIV-infected patients (Table 1). Average log viral load and nadir CD4 count were higher amongst HIV positive participants not yet on ART ($p<0.001$ and $p=0.01$). CD4 count and CD4 % were higher amongst those on ART ($p<0.001$ and $p<0.001$) (Table 2). The distribution of HIV stage differed based on ART use ($p<0.001$) with more participants on ART being WHO stage 3 or 4. Average time on ART was 1262 days (range 15–4084 days, SD = 775). Zidovudine (AZT) use occurred in 56% of the participants on ART for an average of 239 days (range 15–2080 days, SD 370).

Echocardiography

Traditional echocardiograms revealed abnormalities in 8.6 % (19/222) of HIV-infected participants, including 2 children with more than one abnormality. 7 (9.6%) of HIV+ children not yet on ART and 12 (8%) of HIV+ children on ART were abnormal. 6 children had mildly decreased ejection fraction, 5 had diastolic dysfunction, 4 had increased LV Mass

Index, 3 had pericardial effusions (all small), 1 had moderately increased pulmonary pressure, 1 had a mildly dilated aortic root, 1 had a moderate patent ductus arteriosus, and 1 was found to have borderline rheumatic heart disease (RHD). This is compared to a single HIV-uninfected child (1/77, 1.3%) with an abnormality (borderline RHD). There was no significant difference in the proportion of children with any traditional echocardiographic abnormality between the 3 groups ($p = 0.06$) by Chi-squared analysis. There was no difference between EF ($p = 0.21$) and SF ($p = 0.98$) among groups (Table 3).

Before adjustment for age, sex, and BMI, there was a significant difference in global circumferential strain (GCS) between cohorts ($P=0.04$). Both HIV+ groups tended to have worse GCS than the control group. There was no difference between groups in terms of circumferential strain rate, global longitudinal strain, or longitudinal strain rate. After adjusting for age, BMI and sex, there were no statistically significant differences between groups for EF, SF, or any strain or strain rate measurement.

Exercise Performance

The HIV uninfected group performed better (walked a longer distance) on the 6 minute walk test than the HIV infected group on ART and the HIV infected group not yet on ART ($p<0.001$ before adjusting for age, sex, and BMI, $p = 0.01$ after adjustment) (Table 3, Figure 1). Children who are HIV-infected and not yet on ART walked a lower average distance of 25.8 m (adjusted for age, sex, and BMI), compared to HIV uninfected children (95% CI= -51.6, -0.01; $p=0.05$) while the HIV infected children on ART walked a lower average distance of 25.9 m (adjusted for age, sex, and BMI) compared to the HIV-uninfected group (95% CI=-47.8, -4.1; $p=0.015$). P-values are Tukey's adjusted p-values. The average distance is 0.12 m lower among the HIV-infected with ART group compared to the HIV-infected not yet on ART group, but is not statistically significant (95% CI=-22.22, 21.98; $p=0.999$).

Longitudinal strain is associated with 6 minute walk distance in both unadjusted and adjusted analyses. As longitudinal strain increases (worsens) by 1%, the 6 minute walk distance decreases by 3.6 meters ($p=0.02$) in unadjusted analyses and decreases by 4 meters in adjusted analyses (adjusting for group, age, BMI, gender). Circumferential strain ($p=0.11$), circumferential strain rate ($p=0.07$), and longitudinal strain rate ($p=0.6$) are not associated with 6 minute walk in unadjusted or adjusted analyses.

HIV Specific Measures (Table 4)

Among the HIV-infected group, EF and SF were not significantly associated with CD4 count or log VL. In the HIV infected group, there were several HIV specific measures associated with strain, even after adjusting for age, BMI, and sex. For a one-unit increase in log viral load the average circumferential strain increased (worsened) by 0.62% ($p=0.05$). Average circumferential strain is 1.39 % lower (better) for those on ART compared to those not on ART ($p=0.046$). For a one-percentile increase in CD4 % the average longitudinal strain decreases (improves) by 0.06% ($p=0.009$). For a one-percentile increase in CD4 % the average distance covered in a 6 minute walk increases (improves) by 1.21m ($p=0.011$). Time on ART was not significantly related to any immunologic measures.

DISCUSSION

HIV-infected and HIV-uninfected children had similar traditional function (EF and SF) and strain measures regardless of ART status. Some HIV-infected children were found to have mild cardiac abnormalities detected by traditional echocardiography. We also found that the HIV infected group (both on and not yet on ART) had decreased exercise performance compared to the HIV-uninfected group.

Because it is a relatively new technology, there are limited established normal range values for strain and strain rate in this age group using QLab software; therefore, it is difficult to assess which of the strain values should be considered abnormal on their own. That is why we chose to compare to a group of HIV uninfected peers.

After correcting for age, sex, and BMI, there was no difference in traditional (EF and SF) or strain function measures between the HIV uninfected and HIV infected groups. It is possible, however, that the difference between groups would be significant if our study had a larger sample size and greater statistical power as we did find a difference in global circumferential strain between groups before adjustment. Our current results are different from what was found in a previous study of a group of 28 HIV infected children and young adults with HIV acquired at a young age (ages 7 to 32 years) followed at the National Institutes of Health. Compared to healthy controls, the HIV infected group in that study had abnormal strain and strain rate, despite having normal traditional measures of cardiac function. In that smaller study, the participants were from a different era of HIV care and had exposures to many different regimens of antiretroviral medications. Nevertheless, the previous results were noteworthy in that the study population was significantly more likely than healthy controls to have subclinical cardiac dysfunction ($p<0.05$), which was not previously detected by traditional measures of cardiac function (24).

Another group also found that HIV infected children and young adults had decreased longitudinal strain and strain rate when compared to controls, and their average age was 17 years (25), which is much older than our average age of 9.5 years. It is notable that both of these studies were performed in high-resource settings.

HIV-uninfected children performed better on the 6MWT when compared to the HIV infected children. The 6MWT has been shown to be a reliable and reproducible measure for assessing exercise tolerance and endurance in children (11). While our results do not necessarily reflect cardiac function, they do represent a difference in exercise capacities between children. HIV infected children often have repeated lung infections, and this may play a role in their decreased performance on the 6-minute walk test. Our 6MWT results agree with a study performed in the U.S., which has shown that HIV-infected children had, in general, lower measures of fitness compared to uninfected children (26).

The only strain measure that was associated with 6MWT distance was longitudinal strain. Children with a “better” longitudinal strain walked farther on the 6MWT. This is an interesting association that should be explored further.

Amongst the HIV infected group (after adjusting for age, sex, and BMI), there were several HIV measures that were associated with outcomes (Table 4). These associations were evaluated for exploratory purposes only. With an increase in VL, there is worse circumferential strain. Also, circumferential strain was better in children on ART compared to those not on ART. An increase in CD4 percentage is associated with better longitudinal strain. Finally, a higher CD4 percentage is associated with better performance on the 6MWT. Thus, differences in CD4 percentage and VL were reflected in certain strain measurements and 6MWT performance.

Our study is limited in that it was a cross sectional study looking at one point in time, therefore causal inferences cannot be made. In addition, we included HIV-uninfected siblings in our control group, which may have resulted in underestimated variances for response measures. However, only 21 HIV uninfected siblings were included in this study, and results are not likely to be different if completely independent measures had been observed. We thought this was valid because a previous study has shown that cardiac function was similar in HIV uninfected but exposed infants after 2 years of age (27). In addition, only children aged 4 years and older were considered for our study because of limitations of the size of the echocardiography probe that was used.

Frame rate differences may have the potential to alter strain results at very low or high frame rates, but all studies were performed within a range where this was unlikely to happen (60–80 frames/sec)(28). We did not have a second reader for this study because strain has been shown to be reader-independent and we have demonstrated that there was no significant inter and intra- observer variability in the past (24, 29).

Our study's strengths include that it is the largest study to date analyzing strain in HIV infected children, and to our knowledge this is the only study that has included a functional measure such as the 6MWT. In addition, our study was performed in Africa; therefore our results are applicable to those most affected by the HIV epidemic. Many children in Africa are started on ART later than in the U.S., so our study was also able to include a large number of HIV infected but ART-naïve children. Reasons for delayed initiation of ART at the time of the study included a delay in HIV diagnosis and country-specific policies that required that any HIV infected individual qualify for ART based on CD4 count or WHO stage. Another strength to our study is that all data was obtained prospectively.

Prolonged exposure to ART improves survival but may also increase the risk of cardiovascular events (6). This may explain why other studies have seen difference in strain when comparing older HIV infected to HIV uninfected children, but we did not observed a difference amongst our current, significantly younger, population after adjusted analysis.

CONCLUSION

It is likely that the HIV positive group studied is too young to manifest early signs of cardiac dysfunction. Strain imaging may be a helpful predictor of early cardiac dysfunction in an older population of HIV-infected youth. We do advocate for routine echocardiographic

monitoring in this population as many cardiac abnormalities were detected through traditional echocardiography.

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Figure 1.
6 Minute Walk Test Distance

Table 1

Demographics

	All participants N=299	HIV – N=77 (26%)	HIV + no ART N=73 (24%)	HIV + ART N=149 (50%)	p-value
Demographics					
Age at enrollment (years), Mean (SD)	9.4 (3.6)	10.2 (4.2)	8.8 (2.9)	9.3 (3.4)	0.045
Male, N(%)	154 (52%)	37 (48%)	42 (58%)	75 (50%)	0.47
BMI, Mean (SD)	15.8 (2.1)	16.3 (2.8)	15.4 (1.7)	15.7 (1.8)	0.033

Table 2**Lab Values Of HIV+ Participants**

HIV measures	All participants	HIV+ no ART	HIV+ on ART	p-value
log Viral Load, Mean (SD) N=215	8.75 (2.90)	11.67 (1.45)	7.32 (2.29)	<0.001
CD4 count, Mean (SD) N=215	792 (504)	551 (309)	911 (540)	<0.001
Nadir CD4 count, Mean (SD) N=220	403 (256)	466 (278)	373(239)	0.01
CD4 %, Mean (SD) N=208	28.3 (11.2)	21.8 (9.3)	31.4 (10.7)	<0.001
Average time on HAART	-	-	1262 days (775)	-
AZT use	-	-	56%, avg 239 days (370)	-

Table 3

Outcomes (unadjusted)

Walk Test	All Patients	HIV negative	HIV + No ART	HIV + on ART	p-value
6 minute walk distance (m), Mean (SD) N=267	489 (78.9)	521 (82.3)	475 (71.4)	480 (76.6)	<0.001
Traditional Echo Measurements					
Ejection Fraction (%), Mean (SD) N=295	61.5 (5.6)	62.3 (5)	60.6 (6.6)	61.5 (5.3)	0.21
Shortening Fraction (%), Mean (SD) N=282	34.3 (4.8)	34.4 (4.6)	34.4 (5.5)	34.3 (4.6)	0.98
Strain Measurements					
Global Circumferential Strain (%), Mean (SD) N=264	-23.7 (4.2)	-24.1 (4)	-22.5 (4.4)	-24 (4.1)	0.04*
Global Longitudinal strain (%), Mean (SD) N=282	-19.5 (3.1)	-19 (3.2)	-19.5 (2.8)	-19.4 (3.2)	0.75
Circumferential Strain rate (1/s), Mean (SD) N=262	-1.54 (0.29)	-1.50 (0.28)	-1.55 (0.27)	-1.56 (0.31)	0.46
Longitudinal strain rate (1/s), Mean (SD) N=282	-1.22 (0.27)	-1.20 (0.28)	-1.28 (0.32)	-1.21 (0.24)	0.14

Global Circumferential Strain (%)	Coef.	[95% Conf. Interval]	P>t
HIV – Reference			0.1039
HIV+ no ART	1.324682	-0.12852 2.77788	0.074
HIV+ ART	-0.01662	-1.18928 1.156034	0.978

* After Adjustment for Age, BMI, and Gender

Table 4
HIV specific measures associated with outcomes (adjusted for age, BMI, gender, and ART)

For a one unit increase in log viral load, global circumferential strain increases (worsens) by 0.27%. Global circumferential strain rate is 1.39 u lower (better) for those on ART compared to those not on ART. For a one %ile increase in CD4 %, global longitudinal strain decreases (improves) by 0.06%. For a one %ile increase in CD4 % the average distance covered in a 6 minute walk increases (improves)by 1.21m.

Global Circumferential strain	Coef.	[95% Conf.	Interval]	P>t
Log viral load	0.27	−0.0002	0.54	0.05
Global Circumferential Strain Rate	Coef.	[95% Conf.	Interval]	P>t
ART use	−1.39	−2.75	−0.03	0.046
Global Longitudinal Strain	Coef.	[95% Conf.	Interval]	P>t
CD4 percentage	−0.06	−0.1	−0.01	0.009
Distance covered 6 min	Coef.	[95% Conf.	Interval]	P>t
CD4 percentage	1.21	0.29	2.13	0.011