Published in final edited form as:

Ear Hear. 2016 ; 37(4): 443–451. doi:10.1097/AUD.0000000000000276.

Auditory impairments in HIV-infected children

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Abstract

Objectives—In our cross-sectional study of human immunodeficiency virus (HIV)-infected adults, we showed lower distortion product otoacoustic emissions (DPOAEs) in HIV+ individuals compared to controls as well as findings consistent with a central auditory processing deficit in HIV+ adults on anti-retroviral therapy. We hypothesized that HIV+ children would also have a higher prevalence of abnormal central and peripheral hearing test results compared to HIV– controls.

Design—Pure-tone thresholds, DPOAEs, and tympanometry were performed on 244 subjects (131 HIV+, and 113 HIV–, subjects). Thirty-five of the HIV+, and 3 of the HIV–, subjects had a history of tuberculosis treatment. Gap detection results were available for 18 HIV– and 44 HIV+ children. Auditory brainstem response (ABR) results were available for 72 HIV– and 72 HIV+ children. Data from ears with abnormal tympanograms were excluded.

Results—HIV+ subjects were significantly more likely to have abnormal tympanograms, histories of ear drainage, tuberculosis, or dizziness. All audiometric results were compared between groups using a two-way ANOVA with HIV status and ear drainage history as grouping

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Conflicts of Interest

No conflicts of interest.

Statement of author contribution: I.I.M., was primarily responsible for overseeing the study in Tanzania, and assisted with the design of the experiment and data collection. M.B., M.M., and N.M. assisted with the designing the experiment, overseeing data collection in Tanzania, and analyzing data. O.H.C. and J.C.W. assisted with the development of the audiological tests, design of the experiment, and analyzing data. J.G. provided statistical analysis. A.M.F., C.C.R., B.G.J., and J.E.M. assisted with experiment design, test development, data analysis, and data collection. F.E.M., R.D.W., C.F.V. and P.P. assisted with design of the experiment, data analysis, and critical revision. J.C.B. oversaw the study's progress, and assisted with experiment design, data analysis, and paper writing.

variables. Mean audiometric thresholds, gap detection thresholds, and ABR latencies did not differ between groups, although the HIV+ group had a higher proportion of individuals with a hearing loss >25 dB HL in the better ear. The HIV+ group had reduced DPOAE levels ($p<0.05$) at multiple frequencies compared to HIV– subjects. No relationships were found between treatment regimens or delay in starting treatment and audiological parameters.

Conclusions—As expected, children with HIV+ were more likely to have a history of ear drainage, and to have abnormal tympanograms. Similar to the adult findings, the HIV+ group did not show significantly reduced audiometric thresholds, but did have significantly lower DPOAE magnitudes. These data suggest that: (a) HIV+ children often have middle ear damage which complicates understanding the direct effects of HIV on the hearing system, and (b) even when corrected for confounders DPOAEs were lower in the HIV+ group. Previous studies suggest ototoxicity from anti-retroviral drugs is an unlikely cause of the reduced DPOAE magnitudes. Other possibilities include effects on efferent pathways connecting to outer hair cells or a direct effect of HIV on the cochlea.

Keywords

HIV; DPOAEs; audiometry; gap detection testing; auditory brainstem response

Introduction

HIV could affect hearing in children in many ways. HIV infection of relevant structures (such as the cochlea or central nervous system) can be hypothesized to result in detectable hearing deficits. HIV has been shown to infect the cochlea (Michaels et al. 1994; Pappas et al. 1994; Soucek et al. 1996). The central nervous system can serve as a reservoir for HIV (Mirza et al. 2012) so central auditory pathways could also be affected by HIV, which could lead to central auditory processing deficits (Maro et al. 2014), or effects on efferent pathways from the brainstem and other areas to the cochlea. In addition, the presence of enhanced generalized inflammation in both untreated as well as treated HIV-infected individuals may contribute to auditory deficits if this inflammation were in the cochlea or auditory pathways. HIV treatment also involves many medications, some of which may be ototoxic, or could increase the sensitivity to other insults such as noise (Bektas et al. 2008). Children with HIV are more susceptible to ear infections, which could lead to middle ear problems, and potentially, cochlear damage (Hallbauer et al. 2014; Jose et al. 2013; Taipale et al. 2011; Tshifularo et al. 2013).

Overall, however, the prevalence of HIV-related hearing problems in HIV+ children is not firmly established and the primary pathology leading to hearing difficulties in these children is not known. To determine which of the potential causes of hearing problems in HIV+ children is the most significant, we have performed a cross-sectional study of both HIV+ and HIV– children in Tanzania using an audiological test battery.

HIV infection might affect hearing in multiple ways. The hearing deficits can be either sensorineural or conductive and can arise in different locations along the auditory pathway. The loss could result from: (a) infections such as otitis media, mastoiditis, meningitis, etc., (b) ototoxic drug effects on the peripheral hearing system (either anti-retroviral drugs or

anti-tuberculous drugs), (c) the direct effect of HIV and other viruses on neural pathways and structures or (d) the effects of underlying inflammation. Other effects, such as the interaction of anti-retroviral drugs with environmental factors (e.g., noise) or central nervous system neoplasms could also contribute. Otitis media is a common finding in HIV+ patients (Chandrasekhar et al. 2000; Jose et al. 2013) and patients with HIV take a variety of medications. When treated for disseminated tuberculosis (TB), patients with HIV may be at significant risk for hearing loss, since the aminoglycoside antibiotic streptomycin is known to be ototoxic and is used in the treatment of recurrent mycobacterial disease.

The HIV virus can affect both peripheral and central neural pathways involved in hearing (Gurney et al. 2003) and can affect the brain parenchyma (e.g., AIDS dementia), which could also affect the pathways involved in the central processing of auditory information. HIV-infected children, especially those who are untreated, experience a high rate of HIV-associated encephalopathy and neurodevelopmental delay (Donald et al. 2015). Even in the treated state, substantial concern exists for continued central nervous system (CNS) involvement given its compartmental nature and differential pharmacokinetic distribution of antiretroviral drugs. Neurocognitive deficits are still seen in HIV+ individuals despite active antiretroviral therapy (ART) (Mirza and Rathore 2012). Taken together, existing data suggest multiple potential ways that HIV could affect the hearing system.

Existing cross-sectional audiometric studies of adults and children with HIV show conflicting results. In our study with 449 HIV+ adults no differences in threshold audiometry were seen between the HIV+ and HIV- groups (Maro et al. 2014). Luque et al. (Luque et al. 2014) also showed no major differences in audiometric thresholds between HIV+ and HIV- adults in their study of 278 HIV+ adults. In contrast, Torre et al. (P. Torre, 3rd et al. 2015b) showed worse low frequency (250, 500, 1000 and 2000 Hz) and high-frequency (3000, 4000, 6000, 8000) pure tone averages in 222 HIV+ adults compared to a HIV- control group. In a study of HIV+ children, Torre et al. (P. Torre et al. 2015a) showed significantly higher thresholds in the worse ear compared to uninfected children, although the differences were not significant for the better ear.

Also, DPOAE measurements have also not shown consistent findings. Our previous study in adults showed significantly lower DPOAE magnitudes in HIV+ adults on ART compared to HIV- controls. Torre et al. (P. Torre, 3rd et al. 2014), however, did not see a greater number of DPOAE non-responders (DPOAE <-15 SPL, or <6 dB above the noise floor) among 150 HIV+ adults compared to an HIV- control group. Similarly, in studies HIV+ children, Torre et al. (P. Torre, 3rd et al. 2015c; P. Torre et al. 2015a) did not see lower DPOAE magnitudes in the HIV+ group.

These studies, however, all share certain limitations, some of which are inherent in cross-sectional designs. Finding an HIV- control group that matches the HIV+ group on all key parameters (such as noise exposure and socioeconomic status) is often difficult. Also, in addition to any direct effects that HIV infection might have on the hearing system, it is already well established that HIV+ individuals are much more likely to have otitis media and other otologic complications (Taipale et al. 2011). Such effects could damage hearing, although they are not direct effects of the HIV virus.

Determining the prevalence and nature of hearing problems in HIV+ children requires comparing the results of a comprehensive audiological assessment in HIV+ children to a matched HIV– control group. As mentioned above, potential confounders such as noise exposure or chronic otitis need to be assessed. We are currently conducting a National Institutes of Health funded study in Tanzania to measure hearing parameters in a cohort of HIV+ and HIV– children (Maro et al. 2014). The overall study includes cross-sectional results with the longitudinal study ongoing. The purpose of the study was to determine the nature of hearing deficits in these children. We hypothesized that HIV+ children would have a higher prevalence of both abnormal central and peripheral hearing test results compared to HIV– controls, which would not be related to abnormal tympanometry or previous ear infections.

Materials and Methods

Participants

The HIV+ children were recruited primarily from the DarDar Pediatric Program (DPP) at the Infectious Disease Center in Dar es Salaam, Tanzania. The eligibility criteria for the HIV+ children were that they be <18 y of age, and have two positive enzyme-linked immune sorbent assay (ELISA) antibody tests for HIV. In Tanzania, most HIV+ children are infected perinatally, and currently are almost always started on ART after birth. Older children in the cohort often experienced diagnosis later in childhood and/or delayed initiation of ART due to cluster of differentiation 4 glycoprotein (CD4) counts above existing CD4 treatment thresholds. HIV– subjects were recruited from among the family members of the HIV+ subjects. Their HIV– status was also confirmed with two negative antibody tests for HIV.

Testing System and Tests

The testing system has been described in a previous publication from our group (Maro et al. 2014). The laptop-based hearing testing system included: (a) a questionnaire administered to gather data on self-reported hearing and exposure to noise, drugs and toxins, (b) tympanometry to assess middle ear function, (c) threshold audiometry (either Békésy or a modified Hughson-Westlake protocol) to measure hearing sensitivity, and (d) distortion product otoacoustic emission (DPOAE) testing to assess cochlear function. Initially, the plan was to use a gap detection test to assess central auditory processing in children over 12 y and auditory brainstem response (ABR) testing for children <12 y, or for children who had difficulty completing the gap detection test. Over time, however, the protocol was changed to perform ABR testing on all children, but not all children have been tested using this new protocol since some did not return for repeat evaluation. Testing was done with passive noise-attenuating earmuffs over the ears (David Clark Model 19 A, David Clark Company, Worcester, MA or 3M Peltor 97023 Junior Hearing Protector Ear Muffs, St. Paul, MN) and in-ear noise measurements were used to confirm that sound levels in the ear canal were quiet enough for testing as described previously (Buckey et al. 2013). Data were stored in a relational database management system (Microsoft Access 2010, Microsoft, Redmond, WA).

Questionnaire—We used questionnaires to gather data regarding the participants hearing (hearing status questionnaire) and general health status (health history

questionnaire). Initially, all children or their parent or guardian answered a pediatric hearing status questionnaire. Later in the study, we asked children aged 12 and over to complete the adult hearing status questionnaire. Both questionnaires have significant overlap. All items were translated into Kiswahili by the study team in Tanzania. In order to ensure consistency, the hearing questionnaire was recorded in the native language and presented via video, audio and text on a laptop computer. Children were asked about noise exposure, tinnitus, ear drainage, ear infections, chemical exposure, and balance problems.

The health history questionnaire was the same for all participants and was completed by the test operators with the child and the parent or guardian. The questionnaire included questions about past or current tuberculosis (TB) treatment, HIV treatment, gentamicin exposure, and the use of anti-malarials, aspirin, and diuretics.

Tympanometry—Tympanometry at 226 Hz was performed using a Madsen Otoflex 100 (GN Otometrics, Denmark). The device provided measurements of ear canal volume, static admittance, tympanometric peak pressure, tympanometric width, and tympanogram type (A, As, Ad, B, C). The middle-ear analyzer (OTOflex 100) determined tympanogram type from the location (pressure and static admittance) of the peak of the tympanogram. The pressure limits for type A were -100 to $+50$ daPa, and the static admittance limits for type A were 0.3 – 1.7 mmho. Tympanograms with static admittance levels <0.3 mmho with a discernable peak were classified as type As, those with levels <0.3 with no discernable peak were classified as Type B. Those with static admittance levels >1.7 mmho were classified as type Ad. Tympanograms with pressures outside the Type A range, but within the static admittance limits, were classified as Type C.

In-ear noise measurement—For the in-ear noise measurement, the noise spectrum from the DPOAE probe microphone was analyzed, and displayed as one-third octave bands on the operator's screen. Levels below 30 dB SPL for frequencies <1000 Hz, below 25 dB SPL for frequencies between 1000–2000 Hz, and below 20 dB SPL for frequencies >2000 Hz were considered acceptable (gray). The operators were instructed to attempt to have all the bars gray, but if this was not possible, to focus on achieving gray bars at 2000 Hz and above.

Threshold audiometry—Thresholds were measured at frequencies of 500, 1000, 2000, 4000, 6000 and 8000 Hz. A Békésy-like tracking procedure was used for older children; younger children were tested using a modified Hughson-Westlake technique. The stimuli for the Békésy procedure have been described previously (Maro et al. 2014) (pulsed tones with a duration of 250 msec, rise and fall time of 20 msec and an interstimulus interval of 500 msec). When the subject pressed the response button the tone reduced in 4-dB steps until the first reversal, then 2-dB steps were used. The Békésy-like parameters were not adjusted for age. Subjects who could not perform the Békésy-like test easily were tested instead with a modified Hughson-Westlake procedure, where the operator administered the test. For the Hughson-Westlake approach, tones presentations started at 40 dB HL. Tones were increased by 15 dB until the subject responded, then decreased by 10 dB until the subject no longer responded. At this point, tone levels increased in 5 dB steps until a response was obtained and decreased by 10 dB until no response again. This process was repeated until we found

the lowest level at which the subject could hear the tone in 2 out of 3 presentations at that level.

Because the probe used for this study did not have published reference equivalent threshold sound pressure levels (RETSPLs), we determined these levels in a separate study with 10 normal hearing subjects (Buckey et al. 2013). The protocol followed the guidelines outlined in Annex D of ANSI S3.6-2004—"Specification for Audiometers" (American_National_Standard 2004). To maintain a consistent calibration for all devices, each probe and sound card (ECHO Indigo IO, Echo Digital Audio Corporation, Santa Barbara, CA), were calibrated as a pair, and the calibration curve for that pair was stored on the laptop. Before testing each subject on the system, the calibration was checked with a 2 cc coupler. If the calibration did not match a pre-recorded baseline, the subject was tested with another system and the problem was investigated. Due to the maximum sound output from the speakers in the probe being limited, the system could not measure a hearing loss in excess of 70 dB HL.

DPOAEs—DPOAEs were performed at f_2 values of 1496, 1701, 2002, 2196, 3003, 3197, 4005, 4198, 5997, 6201, 7795, and 7999 Hz with an f_2/f_1 ratio of 1.2. Data were collected twice, first using L1/L2 values of 65/55 dB SPL and then 70/70 dB SPL. Subjects were instructed not to swallow during testing and an adaptive noise-rejection algorithm was used to discard noisy segments during the data collection. Each f_1 and f_2 frequency pair was presented for a minimum of 4 s. If after 4 s the difference between the DPOAE level (DP) and averaged noise floor level (NF) was <10 dB SPL, data collection continued until either a DP-NF value of 10 was reached or 10 s had elapsed. The speaker output was not adjusted in the ear canal (i.e., an in-ear calibration was not used). In some subjects, unrealistically high values for DPOAEs and NFs were measured, likely due to resonances in the ear canal. These values were discarded for the data analysis (see below). For each system, the level of harmonic distortion was also determined using a condenser microphone (Brüel and Kjær Type 4157 Ear Simulator/Artificial Ear, Brüel and Kjær Type, Nærum, Denmark).

To assist with proper probe placement a frequency sweep (chirp) was presented in the ear canal before DPOAE testing. The results from three chirps (500–5000 Hz) at 65 dB SPL were averaged, smoothed, and displayed to the operator. A measured level below 20 dB SPL at 500 Hz was used to indicate a bad probe seal. In the case of a bad seal the probe was resealed and the chirps were repeated.

Gap detection testing—To determine an individual's gap detection threshold, the participant was trained to press a button when a short gap in noise was heard. The gaps were placed randomly in the middle portion of 4.5 s of white noise delivered at 65 dB SPL. Details of the gap detection test have been published previously (Maro et al. 2014). To avoid confusion, no gaps were presented in the first or last second. Upon hearing the gap, the subject pressed a pushbutton, and the response time was recorded. Response times of more than 150 ms and less than 1000 ms were considered acceptable; otherwise, the response was considered a missed detection. If the subject correctly identified two gaps in a row then the gaps become shorter, if two gaps in a row were missed then the gaps become longer. If the subject received 5 presentations at a given gap length without getting either 2 correct or 2

wrong in a row, the algorithm scored this as a miss and increased the gap length. The allowable gap lengths were: (70, 60, 50, 45, 40, 35, 30, 25, 20, 16, 13, 10, 7, 4, 2, 1). A reversal occurred when the progression of gap lengths reversed from shorter to longer or vice versa. The test started at a gap length of 20 msec. and continued until the subject either completed 10 reversals or 120 presentations.

The test yielded a value that represented the average of the last eight reversals (a reversal occurred when the subject missed two gap presentations in a row, and the gap lengths presented became longer). In addition, to compensate for instances where the subject may have lost focus during the test, the three reversals with the shortest gap lengths were averaged. Not all children could complete the gap test successfully, usually because they were too young to understand and perform the test.

Auditory brainstem response (ABR) testing—A clinical instrument (GSI AUDIOScreener, Grason-Stadler, Inc., Eden Prairie, MN) was used. The primary stimuli were clicks presented at 32/s at 60 dB and 40 dB nHL. The neural response was detected using skin electrodes placed on each mastoid process and the forehead. The measured voltage was filtered from 30–1500 Hz. Whenever possible children were measured lying down.

CD4+ T-helper cell levels—When HIV+ children arrived for testing they had blood drawn to measure the level of CD4 positive T-helper cells in their bloodstream using a commercial system (Becton Dickinson FACSCalibur system, BD Biosciences, San Jose, California). This served as a marker of the severity of the HIV infection. The normal range for a CD4 count ranges from 600–1500 cells/microliter. ART is usually started when the counts are lower than 350 cells/microliter.

Testing Protocol

All tests and procedures were approved by the Committee for the Protection of Human Subjects at Dartmouth, and the Institutional Review Board at the Muhimbili University of Health and Allied Sciences. Informed consent was obtained from the parent or responsible adult. After informed consent was obtained, the subjects were asked if they had had any significant noise exposure within the preceding 14 h or if they had taken any large dose of aspirin. If the subjects answered yes, they were scheduled for testing on a different day. An otoscopic exam was performed. If the subjects had any significant ear pathology that would preclude testing on that day (e.g., draining ear, external otitis, wax impaction), they were referred for treatment and scheduled for testing on another day. Cerumen that prevented visualization of the ear canal was removed before testing.

After the otoscopic exam the children had tympanometry performed on both ears. The subjects then completed the two questionnaires (the Hearing Status and Health History Questionnaires). After this, depending on their age, they received training in how to perform the Békésy-like threshold and gap-detection tests. The subjects were given the opportunity to practice both the Békésy and gap detection tests before actual data collection. Once training was complete, a calibration check on the system was performed, and the probe was placed in the subject's right ear. The probe placement was checked using the frequency sweep check,

and the insertion was adjusted as needed. The subject's ears were then covered with a passive noise-reducing headset (Model 19A, David Clark Company, Worcester, MA or 3M Peltor 97023 Junior Hearing Protector Ear Muffs, St. Paul, MN), and in-ear noise levels were checked. If the noise levels were above the acceptable range, the headset was repositioned until the in-ear noise check was passed.

DPOAE testing was performed next, first with L1/L2 at 65/55 dB SPL and then with L1/L2 at 70/70 dB SPL. DPOAE testing was followed by audiometry. If the subject had difficulty with Békésy-like audiometry (e.g., excursions greater than 20 dB, or an inability to understand the test), the Hughson-Westlake protocol was used instead. The operator made this determination on a case-by-case basis. In older children, gap-detection testing followed threshold audiometry. Before starting the gap detection test, the subjects received a refresher in how to perform the test, and were given sample gap presentations. Once the operator was satisfied that the subject understood the test, the test was administered. Following completion of the above testing in the right ear, the probe was switched to the left ear and the testing protocol was repeated. ABR testing was done last. In younger children, ABR testing was conducted instead of the gap detection test. Over the course of the study, however, the protocol was changed and ABR testing was completed on all children.

Data Analysis

Previous studies and this study have shown the children with HIV are more likely to have ear infections and abnormal tympanometry. Since the objective in this study was to evaluate the effect of HIV on hearing parameters independently from middle ear problems that might exist, we eliminated audiometry, DPOAE, gap, and ABR data from ears with abnormal tympanograms (Type B and Type C). Although this limited the power of the analyses, it removed the confounding effect of abnormal middle ear status on the Békésy, DPOAE, gap, and ABR tests.

Questionnaire data—The proportion of subjects that provided particular answers to specific questionnaire questions (e.g., Do you have a history of ear drainage?) were compared between groups using chi-square and Fisher's exact tests. A p value of <0.05 was considered significant.

Audiometry data—All cases with thresholds that could not be detected by the Békésy algorithm were reviewed individually, and classified either as unreliable or as no response at the maximum output level of the system. Unreliable values were discarded, otherwise the values were set to 70 dB HL (the maximum value the system could detect). The data from both ears were averaged for the analysis. The number of individuals with hearing loss as defined by the WHO were calculated both before and after removing ears with abnormal tympanometry. The audiometry results between groups were compared using a two-way ANOVA with HIV status and history of ear drainage as grouping factors.

DPOAE data—For the DPOAE data, a frequency histogram of all the DPOAE values was bimodal. It was a mixture of one Gaussian distribution with a mode at approximately 7 dB SPL and a second mode at approximately 60 dB SPL. The second mode consisted of

those values where the DPOAE algorithm returned erroneously high values because of resonances within the ear canal. A clear division between the two distributions existed at 35 dB SPL. For data analysis DPOAE values > 35 dB SPL were omitted.

The harmonic distortion testing on the systems revealed that for the testing frequencies of 1500, 1700, 2000, 2200, 6000, 6200, 7800, 8000 Hz, DPOAE levels above -10 dB SPL could be measured reliably. For the frequencies of 3000, 3200, 4000, 4200 values above -20 dB SPL were reliable. For data analysis, DPOAEs values were included if (a) they were >6 dB above the noise floor, (b) were above the harmonic distortion limit for that frequency, (c) were not >35 dB, and (d) were from an ear that had a type A, As or Ad tympanogram. Otherwise, the DPOAE value was not included in the overall analysis. Average results from the L1/L2 at 65/55 and 70/70 dB SPL did not differ, so only the L1/L2 65/55 dB SPL data are presented. As with the audiometry data, the main analysis was a two-way ANOVA with HIV status and ear drainage history as grouping factors. To compensate for the multiple comparisons made with the DPOAE tests (12 frequencies) the Benjamini-Hochberg false discovery rate method was used.

Gap detection data—For the gap detection data, previous analysis showed that performance on the test improved over time (Maro et al. 2014). To compensate for this, gap detection data from the second visit were used. Also, since performance could improve between ears (the right ear was tested first) or if they were distracted later in the visit, the lowest gap score of the two ears using the average of the best 3 reversals, were used. Data were analyzed using ANOVA as described above.

ABR data—Each ABR tracing was reviewed by the study audiologist (CCR). An ABR tracing was considered to be interpretable if there was a detectable wave V that repeated on the 2 presentations of the clicks (a sample waveform is included as Figure 1). To be conservative in the analyses, for a given subject only data from ears with a type A, As or Ad tympanogram was used and the best ABR (shortest latency) for each subject was chosen. Data were analyzed using ANOVA as described above.

Treatment delay and drug regimens—Since treatment delay is a continuous variable, analysis of the relationship between treatment delay and audiological parameters was done using a linear regression model with ear drainage as a confounder. Comparisons within the HIV+ group between drug regimens were done using an ANOVA with the drug regimen and ear drainage as grouping factors. The analyses were performed using *R: A Language and Environment for Statistical Computing* (<http://www.r-project.org/>) and with Matlab version 2015b (The Mathworks Inc., Natick, MA).

Results

Subject Characteristics

Table 1 shows the characteristics of the subjects in the different HIV+ and HIV- groups. The groups did not differ in age and gender composition, although there was a tendency for fewer males in the HIV- group. The HIV+ group was significantly more likely to report a history of ear drainage or dizziness/imbalance ($p < 0.001$ for both, Fishers exact test), and

also had a significantly higher number of individuals with abnormal tympanograms ($p=0.01$, Fishers exact test) and TB ($p<0.001$, Fishers exact test). The questions about ototoxic drug exposure revealed that 2 (1.5%) HIV+ children had a history of exposure to streptomycin. One of these 2 had abnormal tympanometry and so was not included in the DPOAE analysis. Three (2.7%) HIV- and 5 (3.8%) HIV+ children reported a history of gentamicin exposure. Children were also asked if they had received intravenous drugs for the treatment of fever (which often means they received intravenous gentamicin, although this could not be confirmed). Eleven (9.7%) HIV- and 11 (8.4%) HIV+ children reported this exposure. Because of the small numbers of children with these specific ototoxic exposures, they were not incorporated into the statistical analysis.

HIV group comparisons

Figure 2 shows the threshold values from both ears combined for the different groups. No differences were found between the HIV- (open circles, $n=73$) and HIV+ (solid circles, $n=75$) groups, although there was a trend for thresholds to be slightly better for the former subjects. Without compensating for abnormal tympanometry the HIV+ group had a significantly higher proportion of individuals with a pure tone average (PTA) of frequencies 500, 1000, 2000 and 4000 Hz greater than 25 dB HL (16% HIV+ vs. 6% HIV-, $p=0.039$) (Table 2). When the ears with abnormal tympanometry were excluded this difference was still marginally significant (17% HIV+ vs. 7% HIV-, $p=0.054$).

Figure 3 shows the averaged right and left ear DPOAE results at L1/L2 of 65/55 for the two groups. The HIV- group had 103 individuals, and the HIV+ group included 97 individuals. The DPOAE magnitudes were significantly lower in the HIV+ (solid circles) group at most of the frequencies tested ($f_2=1496$ Hz, $F=4.7$, $p=0.03$, $f_2=1701$ Hz, $F=5.7$, $p=0.018$, $f_2=2002$, $F=4.8$, $p=0.029$, $f_2=2196$, $F=7.6$, $p=0.006$, $f_2=3003$, $F=9.0$, $p=0.003$, $f_2=3197$, $F=8.0$, $p=0.005$, $f_2=5997$, $F=6.0$, $p=0.015$, $f_2=6201$, $F=5.7$, $p=0.019$, $f_2=7999$, $F=4.2$, $p=0.04$). There was no effect of a history of ear drainage on the results. When these results are corrected for multiple comparisons (12 frequencies) using the Benjamini-Hochberg false discovery rate method, the adjusted p values are less than 0.05 except for $f_2=7999$ Hz (adjusted $p=0.053$).

Figure 4 shows the gap detection threshold results for the HIV- (left) and HIV+ (right) groups for the subset of individuals who completed this test at two visits (HIV- =19, HIV+ =48). The individuals who completed the test twice were older (age range 7.4 – 18 y), compared to those who did not (age range 1–17 y). The mean gap detection thresholds were not significantly different between the groups (3.7 ms HIV- vs. 3.7 ms HIV+). The median gap detection thresholds were also not different (3.2 ms HIV- vs. 3.5 ms HIV+). Figure 5 summarizes the results from ABR testing for the individuals who completed the ABR test (74 HIV- =74, HIV+=90). The overall group averages were computed from the best (shortest) ABR latency from either ear from each subject. Similar to the gap results, no significant differences were seen between the means of the HIV- and HIV+ groups (5.9 msec HIV- vs. 5.9 msec HIV+). The results were also not significant if the data were analyzed by ear, rather than by using the shortest latency for each subject.

Effects of Medications

The 2 most common HIV treatment regimens within the cohort were zidovudine-lamivudine-nevirapine (48 children) or stavudine-lamivudine-nevirapine (42 children). There were a total of 15 children on a regimen containing efavirenz rather than nevirapine. No significant differences were detected in audiological parameters (pure tone average, average DPOAE, gap detection thresholds, ABR latencies) between children on the different drug regimens. Twenty-two children were not on ART at the time they were tested. No significant differences in audiological parameters were found between the children on and not on ART.

Effects of Time

For those children who were on ART, there was a wide range of age at initiation (median age 7 y, range 2 mo-14 y). Treatment delay was not significantly related to pure tone average (PTA) of audiometric thresholds, DPOAE magnitudes, ABR latencies, or gap detection thresholds.

Discussion

Results from this cross-sectional study show that HIV+ children had reduced DPOAE magnitudes compared to HIV- children, but their hearing thresholds were similar. The HIV+ group, however, did have a significantly higher proportion of subjects who had a hearing loss >25 dB HL in the better ear, although this may have been related to the higher rate of abnormal tympanometry and history of ear drainage in this group. These threshold audiometry and DPOAE findings are similar to what was found in the adult cohort reported previously (Maro et al. 2014). Also, as with the adult findings, no association between drug regimens and audiometric findings was observed. In contrast with what was seen in our previous study with adults, however, the HIV+ children did not have significantly higher gap detection thresholds. Also, ABR latencies did not differ significantly between the groups.

Threshold audiometry results

Although the threshold audiometry results in our pediatric cohort matched those from the adult cohort (i.e., no significant difference between the HIV- and HIV+ groups), other investigators have seen worse audiometric thresholds in HIV+ individuals compared to an HIV- control group. van der Westhuizen (van der Westhuizen et al. 2013) demonstrated poor audiometric thresholds in HIV+ individuals as did Torre et al (P. Torre, 3rd et al. 2015b). Part of these differences are likely due to the difficulty of assembling HIV+ and HIV- cohorts that are well matched on key variables. Differences in race, socioeconomic status, noise exposure, history of previous ear infections, and other factors can confound any cross-sectional analysis, which may not be completely compensated for using statistics. Analysis of hearing data in HIV+ children is complicated by the fact that these children are significantly more likely than HIV- children to have abnormal ear, nose and throat (ENT) findings, which is likely due to the increased susceptibility to infection in this group (Taipale et al. 2011). Consistent with what has been seen in other studies, the HIV+ children in our cohort were much more likely than the HIV- children to have a history of ear drainage or of abnormal tympanometry.

In the study by van der Westhuizen et al., the investigators were examining auditory and otological problems in HIV+ patients overall, and did not try to separate effects due to ear infections or abnormal tympanometry, from direct effects of HIV on the hearing system. So, the fact that auditory thresholds were significantly lower in the HIV+ group in this study is consistent with the overall higher rate of otological complications in the HIV+ group. Similarly for the Torre et al. study (P. Torre, 3rd et al. 2015b), previous ear infections or abnormal tympanometry were not included as potential confounders in the statistical analysis, so the overall result could include both direct and indirect effects of HIV on the hearing system.

DPOAE results

In our previous study with adults, we saw significantly reduced DPOAE magnitudes in the HIV+ group compared to the HIV– controls. This difference did not appear to be due to ART, because DPOAE magnitudes did not differ between individuals taking and not taking ART. In the current study, DPOAEs were only included if they were greater than 6 dB above the noise floor, were above the harmonic distortion limit of the device, and were not above 35 dB SPL. Also, DPOAEs from ears with Type B and C tympanograms were excluded. Despite this, DPOAE magnitudes were still significantly lower in the HIV+ group compared to the HIV– group.

One possible explanation is an effect due to ART in the HIV+ group. This seems unlikely since no difference between the ART– and ART+ groups was seen in the adult study. In the pediatric cohort, there are too few subjects not taking ART to make a meaningful comparison between the ART+ and ART– groups, so this finding from the adult study cannot be confirmed for this pediatric cohort. Ototoxicity from other drugs also did not seem to be a possibility. Only 2 HIV+ children had a history of streptomycin exposure, and only one of these had normal tympanometry. The exposure to gentamicin (as best as it could be determined) was similar between groups.

Another possible explanation is a direct effect of HIV infection on the cochlea. The possibility that HIV might directly affect cochlear elements was suggested by Michaels and colleagues in the mid-1990s (Michaels et al. 1994; Soucek and Michaels 1996). Pappas et al. (Pappas et al. 1994) demonstrated the presence of viral-like particles consistent with HIV in the cochlea of HIV infected patients. It's possible that the cochlea, like the brain, may serve as a reservoir for HIV and that this could affect outer hair cell function. Latent HIV infection exists in the brain, although no studies have confirmed that latent infection might exist in the cochlea as well. Nevertheless, it is an intriguing possibility that the lower DPOAE magnitudes might be a manifestation of this.

DPOAEs are produced by the outer hair cells in the cochlea. Outer hair cells receive efferents from the medial olivary complex and this innervation is important for the maintenance and function of outer hair cells (Liberman et al. 2014). Damage to efferent pathways to the cochlea due to HIV infection in the brain could potentially allow for increased damage or degeneration of outer hair cells in HIV+ individuals compared to uninfected controls.

Differences between adult and pediatric findings

The adult study had several major findings: lower DPOAE magnitudes and elevated gap detection thresholds in the ART+ group along with a report of difficulty in understanding speech in noise. The latter findings suggested a central auditory processing deficit in this group. In the current study, two measures were included that could detect central auditory problems—the gap detection test and ABR measures. Neither of these measures were significantly different between the HIV+ and HIV– groups.

Part of the reason for the lack of significant findings could be due to limitations in the equipment and the study design. At the outset of the study, we chose ABR equipment that was portable and battery-powered to provide testing flexibility. This also limited the sound level we could use for ABR testing (60 dB nHL), which may have reduced our ability to detect differences between groups. The initial study design also proposed using the gap detection test for older children and the ABR test for younger children. After we began the study, however, the number of potential enrollees decreased and this limited the number of children who had completed the gap detection test. Although the protocol was changed to perform ABR testing on all children to increase the number of individuals who had ABR data, the power to detect changes with either the gap detection test or the ABR test was still much lower than for threshold audiometry and the DPOAE tests. Some children were lost to follow-up so, we were not able to retest all children in the study with ABR.

Studies where ABRs have been measured in HIV+ subjects have shown abnormalities in auditory evoked potentials consistent with a higher rate of central processing defects compared to HIV-negative subjects (Bankaitis et al. 1995; Pagano et al. 1992; Reyes-Contreras et al. 2002). In an early ABR study, Pagano et al. showed prolonged ABR latencies in a group of 35 HIV+ individuals, which they attributed to effects of the HIV virus on the central nervous system. Matas et al. performed evoked potential testing (ABRs and Auditory Middle Latency Response (AMLR)) in 56 HIV+ individuals both on (n=32) and off ART (n=24). Similar to the results of our cross-sectional study in adults, Matas et al. showed a higher rate of abnormal tests in the ART+ group. Approximately 63% of those in the ART+ group had abnormal evoked responses, and only 29% of those abnormal responses could be explained by deficits in the peripheral hearing system (Matas et al. 2010).

One possible explanation for the divergent findings between these studies and our results could be due the difference in HIV treatment between adults and children. In adults, the decision to start ART is based on symptoms and CD4 count. Individuals either needed to have had a low enough CD4 count, or to have significant enough clinical symptoms, to warrant treatment. So, individuals need to reach a certain severity with their disease before starting ART. This means that they could experience adverse CNS effects from their established HIV infection prior to starting ART. In the pediatric population, ART is usually started right after birth, although that was not often the case in the cohort studied here. Nevertheless, it's possible that in the pediatric population the severity of HIV infection never reached the level achieved in the adults, which may have eventually led to fewer central hearing manifestations of the HIV infection.

Limitations

The gap detection results can be influenced by training, attention, or fatigue. To minimize the effect of training, we used the gap results from the second visit to the testing center. All of the subjects had performed the gap detection test at least once before the test that was used for the data analysis. Also, the results from the lowest score from both ears were used, because analysis of our previous results had shown that left ear gap detection threshold tended to be lower than the right ear, which we ascribe to a training effect. Nevertheless, we still found that many children could not complete the gap detection test successfully. In those that did complete the test, to compensate for any loss of attention that might have occurred during the test, we used the best three reversals from each test.

The ABR system used for the testing had limitations as mentioned above, and the environment was not ideal for ABR testing. This limited the number of high-quality tests that could be used in the analysis. Also, for the ABR tests, to compensate for the potential effect of previous ear infections, we used the best result from either ear. This approach to data analysis, while conservative, also minimizes the chance of seeing a small or asymmetric effect that might be due to HIV infection.

Lastly, the pediatric questionnaires were mainly focused on asking about peripheral hearing function, and did not have specific questions relevant to central auditory processing.

Conclusion

On average, the HIV+ group had lower DPOAE values than the HIV– group suggesting possible cochlear damage in this group. These changes could be due to the HIV virus, or perhaps damage to the medial olivocochlear efferent system; our previous data from adults do not suggest TB medications or ART as potential causes of reduced DPOAE magnitudes in HIV-infected individuals.

In contrast to the adult study, no evidence of a central auditory processing deficit was seen in the gap detection and ABR data (although if medial olivocochlear efferents were involved, this could be a central effect). This could be due to the limited number of individuals who completed the gap and ABR tests. Additionally, it's possible that this pediatric cohort did not develop significant central nervous system consequences of HIV exposure, and so have a low rate of central auditory processing deficits. Longitudinal measurements are ongoing to determine the stability of these measures over time, and to assess whether new hearing problems arise.

Acknowledgments

Source of Funding:

This work is supported by grant R01DC009972 from the National Institute on Deafness and Other Communication Disorders (NIDCD). Dr. von Reyn and Dr. Waddell receive support from the Fogarty International Center, D43-TW006807. Dr. Gui receives support from NIH grant R01LM012012.

We gratefully acknowledge the assistance from Dr. James Saunders for the initial testing of the system in Nicaragua. We thank Dr. Brenda Lonsbury-Martin for her help with the proposal and with the protocol for DPOAE measurements. We would like to acknowledge the contribution of Stephanie Nagle to the development of the gap

detection test. We thank the team at the DarDar clinic in Dar es Salaam, Tanzania who collected these data (Esther Kayichile, Kissa Albert, Safina Sheshe, Claudia Gasana, Mariane Mpressa, and Joyce Ghatty). We thank the team at Creare, Inc. that assembled and tested the hearing testing systems, particularly Nathan Brown. We appreciate the support of Erika Kafwimi and Sabrina Yegela who helped with building the video questionnaire and translating the questions.

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Short Summary

In this cross-sectional study, an audiological assessment was performed on a cohort of HIV+ and HIV– children in Tanzania. Compared to HIV– subjects, HIV+ children were more likely to report a history of dizziness and ear drainage. Audiometric thresholds, gap detection thresholds and auditory brainstem latencies were not significantly different between the groups. Even when adjusted for tympanometry status and history of ear drainage, DPOAE levels were significantly lower in the HIV+ group. Previous studies suggest ototoxicity from drugs is unlikely. Other possibilities include effects on cochlear efferent innervation or a direct effect of the HIV virus on the cochlea.

Figure 1.
Sample ABR tracing from the GSI AUDIOScreener used in the study.

Figure 2.

Hearing threshold data (average of both ears) comparing the HIV+ (solid circles) and HIV- (open circles) groups. Error bars show the standard error of the mean (SEM). There were no statistically significant differences between groups.

Figure 3.

DPOAE results from the HIV+ and HIV- groups (average $\pm$ SEM). The data from both ears have been averaged. DPOAEs were significantly lower in the HIV+ group at multiple frequencies.

Figure 4.

Gap Detection Thresholds for the HIV+ and HIV- groups. The results from the left ear using the average of the lowest 3 reversals are shown. The difference between the HIV- (3.6 msec, n=19) and HIV+ (3.7 msec, n=48) is not significant. The target symbol on the boxplot shows the median value, which was the same for both groups (3.0 msec).

Figure 5.

ABR latencies for the HIV+ and HIV- groups. The latency from the better ear at 60 dB nHL was used. The difference between the HIV- (5.8 msec, n=74) and HIV+ (5.9 msec, n=90) is not significant.

Table 1

Characteristics of the groups. The HIV + group was significantly more likely to have a history of TB ($p<0.001$), an abnormal tympanogram ($p=0.01$), a history of ear drainage ($p<0.001$), or dizziness/imbalance ($p<0.001$). Asterisks (*) indicate statistically significant findings.

	N	Male N(%)	Female N(%)	Average Age (years)	TB History	CD4 Count	Abnormal Tymp During Testing	Ear Drainage History	Dizziness Imbalance
HIV-	113	48(42%)	65(58%)	10.1	3(3%)		14(12%)	8(7%)	1(1%)
HIV+	131	67(51%)	64(49%)	10.1	35(27%)*	842	33(25%)*	34(26%)*	20(15%)*

Table 2

Prevalence of hearing loss. Not all subjects could complete an audiogram (usually because they were too young to complete the test). The HIV+ group had a significantly higher proportion of subjects who had a hearing loss >25 dB HL in the better ear.

	N	Had audiogram on either ear N(%)	PTA <25 dB HL better ear N(%)	PTA 26–40 dB HL better ear N(%)	PTA >40 dB better ear N(%)
HIV–	113	80(71%)	75(66%)	2(2%)	3(3%)
HIV+	131	97(74%)	81(62%)	9(7%)	7(5%)