
# The Prevalence of Hearing Loss in HIV-infected South African Adolescents on Antiretroviral Therapy

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Dissertation submitted in partial fulfilment of the requirements for the degree

MASTER OF PUBLIC HEALTH

in Epidemiology

in the

School of Public Health and Family Medicine

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13 March 2017

# PREAMBLE

To my grandfather, Peter Doice Tayengwa Banga,

in memorium; *ab ove maiori discit arare minor.*

## Declaration

I, Agatha Banga (BNGAGA001), hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Date: 13 March 2017

## Dissertation

### Structure

This mini-dissertation is presented in four parts. Part A ('Protocol') provides a background and justification for the study, as well as describing the methodology of the cross-sectional analysis. Part B ('Literature Review') illustrates and summarises literature relevant to the objective of the cross-sectional analysis. Part C (the journal-ready 'Manuscript') presents the methodology, findings and discussion based on the cross-sectional analysis. Part D ('Appendices') contains tables, figures and supporting documents for the dissertation as a whole.

### Background

The use of Antiretroviral Therapy (ART) has increased longevity amongst HIV-infected populations. As a result, children perinatally infected with HIV (PHIV+) are living into adolescence for the first time in the history of the global HIV epidemic. PHIV+ adolescents are a unique population as they bear the combined effects of chronic HIV-infection and the prolonged use of ART. Sub-Saharan Africa continues to make a significant contribution to the global HIV burden, and concurrently has one of the highest prevalences of hearing loss in the world. Although it is known that hearing loss is common among PHIV+ adolescents, there remains sparse evidence about predictors for hearing loss in this population, especially in the developing world. Research is highlighting the importance and need for further investigation into this unique population with inimitable health needs.

In South Africa, the Cape Town Adolescent Antiretroviral Cohort (CTAAC) - a prospective, descriptive cohort study - began in July 2013, looking at 625 adolescents (515 PHIV+ and 110 HIV-non-infected (HIV-)). The overall objective of the CTAAC was to investigate the progression of chronic HIV-infection among PHIV+ adolescents on ART. This dissertation is

based on a cross-sectional analysis of audiometric data from the CTAAC participants. The objective of this cross-sectional analysis was to investigate hearing loss among PHIV+ adolescents on ART, and HIV-adolescents in Cape Town, South Africa.

**Methods**

A cross-sectional analysis was carried out to describe the prevalence, nature and predictors (demographic, past medical history, clinical findings) of hearing loss in adolescents between 9 and 14 years of age. Screening pure-tone air-conduction (AC) thresholds above 30 decibels (dB) were considered to be indicative of debilitating hearing loss. Statistical analysis included univariate analysis and multivariate logistic regression.

**Results**

The cross-sectional analysis included data from 540 participants. 225 (52%) were male, with a median age of 12 years. A varying degree of hearing impairment was observed in 19% of all the adolescents in the study. Multivariate analysis showed the following predictors for any hearing loss: an unmarried primary caregiver (odds ratio (OR) 0.59; 95% confidence interval (CI), 0.39; 0.91, p = 0.015), being female (OR 1.67; 95% CI, 1.12; 2.51; p = 0.013) and reports of being troubled by ear pain or discharge in the last month (OR 2.54; 95% CI, 1.55; 4.17; p = <0.001) after adjustment. Univariate analysis showed an association between hearing loss and a longer duration on ART among PHIV+ adolescents (OR 1.80, 95% CI 1.17; 2.75, p = 0.007).

**Discussion**

Data showed that the prevalence of hearing loss appears to be comparable between PHIV+ and HIV- adolescents in Cape Town. In low resource settings, a history of ear pain or

discharge within the last month may be used as a screening tool for a hearing assessment, and guide referral for formal hearing tests.

## Acknowledgements

I would like to express my gratitude to my supervisor - Professor Landon Myer - who suggested this dissertation topic, and supported and encouraged me through the journey of transforming an idea into a tangible piece of academic work. I will not forget his patience and his careful attention to detail during the review of this dissertation.

I would also like to thank my husband - Robert Jacobsz - for his continued support and companionship as I worked on this dissertation. I would like to thank Leila Hall for her invaluable editorial input.

I am thankful to a number of women in research who, in different ways, have contributed to this work. Among these:

Dr Sana Mahtab - the Cape Town Adolescent Antiretroviral Cohort (CTAAC) Project Coordinator at the Red Cross War Memorial Children's Hospital (RCWMCH) - for ensuring that I received essential information required to carry out work on this thesis.

Dr Nana Akua Asafu-Agyei - consultant paediatrician - for providing much needed insight into the CTAAC study and providing logistical support even during out-of-office hours.

Dr Shazia Peer - specialist paediatric otolaryngologist at the RCWMCH - for her invaluable input to my understanding of the research topic.

Silva Kuschke - Chief Audiologist and Head of the Audiology Department at RCWMCH - for providing extraordinary insight into the finer details surrounding audiology. Professor

Heather Zar - Co-Principal Investigator of the CTAAC, Head of the Department of Paediatrics and Child Health at RCWMCH at the University of Cape Town - for allowing me to carry out research using unpublished data. Many thanks to the CTAAC team who have made it possible to carry out this work.

## List of Abbreviations and Acronyms

| | |
|---|---|
| **AC** | Pure-tone Air-conduction |
| **AIC** | Akaike Information Criterion |
| **AIDS** | Acquired Immune Deficiency Syndrome |
| **AMP** | Adolescent Master Protocol |
| **ANOVA** | Analysis of Variance, a collection of statistical methods |
| **ART** | Antiretroviral Therapy |
| **ASD** | Adult Spectrum Disease |
| **BAEP** | Brainstem Auditory Evoked Potential |
| **BC** | Bone Conduction |
| **BIC** | Bayesian Information Criterion |
| **BMI** | Body Mass Index |
| **CDC** | Centres for Disease Control |
| **CD4** | Cluster of Differentiation 4 |
| **CI** | Confidence Interval |
| **CONSORT** | Checklist of items to include when reporting randomised trials |
| **CRF** | Case Record Forms |
| **CRU** | Clinical Research Unit |
| **CTA** | Copyright Trademark Agreement |
| **CTAAC** | Cape Town Adolescent Antiretroviral Cohort |

| | |
|---|---|
| **DOI** | Digital Object Identifier |
| **DNA** | Deoxyribonucleic Acid |
| **FWF** | Austrian Science Fund |
| **GBD** | Global Burden of Disease |
| **GSI** | Audiometer |
| **HAART** | Highly Active Antiretroviral Therapy |
| **Hinari** | Platform which provides access to low-cost online journals |
| **HIV** | Human Immunodeficiency Virus |
| **HIV-** | HIV-uninfected |
| **HPTA** | High Frequency Pure Tone Average |
| **HREC** | Human Research Ethics Committee |
| **HSRC** | Human Sciences Research Council |
| **HTML** | HyperText Markup Language |
| **Hz** | Hertz |
| **IDC** | Infectious Diseases Clinic |
| **ICMJE** | International Committee of Medical Journal Editors |
| **IeDEA-SA** | International Epidemiological Databases to Evaluate AIDS-South Africa |
| **IMRD** | Introduction Methods Results Discussion |
| **IQ** | Intelligence Quotient |
| **IQR** | Inter-Quartile Range |

| | |
|---|---|
| **kHz** | Kilohertz |
| **LPTA** | Low Frequency Pure Tone Average |
| **MACS** | Multicentre AIDS Cohort Study |
| **MBBS** | Bachelor of Medicine, Bachelor of Surgery |
| **MDR-TB** | Multidrug resistant tuberculosis |
| **MRL** | Minimum Response Level |
| **MW** | Malawi |
| **NHANES III** | National Health and Nutrition Examination Survey |
| **NRTI** | Nucleoside reverse-transcriptase inhibitor |
| **NNRTI** | Non-nucleoside reverse-transcriptase inhibitor |
| **OAA** | Open Access Agreement |
| **OR** | Odds Ratio |
| **PDF** | Portable Document Format |
| **PHACS** | Pediatric HIV/AIDS Cohort Study |
| **PHIV+** | Perinatally HIV-infected |
| **PI** | Protease Inhibitor |
| **PMTCT** | Prevention of Mother to Child Transmission |
| **PRISMA** | Checklist for systematic reviews and meta-analyses |
| **PTA** | Pure-Tone Average |
| **RCWMCH** | Red Cross War Memorial Children's Hospital |

| | |
|---|---|
| **RCUK** | Research Councils UK |
| **SA** | South Africa |
| **SD** | Standard Deviations |
| **SQL** | Structured Query Language |
| **SSA** | Sub Saharan Africa |
| **STARD** | Checklist of items for reporting studies on diagnostic accuracy |
| **STATA 13** | Statistical Software |
| **TB** | Tuberculosis |
| **TMIH** | Tropical Medicine & International Health |
| **TREND** | Checklist for standardised reporting of nonrandomised controlled trials |
| **TX** | Texas |
| **UCT** | University of Cape Town |
| **UNAIDS** | United Nations Programme on AIDS |
| **UNICEF** | United Nations International Children's Emergency Fund |
| **WALS** | Wiley Author Licensing Service |
| **WHO** | World Health Organization |
| **WIHS** | Women's Interagency HIV Study |
| **YLD** | Years Lost due to Disability |

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# PART A: PROTOCOL

## Protocol Summary

This protocol represents a study aimed at investigating hearing loss among perinatally HIV-infected (PHIV+) adolescents on antiretroviral therapy (ART), and HIV-non-infected (HIV-) adolescents in Cape Town, South Africa.

The study consists of a secondary analysis of pure-tone audiometry results, clinical history, physical examination findings, HIV-related blood sample results, demographics, health-related and socio-economic status indicators, collected between July 2013 and February 2015, during the first visit for participants enrolled in the Cape Town Adolescent Antiretroviral Cohort (CTAAC) Study, on a cross-sectional sample of 540 adolescents (432 with perinatal HIV-infection on ART and 108 HIV-uninfected adolescents).

South Africa remains a large contributor to the global HIV burden, and is a part of the developing world where the burden of hearing loss is highest. ART has increased longevity in HIV-infected populations, resulting PHIV+ children living to their adolescence with chronic HIV-infection and prolonged use of ART. Various studies have suggested that PHIV+ adolescents have higher rates of hearing impairment compared to their HIV- uninfected counterparts. Few studies have been done in South Africa including a longitudinal follow-up of a cohort as large as the CTAAC Study. Knowledge about hearing loss in PHIV+ adolescents and the specific risk factors is scarce. There is a consensus that research into hearing loss seen among PHIV+ adolescents needs to be up-scaled, particularly in low-resource settings.

In this study, a cross-sectional analysis will be carried out, descriptive analyses and relevant methods for comparing HIV-infected and HIV- adolescents will be instated, and logistic regression models will be established to identify predictors for hearing loss in PHIV+ adolescents in the South African adolescent population.

## 1. Background

There are over 36 million people living with HIV worldwide, of which 19 million live in East and Southern Africa (1). South Africa is one of six countries that make up for half of the world's adolescent population living with HIV (2).

Hearing loss is common amongst children and adolescents who had perinatal exposure to HIV. HIV positive children and adolescents show higher rates of hearing loss compared to their HIV uninfected counterparts (3).

According to the World Health Organisation (WHO), hearing thresholds above 30 decibels (dB) in the better ear are indicative of disabling hearing loss (4). In children and adolescents, hearing is essential to spoken language, academic performance and social engagement (5). Therefore, hearing loss becomes an immediate challenge towards social integration and education (5). The effect of hearing loss in the long run has a negative impact on various aspects of an individual's life, including attitude, social skills, self-esteem and job performance. This in turn can affect the economic and social development of communities and the country at large (5).

The majority of the 360 million people worldwide who live with debilitating hearing loss come from low and middle income countries, with children making up to 10% of those affected by hearing loss (5). Seventy-five percent of childhood hearing loss is preventable in low income countries, with over a third being due to infection (5).

Some low income countries report levels of hearing loss in HIV-infected children to be nearing 40% (6). A systematic review exploring the burden of disability in Sub-Saharan Africa showed a significant increase in hearing impairments amongst those living with HIV, with a median prevalence of 24.1% (95% CI: 19.2–29.0% (7)). Another study in an HIV paediatric clinic in Johannesburg showed similar figures of 26% (8). Previously, outside of

Africa, the largest cohort study (231 participants) of diagnostic audiology testing that looked at hearing loss in HIV infected children and HIV uninfected but exposed children showed significantly higher levels of hearing loss in these populations compared to their healthy counterparts (3). In Cape Town, a similar study used a smaller sample, showing a 21.6% prevalence of hearing loss amongst HIV infected children. Despite this figure not being statistically significantly different to that of hearing loss amongst uninfected children, the study pointed to a higher level of hearing sensitivity in HIV infected children (9). We are already seeing that this population has problems of hearing loss beyond and above those seen in HIV non-infected children and adolescents (10, 11).

Despite the widespread and heavy burden of HIV in Sub-Saharan Africa, and evidence suggesting that HIV is associated with impairment, there is still insufficient research in this area (7, 11, 12). Although some previous studies have been conducted in South Africa, larger studies have been advocated for in this population group (8). The specific risk factors for the increased rate of hearing loss in HIV-infected children and adolescents are unknown. Future studies should therefore evaluate specific risk factors for hearing loss in order to suggest meaningful avenues for people living with HIV to manage these challenges (3, 7, 9). Early identification of hearing loss in children can provide immense benefits, yet many children and adolescents remain unreached (5, 6, 13). This emerging population of children and adolescents living with HIV have unique needs which need to be addressed.

## 2. Aim and Objectives

The overall aim of this study is to investigate hearing loss in adolescents in South Africa, focusing on perinatally HIV-infected adolescents. This study is nested in the Cape Town Adolescent Antiretroviral Cohort (CTAAC) Study, whose overall aim is to look at chronic disease markers and disease progression in this same population.

These are the specific aims of the study:

1) To describe the prevalence of hearing loss in HIV-infected and HIV non-infected adolescents in Cape Town, South Africa using screening tools.
2) To investigate the patterns in the association between hearing loss and perinatal HIV infection in adolescents.

## 3. Methodology

### 3.1. Study Design

This is a cross-sectional, descriptive analysis, nested in the CTAAC Study (protocol attached).

### 3.2. Characteristics of the Study Population.

Five hundred and fifteen perinatally HIV-infected adolescents who have been on antiretroviral therapy (ART) for at least 6 months, and 110 HIV non-infected adolescents, all between 9 and 14 years of age, will be included in the analysis. The inclusion and exclusion criteria are as per the CTAAC Study as stated below, as well as the adolescents having undergone pure-tone air-conduction (AC) testing, and having consistent and available results.

*Inclusion Criteria*

- The inclusion criteria will be made broad so as to provide a generalisable sample of participants.
    - The participants will all be between the ages of 9 and 14 years at last birthday.
    - Receiving ART for a minimum of 6 months at recruitment (factors that were not considered included exposure to ART via Prevention of Mother to Child Transmission (PMTCT), previous ART exposure, and level of viral load suppression at enrolment).
    - Child providing assent to participate in the study with the parent/ caregiver/legal guardian.
    - Parent/caregiver/legal guardian consent provided.
    - Pure-tone air conduction testing results available.
- It is anticipated that these criteria will make it possible for >90% of all children in the target age range to be eligible participants in the study.

*Exclusion Criteria*

- Failure to have met any of the inclusion criteria listed above.
    - Inability to get informed consent from a parent/caregiver/legal guardian (including absence, incapacitation, or refusal regardless of child assent).
    - Child dissent despite parent/caregiver/legal guardian informed consent.
    - No pure-tone air conduction testing results available.

### 3.3. Recruitment, Enrolment and Follow-up Care

Adolescents will be recruited in Cape Town over a duration of 1 year from 8 sites, which include 4 established services: Gugulethu Community Health Centre, Infectious Diseases Clinic (IDC), Red Cross War Memorial Children's Hospital (RCWMCH), G26 HIV/AIDS Service/KidzPositive, Paediatric Infectious Diseases Clinic/KID-Cru, Tygerberg Children's

Hospital and smaller Community Health Centres. Participants will be enrolled at the Clinical Research Unit (CRU) at the RCWMCH and other associated facilities. Follow-up visits will be ongoing at these centres, where routine HIV care and follow up care will be provided.

### 3.4. Research Procedures and Data Collection Methods

This cross-sectional analysis will include all adolescents in the CTAAC study who had received an audiometric screening examination at enrolment. Baseline visit information (including demographic assessment and medical history), laboratory parameters, and clinical examination (including examination of the external ear and otoscopy) will be included.

### 3.5. Baseline Visit Information

Questionnaires will be administered to adolescents and their caregiver/guardian. Interviews will be run in private by trained, well-experienced counsellors at the CRU at the RCWMC.

Demographic assessments will be conducted by collecting data on the following: household and family structure; caregiver/social history (foster care/orphan status, living arrangements, educational history and other relevant socioeconomic status questions).

The detailed medical histories of all eligible participants will be recorded. These include early life history (delivery and perinatal history, including any obstetric complications), exposure to PMTCT interventions, HIV related history (ART initiation, regimens, side effects and adherence), hospital admissions, history of opportunistic infections including tuberculosis (TB) (contacts, treatment, site), and use of drugs for prevention of opportunistic infections. ART adherence was deciphered using pill counts and validated tools (visual analogue scale and 3-day recall from the caregiver and adolescent were conducted separately).

Clinical history will be abstracted by trained research assistants on each child using the medical history interview, as well as the medical record documentation from their service

provider site to verify history and dates. In cases where follow-up history or in-depth details of acute admission details are needed, these will be extracted from medical records from the referral facilities.

### 3.6. Clinical Examination

A structured clinical assessment was done on each participant, which consisted of a structured physical examination by a senior study nurse with in-depth training and extensive supervision from members of senior clinical staff.

### 3.7. Laboratory Parameters

Blood samples will be taken by venepuncture for TB screening, HIV viral load testing and CD4 count. HIV viral load testing was done using the Abbott Molecular Real-Time HIV-1 assay at the National Health Laboratory Services laboratory.

### 3.8. Special Tests

**Screening Hearing Test**. The screening hearing test will consist of AC testing for the hearing threshold at frequencies of 250Hz, 500Hz, 1000Hz, 2000Hz and 4000Hz in both the right and left ears. A threshold of greater than 30dB will be considered a fail. The GSI 18 Screening-audiometer (a single channel, manual audiometer) (14) will be used. AC will be performed according to a standard defined protocol by a trained physician/medical officer in a quiet examination room (typically completed at the CRU at the baseline visit or first possible opportunity). The quiet examination room has previously been shown to provide results comparable to those with noise levels of 50-60dBSPL as recommended. This has been shown by comparing participant result in EchoRoom to the quiet examination room. The same threshold of 30dB will be used to indicate failure, in accordance with the WHO grades

of disabling hearing loss (4). The history, examination and select investigations are summarised in Table A1.

***Table A1*** *Summary of Selected Variables to be included in Data Analysis*

| Variable | Type of Variable | How Variable is Measured (coded value) | | Variable Alternative Form For Analysis if used (code of collapsed categories) | |
|---|---|---|---|---|---|
| **Demographics** | | | | | |
| **Sex** | Categorical, nominal | Male | (1) | - | - |
| | | Female | (2) | | |
| **Race** | Categorical, binary | Black | (1) | - | - |
| | | Coloured | (3) | | |
| **Age** | Numerical, continuous | Years | - | <12 years | (1) |
| | | | | ≥12 years | (2) |
| **Caregiver and Socioeconomic Characteristics** | | | | | |
| **Current caregiver** | Categorical, nominal | Mother | (1) | Caregiver is parent | (1/2) |
| | | Father | (2) | Caregiver is not parent | (3/4/5) |
| | | Grandmother | (3) | | |
| | | Grandfather | (4) | | |
| | | Other | (5) | | |
| **Caregiver marital status** | Categorical, nominal | Married | (1) | Caregiver married | (1) |
| | | Single | (2) | Caregiver divorced/single/other | (2/3/4) |
| | | Divorced | (3) | | |
| | | Other | (4) | | |
| **Caregiver tertiary level of education** | Categorical, binary | Yes | (1) | - | - |
| | | No | (0) | | |
| **Dependence on social grant** | Categorical, binomial | Yes | (1) | - | - |
| | | No | (0) | | |
| **Current school attendance** | Categorical, binary | Yes | (1) | - | - |
| | | No | (0) | | |
| **Primary language spoken at home** | Categorical, nominal | IsiXhosa | (1) | IsiXhosa | (1) |
| | | English | (2) | English/Afrikaans/other | (2/3/4) |
| | | Afrikaans | (3) | | |
| | | Other | (4) | | |
| **Medical History** | | | | | |
| **Previous tuberculosis diagnosis** | Categorical, binary | Yes | (1) | - | - |
| | | No | (0) | | |
| **Experienced ear discharge/ear pain** | | Yes | (2) | | - |
| | | No | (1) | | |
| **Antiretroviral regimen (ART)** | Categorical, nominal | 2 X NRTI + NNRTI | (0) | - | - |
| | | 2xNRTI + PI | (1) | | |
| | | Other | (2) | | |
| **Age at ART initiation** | Numerical, continuous | Years | - | 0-2 | (0) |
| | | | | 3-5 | (1) |
| | | | | 6-14 | (2) |

| | | | | | |
|---|---|---|---|---|---|
| **Duration on ART** | Numerical, continuous | Years | - | < 8<br>≥ 8 | (1)<br>(2) |
| **Physical Examination Findings** | | | | | |
| **Height** | Numerical, continuous | Metres (m) | - | - | - |
| **Weight** | Numerical, continuous | Kilograms (Kg) | - | - | - |
| **Body mass index (computed from height and weight)**‡ | Numerical, continuous | - | - | <18.5<br>≥18.5 | (1)<br>(2) |
| **Right and Left ear external ear examination** | Categorical, nominal | Normal<br>Otitis media<br>Other | (1)<br>(2)<br>(3) | Both ears normal<br>Signs of otitis externa/other | (1)<br>(2/3) |
| **Laboratory Parameters** | | | | | |
| **Viral load** | Numerical, continuous | Copies per millilitre | - | ≤50<br>>50 - <1000<br>≥1000 | (1)<br>(2)<br>(3) |
| **Absolute CD4 count** | Numerical, continuous | Cells/$mm^3$ | - | <200<br>≥200 - <500<br>≥500 | (1)<br>(2)<br>(3) |
| **Percentage CD4** | Numerical, continuous | % | - | <25%<br>≥25% | (1)<br>(2) |
| **Pure-tone air-conduction(AC) testing** | | | | | |
| **AC testing done** | Categorical, binary | Yes<br>No | (1)<br>(2) | - | - |
| **Minimum response level (MRL)**§ | Numerical, continuous | Decibels (Db) | - | - | - |
| **Pure-tone average(PTA)**¥ | Numerical, continuous | Decibels (Db) | - | ≤ 30dB<br>>30dB | (1)<br>(2) |
| **Hearing loss** | Categorical, binary | None<br>Any(unilateral or bilateral) | (1)<br>(2) | - | - |
| **Hearing loss** | Categorical, nominal | None<br>Unilateral<br>Bilateral | (1)<br>(2)<br>(3) | - | - |

‡ Body mass index (BMI) computed from height (m) and weight (kg), equation: $\boldsymbol{BMI} = \frac{\boldsymbol{kg}}{\boldsymbol{m^2}}$, § MRL is the hearing threshold in decibels which was recorded at each frequency (250 Hz, 500Hz, 1000Hz, 2000Hz and 4000Hz) for each ear, ¥ PTA calculated by averaging MRL at 500Hz, 1000Hz, 2000Hz for each participant and will be used to decipher best, worst ear and for right and left ear.

### 3.8.1. Data Management

No data containing real patient names will be handled during this study (i.e. patient files, informed consent forms). All data containing documents and files will have an anonymous participant identification number, kept separately from any linking information which will not be required for this study. Patient identity will be safeguarded.

The study manual and standard operating procedure from the CTAAC study will be adhered to. Electronic data required for analysis is kept in password-protected files, and only made available by the entrusted study team members. All data is stored at the University of Cape Town (UCT) within a firewall protected SQL server.

### 3.8.2. Data Analysis

Data will be exported to STATA 13 (STATA for Windows, version 13, STATAcorpLP; College Station, TX). The statistical analysis will be done in this program by the candidate.

General: Data exploration of individual variables will be carried out, examining the distributions and relevant summary statistics (categorical variables: frequencies, percentage frequencies; continuous variables: histograms, means alongside confidence intervals, standard deviations, medians, interquartile ranges). Appropriate measures of central tendency will be instituted, and used to describe normal and non-normal distributions.

Associations will be explored between independent and dependent variables (continuous variables: scatterplots, correlation analysis; categorical variables: contingency tables, cross-tabulation). Visual examination of correlation matrices will be reviewed.

*To address Specific Aim 1:*

The prevalence of hearing loss will be calculated in STATA 13 (STATA for Windows, version 13, STATAcorpLP; College Station, TX) for the HIV-infected and the HIV uninfected adolescents. Ear-specific pure-tone average (PTA) results will be compared by adolescent HIV status using the appropriate parametric (t-test)/non-parametric (Wilcoxon rank-sum test) test for the two independent groups. The minimum response level will be calculated, and every participant will be classified as a pass or fail according to their PTA at 500Hz, 1000Hz, 2000Hz per ear, with respect to the WHO grades of hearing impairment.

*To address Specific Aim 2:*

Clinical and other characteristics will be compared between the two adolescent groups with and without hearing loss, using Fisher's exact test or two-sample t-tests, as deemed appropriate. Potential factors associated with hearing loss will be evaluated using generalized linear models. An appropriate model will be built according to the nature and distribution of the dependent variable, and to the study design. The transformation/link function will be selected according to the independent and dependent variable relationship. The appropriate link function will be selected: an identity link for continuous outcomes, a logit link for binary outcomes.

A manual, backward model selection process will be used to build a model, with hearing loss as the outcome variable of interest. This will be done in the presence of baseline demographic and clinical characteristics deemed significant. The variable selection process will include univariate models for each variable. A conservative p value of 0.2 will be used as a cut-off point for variable inclusion into the multivariate model. The model will be run, and variables excluded according to their significance in the model and model fit.

The final model will be selected using appropriate tools, depending on whether the models being compared are nested or not. Methods of selection will include the log-likelihood test; Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) will also be noted. The desire for parsimony will be considered. The final model will be validated according to the type of model, to ensure that model assumptions have not been violated.

### 3.9. Ethical Considerations

Approval to carry out this analysis as a student mini-dissertation will be obtained from The Human Research Ethics Committee of the University of Cape Town, Faculty of Health Sciences, South Africa. Approval for the CTAAC Study was obtained prior to commencement of the study. The ethics reference number (HREC) is 051/2013 and is due for renewal on the 30th of May 2017. This protocol complied with the following:

- The Declaration of Helsinki, 2008
- The Department of Health: Ethics in Health Research: Principles Structures and Processes, 2004
- Guidelines for Good Clinical Practice in the Conduct of Clinical Trials in Human Participants in South Africa. Second Edition, 2006
- Research Involving Children: Standard Operating Procedure: Human Research Ethics Committee, Faculty of Health Sciences: University of Cape Town, 2013

### 3.10. Description of Risks and Benefits

*Potential Risks*

This study will use secondary data. There is no direct contact with study participants and therefore no invasive procedures will be involved. The potential risks to participants are:

1) Risks due to loss of confidentiality due to handling of secondary data of a sensitive nature e.g. HIV status.

All participants will be informed of all possible risks as part of the informed consent process in the enrolment phase of the CTAAC study. Risks involved with the CTAAC study will be clearly outlined and justified. Relevant measures will be taken to protect against them.

*Protection Against Risks*

The risk of any loss of confidentiality will be minimised in various ways.

1) No data containing real patient names will be handled during this study (i.e. patient files, informed consent forms). All data containing documents and files have an anonymous participant identification number, kept separately from any linking information which will not be required for this study. Patient identity will be safeguarded.
2) The study manual and standard operating procedure from the CTAAC study will be adhered to. Relevant measures include:
    a. Electronic data required for analysis will be kept in password-protected files, and only made available to the entrusted study team members. All data is stored at UCT within a firewall protected SQL server.
    b. No previous incidents of loss of confidentiality have been reported on the CTAAC study or any previous studies carried out by the team following these procedures.

*Benefits*

The study population may not directly benefit from the study. The following are potential benefits that justify any potential and minimal risk to participants:

1) An improved understanding of hearing loss in HIV positive adolescents has the potential to improve future health care for this population, particularly in the targeted screening and identification of associations to look for during routine care. The insight added to this area of currently limited knowledge will affect and possibly improve health care in South Africa and beyond.

### 3.11. Informed Consent Process

No informed consent process will take place for this nested study, as consent/assent has been collected in the CTAAC study prior to commencement of the study, in accordance with the requirements of the 2012 HSRC Guidelines for research with minors in the participant's or caregiver's home language (15).

## 4. Use of Information and Publication

Presentation and/or publication of the study results will be done upon collaboration with the CTAAC study investigators. No funding has been involved, and will not impact the results presented and/or published in any way.

The major stakeholders in this research are the adolescent population in Cape Town living with HIV. Their participation in the study includes continued attendance to follow-up sessions and provision of information and samples.

## 5. Logistics

***Table A2*** *Proposed Timetable*

| TASK | DURATION | | | | | | |
|---|---|---|---|---|---|---|---|
| | Aug '16 | Sept '16 | Oct '16 | Nov '16 | Dec '16 | Jan '17 | Feb '17 |
| **Literature Review** | ■ | ■ | ■ | | | | |
| **Data cleaning & exploration** | | | ■ | ■ | | | |
| **Data analysis** | | | | ■ | ■ | | |
| **Results** | | | | ■ | ■ | | |
| **Discussion** | | | | | ■ | ■ | |
| **Dissertation Draft** | | | | | ■ | ■ | |
| **Final Dissertation Write-up** | | | | | | ■ | ■ |

## 6. Budget

The candidate will perform data management and analysis as a part of The Master of Public Health Degree thesis, and no payment is required. No further activities require payment.

## 7. Conflicts of Interest

There are no conflicts of interest to be declared.

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# PART B: LITERATURE REVIEW

## 1. Introduction

Since the advent of Antiretroviral Therapy (ART), the world has seen an increase in longevity amongst HIV-infected populations. With this, perinatally HIV-infected (PHIV+) children are living into adolescence for the first time in history. This has posed a challenge to understanding the effects of chronic HIV-infection and the prolonged use of ART. South Africa remains a large contributor to the global HIV burden, and is concurrently situated in Sub-Saharan Africa, one of the regions with the highest prevalence of hearing loss in the world. Despite the knowledge that hearing loss is common among PHIV+ adolescents, there remains limited knowledge about predictors for hearing loss in this population, especially in the developing world. Research is highlighting the importance and need for further investigation into this unique population with inimitable health needs.

The aim of this review is to describe the prevalence of hearing loss worldwide in order to place the prevalence of hearing loss in PHIV+ adolescents into a global context. The aim is also to identify previously investigated predictors for hearing loss in literature, particularly those unique to PHIV+ adolescents on ART. A brief outline of basic definitions and concepts associated with hearing loss is included in order to provide a background to the summary of literature.

## 2. Literature Search Strategy

The literature search was conducted using Google Scholar and Pubmed online databases. A search using Boolean operators and the following search terms was run: 'HIV' AND 'hearing' AND 'adolescent'. This also included search word variations in the Pubmed database. The literature search was restricted to English language publications published within the last twenty years. Abstracts and titles of these resulting articles were then reviewed to guide

article selection. The summary of the selected Pubmed articles is presented in Table B2. References of the selected Pubmed publications and existing reviews were searched and examined to ensure that all the literature relevant to the literature review objectives were reviewed and included by the author. The relevant studies from the extended literature search, including Google Scholar search engine results, are summarised in Table B3.

## 3. Concepts and Definitions

### 3.1. Hearing Loss

Hearing status is usually quantified according to the lowest intensity of a sound signal that is audible to an individual, which is known as the hearing threshold, and measured in decibels (dB). The hearing threshold is measured over a range of multiple frequencies that are reported in hertz (Hz), and are typically 250Hz, 500Hz, 1000Hz, 2000Hz, 4000Hz and sometimes 6000Hz and 8000Hz (1). In adults, abnormal hearing sound thresholds are those found to be above 25dB (1,2). In children, the World Health Organisation (WHO) describes a sound threshold above 30dB in the better ear as debilitating hearing loss (2).

### 3.2. Adolescence

The WHO has defined adolescence to be the phase of critical transition that occurs between 10 and 19 years of age (3), which has been endorsed by other organisations that have interest in the concept of adolescence (4). However, it is accepted that this definition does not address the experiences of individuals transitioning from childhood to adulthood, cultural differences in shifting from childhood to adulthood, and historical factors that influence the definition of adolescence (3, 5).

## 3.3. Perinatal HIV Infection

For the purposes of this study, perinatal HIV infection is considered HIV infection via vertical infection (mother-to-child). It is presumed that given the age of study participants, horizontal routes of infection are unlikely but cannot be ruled out completely, therefore only a small percentage of the study participants may have acquired HIV horizontally, eg. iatrogenic or sexual.

## 3.4. Evaluation of Hearing

*Pure-Tone Audiometry*

Pure-Tone Audiometry is considered the gold standard for determining hearing thresholds. Audiometry results are traditionally represented graphically using an audiogram. When used for both ears, pure-tone audiometry enables configuration, type, symmetry and degree of hearing loss to be ascertained (1). Pure-tone audiometry can consist of air-conduction (AC) tests, which represent sound through the entire ear, and bone-conduction (BC) tests, which represent sound conducted through the inner ear and auditory canal. When AC testing and BC testing are used together, the location of a pathological lesion causing hearing impairment can be deciphered. In special cases, like auditory neuropathy, hearing loss may require other advanced tests such as auditory brain stem responses and auto-acoustic emissions for diagnosis (1). There are also other audiologic tests and technologies under investigation(6).

*Classification of Hearing Loss*

Classification of hearing loss enables the determination of further test procedures and the selection of appropriate interventions (1, 4). It is essential to classify any hearing loss as part of an audiometric assessment according to degree, configuration and type. There are three basic types of hearing loss: conductive, sensorineural and mixed hearing loss (1, 4).

Conductive hearing loss occurs when sound is not successfully transmitted through the ear canal to the middle ear, whilst sensorineural hearing loss occurs when there is inner ear damage, or damage to the nerve pathway connecting the inner ear to the brain (4). Mixed hearing loss refers to a combined pathology of conductive and sensorineural hearing loss (1). Configuration is determined by the audiogram generated by the AC threshold across the frequency spectrum. The terms used to describe hearing loss configuration include (but are not limited to): flat, rising, trough, notch, gradually and sharply sloping. These are determined by the shape of the audiogram (1). For each ear, the degree of hearing loss is used as an indication of hearing loss severity. The degree of severity is calculated using the Air-Conduction Pure-Tone Average (PTA) at 500Hz, 1000Hz, 2000Hz (1, 4). When hearing loss affects one ear (right or left) it is termed unilateral hearing loss. When it affects both ears (right and left) it is termed bilateral hearing loss. Hearing loss severity is also measured by metrics such as the symmetry of the severity, the onset progression, and the changing severity of the hearing loss.

*Causes of Hearing Loss*

There are many identified causes of hearing loss, ranging from congenital to acquired causes, that vary by hearing loss type. Table B1. shows selected causes of sensorineural and conductive hearing loss adapted from Mahomed et. al. (1).

***Table B1*** *Showing a summary of Selected Causes of Conductive and Sensorineural Hearing Loss(1)*

| **Type of Hearing Loss** | |
|---|---|
| **Conductive Hearing Loss** | **Sensorineural Hearing Loss** |
| **Wax impaction in ear canal** | Congenital infections (e.g. Cytomegalovirus, Rubella) |
| **Tympanic membrane perforation** | Acquired infections (including TB, HIV) |
| **Otosclerosis** | Ototoxicity (e.g aminoglycosides, cytotoxic drugs, antimalarials, loop diuretics) |
| **Ossicular chain dislocation** | Noise induced hearing loss |
| **Cholesteatoma** | Presbyacusis |
| **Otitis media with effusion** | Genetic conditions |

In light of the scope of this literature review, the causes highlighted in the following summary will focus on those that concern PHIV+ adolescents.

## 4. Summary of Literature

### 4.1. The Prevalence of Hearing Loss

Over 1.3 billion people are affected by hearing loss worldwide, making hearing loss the second highest most prevalent impairment contributing to Years Lost due to Disability (YLD) (7). There has been an almost 30% increase in the global prevalence of hearing loss in the last 10 years (7). Globally, 32 million children are living with disabling hearing loss, with the majority coming from low-income and middle-income countries (8).

In the United States of America (USA), the Third National Health and Nutrition Examination Survey (NHANES III) showed that there was a 14.9% prevalence of hearing loss amongst young people between the ages of 6 - 19 (9). These estimates raised interest amongst other researchers, who re-evaluated the data using alternative methods of analysis (9). Other smaller case record reviews of adults in the USA found that the prevalence of hearing loss was less than 1% in HIV-infected patients. This finding has, however, been questioned, and the very low prevalence of hearing loss in this cohort has been attributed to case records lacking information in audiometric testing (10).

There are various studies worldwide that have investigated hearing loss associated with HIV-infection. The Paediatric HIV/AIDS Cohort Study (PHACS) investigated hearing loss amongst two groups of American adolescents: the first group were HIV-infected, and the second were HIV-exposed but uninfected. It was found that 20% of HIV-infected adolescents had hearing loss, which was significantly higher than the 15% of their HIV-uninfected

counterparts, and higher than the hearing loss prevalence reported in NHANES III (11). Young, healthy, Western populations have shown a percentage of diagnosed hearing loss lower than 10% (12). Even in the early stages of HIV-infection (13), hearing loss is a common problem in HIV-infected populations (13-15). A systematic review of evidence spanning over 30 years found the prevalence of hearing impairment in Sub-Saharan Africa to be 24.1% amongst people living with HIV (14). A Cameroonian study found the prevalence of hearing loss in HIV-infected patients to be 27.2%, which was four times higher than the HIV-uninfected control group in the same study (15). A study conducted in Johannesburg yielded similar results, showing a hearing loss prevalence of 23% among HIV-infected adults and 26% among HIV-infected children (16). PHIV+ children in Cape Town had a higher hearing loss of 21.6%, in comparison to the 8.3% prevalence of hearing loss HIV- controls. It is noteworthy that there is not a significant difference between these two percentages (17). Similar studies done in other countries have reported even higher prevalences of hearing loss amongst study groups of HIV-infected children and adolescents: 34% in India, 38.8% in Peru (18) and 33% in a small cohort of HIV-infected Mexican adolescents (19).

*Projected Increase in the Burden of Hearing Loss*

A projected increase in hearing loss in the USA shows that by 2060 almost twice as many people in the country will have hearing loss: an increase from 44.11 million to 73.5 million hearing impaired individuals. This rate of increase in hearing loss will be faster than the population growth rate itself, and is anticipated to put a significant strain on already stretched resources (20).

## 4.2. Risk Factors for Hearing Loss in Adolescents

Risk factors are noted for hearing loss in adolescents throughout literature, with some specifically related to HIV. The following have been found to be independent risk factors for

hearing loss among Chinese children: low parental educational attainment, an income below the national average and single-headed households (21). Western studies among HIV-infected and HIV-uninfected but exposed groups have revealed caregiver race, caregiver IQ, caregiver education status, family income and who the primary caregiver was in the home to be some of the socio-demographic risk factors for communication related impairments (22). Similar socio-demographic predictors have been examined for neurological sequelae in HIV-infected children in Cape Town, namely Body Mass Index (BMI), ancestry, relationship to caregiver and absence of biological parents (23). In HIV-infected Sub-Saharan African children, a low BMI, being male and tuberculosis (TB) infection were found to be risk factors for hearing loss (24). Previous cerebral infections and a history of seizures in HIV-infected children have also been shown to be risk factors for hearing loss (18).

## 4.3. Hearing Loss and HIV Related Risk Factors

*Type of Hearing Loss in HIV- Infected Populations*

It has not been possible to ascertain a typical profile of hearing loss for HIV-infected people, as varied audiological manifestations have been found throughout literature (13, 14, 19, 25). This heterogeneity is also evident in studies conducted amongst HIV-infected populations in South Africa (26). Various audiometric patterns of hearing loss have been found, including sensorineural (13, 25, 27), conductive (28) and mixed hearing loss. In the past, among HIV-infected populations, children have shown conductive hearing loss secondary to middle ear pathology more commonly than adults who experienced more sensorineural hearing loss due to inner ear pathology (29).

*Aetiology and the Mechanism of Hearing Loss Specific to HIV-Infected Populations*

The aetiology of hearing loss in HIV-infected people has been attributed to ototoxic drugs, the action of the virus itself (25, 30) and opportunistic infections (11). Mitochondrial toxicity

resulting in hearing loss has been proposed as the mechanism through which hearing loss may occur, which may be compounded by the synergistic effect of increasing age, NRTI, and HIV (30-32). Sub-Saharan Africa in particular has seen a shift in the epidemiology of paediatric HIV. PHIV+ are affected by chronic HIV-infection and have long-term ART exposure. The effects of aging combined with HIV exposure have manifested in vast complications that include, but are not limited to, hearing loss (33). Lin et. al. found an increased prevalence of sensorineural hearing loss in HIV- infected people aged 18 to 35, which they attributed to HIV-related accelerated biological aging (34).

*Antiretroviral Therapy and Hearing Loss*

The use of unspecified ART regimens have been implicated in the increase in hearing loss seen among HIV-infected adults in comparison to the lower rates of hearing loss in their HIV-infected but ART-naïve counterparts (27). In Australia and America, HIV-infected adults on a regimen containing a Nucleoside Reverse-Transcriptase Inhibitor (NRTI) were found to have markers of mitochondrial toxicity (32) and associated hearing loss (30). Similarly, NRTI use in PHIV+ adolescents was associated with hearing loss in an Australian longitudinal study (32). Despite this evidence of the positive association between the use of NRTI and the increased risk of hearing loss, the PHACS study did not find an association between ART use in PHIV+ adolescents and hearing loss (11, 17). ART therapy was also not associated with hearing loss among HIV-infected adults enrolled into the Multicentre AIDS Cohort Study (MACS) and the Women's Interagency HIV Study (WIHS) studies (35). Protease Inhibitor (PI) use was not associated with mitochondrial damage in Western populations by Cherry et al. and Crain et al. (31, 32). There exist, however, case reports of ototoxicity linked to protease inhibitors. For example, a case study of a 44-year-old Hispanic patient in Albuquerque showed a possible link between hearing impairment and mitochondrial dysfunction due to the independent use of a PI (36).

Apart from investigating the ART drug regimen as a predictor for hearing loss, research has also been conducted to investigate duration on ART as a predictor for hearing loss. Duration on ART was not linked with hearing loss in a case-control study carried out in Cameroon (15), neither was a longer duration on ART linked to hearing loss in a small cross-sectional analysis by Palacios et al. carried out in Mexico (19). Makar et al. suggested that a longer period of time on ART is protective against hearing loss (37). A study conducted amongst an HIV-infected Kenyan cohort found that there was an initial worsening of hearing during the first six months on ART. However, after this initial period of time, the hearing capabilities of the study participants on ART improved and were comparable to those not on ART (38).

*Opportunistic Infections and Hearing Loss in HIV-Infected Individuals*

Opportunistic infections manifesting as middle ear effusions are more prevalent in HIV-infected individuals, as compared to healthy people. This increased prevalence of middle ear effusions has been attributed to opportunistic infections due to low CD4 counts in HIV-infected people (18, 26, 28), and especially so among HIV-infected children (19, 29). At 23% among HIV+ children in Johannesburg, South Africa, otitis media was a common finding in children suffering from hearing loss (16). Fifteen percent of HIV-infected children had opportunistic infections affecting the nervous system in a Cape Town based study, with a resultant 6% prevalence of sensorineural hearing loss (23). Low CD4 counts were associated with increased hearing impairment in South African adults (26), Peruvian children (18) and Mexican children (19). However, low CD4 counts were not seen as predictors for hearing loss among PHIV+ adolescents in other geographical regions (11, 19, 34, 39). High viral load was associated with poorer cochlear function in PHIV+ children (17). A high viral load was also found to be a predictor for generally poor audiological outcomes, (19) but not among MACS and WIHS cohorts (40, 41).

There is a known relationship between HIV-infection and TB, with a global co-infection rate of 13% (7). In Southern Africa, HIV and multi-drug resistant TB co-infection has been noted to be as high as 66% in Botswana (42), 46% in Namibia (24), and 30% in South African children (43). Culture-confirmed TB diagnosis has been recognised as a risk factor for hearing loss in HIV-infected paediatric populations (43), and previous episodes of TB infection were common among HIV-infected children in South Africa (23). Certain drugs used for the treatment of TB are ototoxic, in particular aminoglycosides like amikacin (24, 42). Ototoxic TB treatment drugs are associated with a high incidence of hearing loss, and have shown a clear dose dependent response between drug dosage, duration of use and hearing loss (42). A quarter of children with multi-drug TB infection had hearing loss associated with TB drug treatment, and continued to worsen after cessation of treatment (43).

### 4.4. Adverse Outcomes of Adolescents with Hearing Loss

Globally, individuals with hearing loss have been found to have delays in speech and language acquisition (11), poorer emotional coping mechanisms, and lower academic achievement (44, 45). During adolescence, these individuals are also less likely to be attending school compared to adolescents who do not suffer from hearing loss (45). Furthermore, adolescents with hearing loss are more likely to have behavioural, psychological and social difficulties (11, 44). The adverse outcomes of hearing loss are vast and have the propensity to negatively affect social and economic development (8). Already, PHIV+ adolescents have been shown to be at a higher risk of mental health issues, to perform more poorly in general cognitive tests and to have slower processing on visuo-spatial tasks than healthy adolescents (46). Rates of learning impairment in HIV-infected and HIV-uninfected but exposed children are over double (40% and 16% respectively) the rates expected in developed nations (22). PHIV+ adolescents who develop hearing loss carry the

heavy burden of the compounded effects of chronic HIV-infection and the many complications associated with hearing loss (33).

## 5. Conclusion

Hearing loss remains an important source of disability. In the developing world where less data exist on hearing loss (47), the problem of hearing loss receives inadequate attention, despite requiring increased attention, especially among adolescents (48). Furthermore, hearing loss is a more frequent occurrence in HIV-infected individuals and HIV-exposed but uninfected individuals, as compared to HIV-uninfected and unexposed individuals (11). Research efforts are intensifying with PHACS, following-up with the Adult Master Protocol (AMP), in order to further understand the first generation of PHIV+ adolescents who have survived into adolescence in the era of ART (49). However, this research is not based in Sub-Saharan Africa, where it has been found that there is possibly the highest double burden of HIV-infection and hearing loss in the world (47, 50). Striking findings in a review of literature highlight that there is scarce data available about PHIV+ adolescents in developing countries, where the double burden of hearing loss and HIV-infection is highest (46).

A review of literature has suggested that audiological manifestations of HIV-infection in developing countries may well differ from manifestations seen in developed countries, due to differences in contextual and cultural factors (47). Therefore, it is of great importance that there is an increase in research conducted about PHIV+ adolescents and hearing loss in Sub-Saharan Africa. It is also essential to tailor programmes that are directed at auditory well-being in PHIV+ adolescents, and to ensure that measures are instated to prevent and manage this unique group of individuals (11, 14, 25, 28, 51). Primary (reducing the number of new cases of hearing loss), secondary (halting the progression of hearing loss) and tertiary (treatment and reduction of the sequelae of hearing loss) prevention strategies are advocated for by researchers who are concerned about the future burden of hearing loss (20, 50).

***Table B2*** *Showing Summary of Pubmed Search*

| In-text Citation | Author, year | Setting | Study design | Population and sample size | Objective | Findings |
|---|---|---|---|---|---|---|
| **(6)** | Peer, 2015 | Cape Town, South Africa | Quasi-experimental study | Twenty-five people aged between 15-80 years, attending the Otolaryngology Clinic at Groote Schuur Hospital | To determine the accuracy of the uHear app on the iPhone as a screening tool for moderate hearing loss at multiple frequencies | The uHear application for iPhone was a reasonable screening tool for disabling hearing loss. It showed high sensitivity and a high negative predictive value |
| **(10)** | McNaghten, 2001 | The United States of America | Medical case record review | Three thousand six hundred and forty-six HIV-infected patients, of median age 37.5 years | To describe the prevalence of hearing loss amongst HIV-infected patients enrolled in the Adult Spectrum Disease (ASD) project | The prevalence of hearing loss among HIV-infected adults was less than 1% (exact percentage is 0.8%), but the low prevalence could be attributed to case records not containing information on audiometric assessment |
| **(11)** | Torre, 2012 | Fifteen sites in Puerto Rico and the United States of America | Cohort study | Two hundred and thirty-one adolescents (145 HIV positive, 86 HIV unexposed) 7-16 years old, enrolled in the Adolescent Master Protocol (AMP) study of the Paediatric HIV/AIDS Cohort Study (PHACS) | Defining prevalence and evaluation of risk factors for hearing loss | HIV-infected participants had increased odds of hearing loss compared to HIV-uninfected but exposed participants (20% versus 10.5%), and both greater than NHANES III estimates. ART regimen was not linked to hearing loss |
| **(13)** | Tshifularo, 2013 | Pretoria, South Africa | Prospective cohort study | One hundred and fifty-three adults and children attending the outpatient's HIV clinic at Steve Biko Teaching Hospital | To ascertain otolaryngological manifestations in HIV-infected patients | Otolaryngological manifestations are common early presentations of HIV infection. Chronic suppurative otitis media was the most common otological presenting complaint. Chronic suppurative otitis media, otitis media with effusion and sensory-neural hearing loss were in the top five otolaryngological manifestations of HIV-disease |

| In-text Citation | Author, year | Setting | Study design | Population and sample size | Objective | Findings |
|---|---|---|---|---|---|---|
| **(15)** | Fokouo, 2015 | Yaounde, Cameroon | Case-control study | One hundred and eighty patients aged 15 to 49 years old | To investigate hearing loss in HIV-infected patients on ART, and HIV-infected patients not on ART | HIV+ participants had more otologic complaints than HIV- participants. The prevalence of hearing loss among HIV+ participants was 27.2%, and 5.6% in the HIV-uninfected participants. Age, sex, CD4 count and duration on ART were not found to be predictors for hearing loss among HIV- participants. Bilateral hearing loss was found in 43% of cases, right sided hearing loss in 21%, and 36% in left sided hearing loss. Sensorineural hearing-loss was the most prevalent type of hearing loss (61.7%). ART naïve patients had worse hearing than patients on ART |
| **(17)** | Torre, 2015 | Puerto Rico and the United States of America | Prospective cohort study | One hundred and seventy-two children aged 7- 16 years old who were born to HIV+ mothers, enrolled in the PHACS study | To determine the association between HIV and distortion product optoacoustic emissions (DPOEs) | PHIV+ children and HIV-exposed but HIV-infected children had similar cochlear function. Higher viral load was associated with poorer cochlear function in PHIV+ children |
| **(18)** | Chao, 2012 | Lima, Peru | Cross-sectional observational study | One hundred and thirty-nine HIV-infected children aged 4 to 19 years | To investigate predictors for hearing loss, and measure the prevalence of hearing loss among HIV+ children in Peru | The prevalence of hearing loss among HIV-infected children was 38.8%. Predictors for hearing loss in HIV-infected children were low CD4 cell count, previous cerebral infection and middle ear disease |
| **(19)** | Palacios, 2008 | Mexico City, Mexico | Cross-sectional study | Twenty-three children between 5 months and 16 years of age | To evaluate vestibular and audiologic disorders in HIV-infected children on ART | Thirty-three percent of children between 12 and 16 years old had hearing loss. Chronic suppurative otitis media was a predictor for conductive hearing loss. Prolonged duration of HIV-infection, higher viral load counts and lower absolute CD4 counts were predictors for abnormal audiological findings. A wide variety of audiological manifestations of HIV infection were seen among HIV-infected children. Duration of ART exposure was not found to be a predictor for hearing loss |

| In-text Citation | Author, year | Setting | Study design | Population and sample size | Objective | Findings |
|---|---|---|---|---|---|---|
| **(22)** | Rice, 2012 | Puerto Rico and the United States of America | Prospective cohort study | Four hundred and eighty-six HIV-infected and HIV-exposed but uninfected participants aged 7-16 years old | To quantify risk of language impairment in children with perinatal HIV-exposure or HIV-infection | There were no differences found between caregiver characteristics (marital status, education, IQ). HIV-infected children came from higher income households than their HIV-uninfected but exposed counterparts, they were also more likely to come from homes where their own parent was their primary care-giver. Children with HIV-infection or HIV-exposure have high rates (40%) of learning impairment compared to the 16% expected in the American population |
| **(23)** | Govender, 2011 | Cape Town, South Africa | Cross-sectional study | Seventy-eight patients aged 2 to 12 years of age | To investigate neuro-behavioural and neurologic complications of HIV1-infected children | Six percent of HIV1-infected children had sensorineural hearing impairment. Previous episodes of TB were common among children infected with HIV1. Fifteen percent of participants had central nervous system opportunistic infections. Over half of the participants had behavioural problems |
| **(24)** | Sagwa, 2015 | Namibia | Retrospective cohort study | Three hundred and fifty-three patients on MDR-TB treatment | To quantify risk caused by Kanamycin and Amikacin in MDR-TB treatment | Forty-six percent of MDR-TB patients were HIV-infected as well. The prevalence of any hearing loss was 58% in this study. Amikacin proved to be a higher risk than Kanamycin for hearing loss. Risk factors for hearing loss in this population were HIV co-infection, a lower BMI and being male |
| **(26)** | Khoza, 2001 | Johannesburg, South Africa | Observational study design | One hundred and fifty HIV-infected adults aged between 18 and 55 years old | To describe the prevalence and type of hearing loss among HIV-infected adults in Johannesburg | The prevalence of hearing loss was 23% among HIV-infected adults in Johannesburg. Conductive and sensorineural hearing loss, with a wide range of severity, was found in this population. Deterioration of immunological status was associated with sensorineural hearing loss. The severity of hearing loss was not range-specific. Opportunistic infections and their treatment appeared to be predictors of hearing loss |

| In-text Citation | Author, year | Setting | Study design | Population and sample size | Objective | Findings |
|---|---|---|---|---|---|---|
| **(27)** | Matas, 2010 | Sao Paulo, Brazil | Case-control study | Fifty-six HIV-infected adults (24 not on ART, 32 on ART) | To compare electro-physical manifestations between HIV-infected adults on ART and HIV-infected adults not on ART | ART exposure was associated with increased abnormalities in the brainstem auditory pathway among HIV-infected adults |
| **(28)** | Obasikene, 2014 | Benin City, Nigeria | Cross-sectional study | Ninety-seven HIV-infected people and non HIV-infected people all between 18-45 years old and attending the HIV clinic at the University of Benin Teaching hospital | To use tympanometry to compare middle ear pathology between HIV-infected adults and HIV-uninfected adults | Middle ear effusion was more common in HIV-infected adults than in HIV-uninfected adults. Middle ear effusion (seen as a type B tympanogram) could be the cause for middle ear pathology seen in HIV-infected populations, although it may not be detected in the absence of clinical features of otitis media. Decreasing CD4 cell counts were correlated with increasing presence of middle ear effusions |
| **(29)** | Matas, 2010 | Sao Paolo, Brazil | Cross-Sectional study | One hundred and forty-eight HIV-infected and HIV-uninfected adults and children between 3-50 years of age | To characterise audiological outcomes and Brainstem Auditory Evoked Potential (BAEP) in adults and children with HIV | There was a high prevalence of hearing loss among HIV-infected adults and children. Assessments showed that adults had a significantly higher prevalence of altered hearing than children in the HIV-infected group. Findings of middle ear pathology (manifesting as conductive hearing loss) were more common in children, whilst inner ear pathology (manifesting as sensorineural hearing loss) was more common in adults in the HIV-infected group |
| **(37)** | Makar, 2012 | India | | Sixty-seven HIV+ participants aged 4-16 years | To describe the nature of speech, language and audiological problems encountered by children with HIV | Thirty-four percent of HIV infected adolescents and children had hearing loss. Communication problems were common among HIV-infected adolescents and children. Those on ART for longer duration had a lower occurrence of communication disorders |

| In-text Citation | Author, year | Setting | Study design | Population and sample size | Objective | Findings |
|---|---|---|---|---|---|---|
| **(38)** | Makau, 2010 | Nairobi, Kenya | Case-control study | Two hundred and seventy-one HIV-infected patients on ART, and 273 who were not on ART | To determine if ART is related to hearing loss | ART use was not associated with hearing loss, despite an initial worsening of hearing in the first 6 months on ART, that was noted to resolve. More patients not on ART (34%) compared to those receiving ART (28%). Increasing age was associated with hearing loss. The most common type of hearing loss was sensorineural hearing loss |
| **(39)** | McHugh, 2016 | Harare, Zimbabwe | Cross-sectional study | Three hundred and eighty-five participants of ages 6-15 years | To determine the prevalence of chronic HIV-related morbidity | Children and adolescents with HIV had a heavy burden of chronic disease. There was no association found between CD4 count and chronic conditions. From self-reporting, 12% of HIV+ children and adolescents had hearing impairment |
| **(42)** | Modongo, 2014 | Botswana | Retrospective cohort study | Four hundred and thirty-seven patients with MDR-TB above 15 years of age attending specialised TB treatment centres | To investigate the effect of Amikacin in treating MDR-TB and risk factors associated with hearing loss in patients receiving Amikacin for MDR-TB treatment | Amikacin was associated with a high incidence of hearing loss. Increasing dose, and increasing duration of Amikacin use increased risk of hearing loss |
| **(46)** | Laughton, 2013 | Global | Systematic review | Multiple studies | To review evidence on neurodevelopmental outcomes of PHIV+ adolescents | There is a paucity of research evidence in developing countries compared to developed nations. Effects of HIV-infection on the developing brain are not well understood. PHIV+ adolescents have worse neurodevelopmental outcomes than their healthy peers |
| **(47)** | Khoza-Shangase, 2010 | Global | Systematic review | Multiple (exact number not stated) | To review literature with the aim of highlighting the need for intensified research on HIV-infection and its effect on auditory function | Audiological manifestations of HIV-infection are heterogeneous. There is a paucity of research on hearing loss in developing countries in the HIV-infected population. It is possible that the results of studies done in developed countries do not correctly mirror findings in the developing nations as contextual factors differ |

| In-text Citation | Author, year | Setting | Study design | Population and sample size | Objective | Findings |
|---|---|---|---|---|---|---|
| **(49)** | Tassiopoulo, 2016 | Puerto Rico and the United States of America | Protocol for a prospective cohort study | Eight hundred young men and women previously enrolled in the PHACS Adolescent Master Protocol (AMP) study, now planned for follow-up in the AMP Up study | To investigate the outcomes of PHIV+ adolescents during long-term follow-up | No outcomes recorded from the study yet |

***Table B3*** *Summary of Studies Included in the Extended Search*

| In-text Citation | Author, year | Setting | Study design | Population and sample size | Objective | Findings |
|---|---|---|---|---|---|---|
| **(7)** | Vos, 2016 | Global | Systematic review and analysis (1990-2015) | Over nine billion estimates as part of the Global Burden of Disease (GBD) Study | To generate assessments and provide trends of disease and injury sequelae, prevalence and years lost to disability for 310 conditions | The second leading cause of impairment in individuals by numbers affected was hearing loss. Hearing loss of greater than 20dB affected 1.33 billion people worldwide.<br>HIV/AIDS was one of the main contributors to disease burden in regions with a low socio-demographic index, and in southern Sub-Saharan Africa, it was found to be the universal leading cause of disability.<br><br>Global tuberculosis incidence was 10.2 million cases in 2015, and higher than the WHO estimate of 9.6 million cases in 2014. 13% had HIV and tuberculosis co-infection, as opposed to WHO estimate of 12% |
| **(9)** | Barret, 2015 | The United States of America | A re-analysis of Third National Health and Nutrition Examination Survey (NHANES III) | Six thousand one hundred and sixty-six children aged 6 to 19 years of age | To make refinements to the original NHANES III analysis by delineating permanent hearing loss from fluctuating hearing loss, and provide an emphasis on best ear and worst ear thresholds | Better ear threshold is a superior gauge for portraying real disability in children. The prevalence of permanent hearing loss was 1.23%, and fluctuating hearing loss was 0.2% at a 16db threshold. Ethnicity and race were found to differ in prevalence of hearing loss |
| **(12)** | Centres for Disease Control (CDC), 2009 | The United States of America | National Survey (hearing Screening and Follow-up Survey) | Three million nine hundred and fifteen thousand three hundred and sixty infants | To screen and diagnose hearing loss in infants | Hearing loss was diagnosed in 8.9% of infants who failed the screening test |
| **(14)** | Banks, 2015 | Sub-Saharan Africa | Systematic review of literature from 1980 to 2013 | Sixty-one articles | To review the prevalence of HIV and disabilities amongst adults and children | HIV was linked to an increased risk of disability, and significantly lower levels of functioning in HIV-infected people. The prevalence of hearing impairment was 24.1% in people living with HIV |

| In-text Citation | Author, year | Setting | Study design | Population and sample size | Objective | Findings |
|---|---|---|---|---|---|---|
| **(16)** | Khoza-Shangase, 2009 | Johannesburg, South Africa | Pilot study | Sixty-two children from 18 months to 6 years old | To describe the prevalence of hearing loss and associated clinical findings in HIV-infected children | The prevalence of hearing loss was 26% in HIV-infected children, and 23% of these participants had otitis media, which was found to be the most likely cause for hearing loss |
| **(20)** | Yun, 2017 | China | A national survey (2006 China National Survey on Disability) | Six hundred and sixteen thousand nine hundred and forty children aged 0-17 years old. | To describe the prevalence of hearing loss and identify risk factors for hearing loss among Chinese children | The prevalence of hearing loss was 17.49 per 10,000 which was significantly lower than American, Canadian and African populations. Children and adolescents between the ages of 6-17 had significantly higher rates of hearing loss than those younger than 6 years of age. Risk factors for hearing loss were low parental educational attainment, low family income, single-head homes |
| **(25)** | Araújo, 2012 | Multi-country | Systematic review | Thirty-eight studies of hearing impairment in HIV infected individuals | To determine the occurrence of hearing loss, as well as characterise hearing loss by degree and type, in HIV infected individuals | Hearing loss varied vastly by configuration and degree in HIV-infected individuals. It included conductive, sensorineural, and mixed hearing loss, in addition to vestibular disorders and tinnitus. Aetiology was attributed to ototoxic drugs, opportunistic infections, and to the virus itself |
| **(30)** | Simdon, 2001 | Denver, United States of America | Case series | Three adult males infected with HIV | To observe the progression of hearing on NRTI | Tinnitus and hearing loss found in patients taking NRTI. Effect of NRTI toxicity could be worse in aging populations in combination with the HIV virus itself |
| **(31)** | Crain, 2010 | The United States of America | Prospective cohort study (1993-2004) | Two thousand nine hundred and thirty-one children perinatally infected with HIV, enrolled in the Paediatric AIDS Clinical Trials Group | Mitochondrial damage in association with ART in perinatally HIV infected adolescents | NRTI (Stavudine and Lamuvidine) associated with mitochondrial damage in perinatally HIV-infected children |

| In-text Citation | Author, year | Setting | Study design | Population and sample size | Objective | Findings |
|---|---|---|---|---|---|---|
| **(32)** | Cherry, 2006 | Australia | Longitudinal study | Sixty-two HIV infected adults, >18 years old, attending Alfred hospital in Melbourne | To use tissue mitochondrial DNA as a marker of NRTI (nucleoside analogue reverse transcriptase inhibitor) toxicity | Current use of NRTI (Stavudine and Didanosine) associated with tissue mitochondrial DNA depletion ($p<0.0001$), but no association with protease inhibitor therapy |
| **(33)** | Lowenthal, 2014 | Sub-Saharan Africa | Systematic literature review | Number unspecified | To discuss HIV infection in adolescents, and the changing epidemiology of paediatric HIV | There is a shift of the burden of HIV-infection from paediatric populations to adolescent populations. PHIV+ adolescents are facing distinct chronic health challenges and psychological issues |
| **(34)** | Lin, 2013 | Taiwan | Retrospective cohort, population-based study | Eight thousand HIV-infected patients and 43800 HIV-uninfected controls aged 18 to 35 years old and appearing on the Taiwan National Health Insurance research database | To investigate sensorineural hearing loss in HIV infected adolescents and young adults | Sensorineural hearing loss was associated with HIV infection in patients between 18 and 35 years of age, but not above 36 years of age. Sensorineural hearing loss was attributed to HIV- related acceleration of biological aging in HIV-infected people. Sensorineural hearing loss was not associated to the severity or progression of HIV infection |
| **(35)** | Torre, 2015 | The United States of America | Cross-sectional analysis of data from cohort study | Two hundred and sixty-two men (enrolled in the Multicentre AIDS Cohort Study [MACS]) and 134 women (enrolled in the Women's Interagency HIV Study [WIHS]) | To investigate pure-tone thresholds in HIV-uninfected and HIV-infected adults. To find HIV-related factors associated to pure-tone thresholds | HIV-infected participants had higher low-frequency (LPTA) and higher high-frequency pure-tone averages (HPTA) in the better ear than HIV-uninfected participants (18% and 12% respectively). The effect of HIV-infection on hearing was the same for best and worst ear. HIV-related factors (viral load, CD4, CD8, ART therapy, AIDS history) were not significantly associated with LPTA and HPTA, although increased age was associated with LPTA and HPTA |

| In-text Citation | Author, year | Setting | Study design | Population and sample size | Objective | Findings |
|---|---|---|---|---|---|---|
| **(36)** | Williams, 2001 | Albuquerque, United States of America | Case report | One 44-year-old, HIV-infected Hispanic man | To report on mitochondrial toxicity | Mitochondrial dysfunction was independently associated with Protease Inhibitor (lopinavir-ritonavor) manifested as hearing impairment |
| **(41)** | Torre, 2016 | The United States of America | Cross-sectional analysis of data from cohort study | Two hundred and sixty-two men (enrolled in the Multicentre AIDS Cohort Study [MACS]) and 134 women (enrolled in the Women's Interagency HIV Study [WIHS]) | To compare speech audiometry measures between HIV-uninfected and HIV-infected adults. To find HIV-related factors associated to speech audiometry measures | Speech recognition thresholds were only borderline significantly worse in HIV+ participants than HIV-participants. HIV-related factors (viral load, CD4, AIDS history) were not significantly associated with speech audiometry results. In combination with findings from this and other research done on this cohort, the aetiology of hearing loss in HIV is likely due to central auditory processing disorders or peripheral auditory neural issues. Speech-in-noise testing may be useful in the future to compare hearing between HIV-infected and HIV-uninfected populations |
| **(43)** | Seddon, 2013 | Cape Town, South Africa | Retrospective cohort study | Ninety-four children, median age of 43 months old | To examine hearing loss in children treated for multidrug-resistant tuberculosis | Prevalence of hearing loss was 25%, and a third were HIV-infected. Culture-confirmed vs presumed TB was a risk factor for hearing loss. In some cases, hearing loss continued to worsen after TB treatment |
| **(44)** | Kotby, 2008 | Egypt | Retrospective case-record review between 2002 and 2006 | Number not stated. Participants seen in the Audiology Unit, Ain Shams University | To determine the public health impact of hearing loss in Egypt | Eighteen percent of newborns failed screening hearing test, of which 4.8% were confirmed to have hearing loss on follow-up. Adverse outcomes of hearing loss include poor language skills, low academic achievement, as well as behavioural, psychological, intellectual and social problems |
| **(45)** | Hrapack, 2016 | Lilongwe, Malawi | Cross-sectional survey | Three hundred and eighty HIV-infected children aged 4-14 years of age | To estimate prevalence and types of hearing loss in HIV-infected children | Prevalence of hearing loss was 24%, Frequent ear infections, ear drainage, TB, severe HIV disease, or low BMI increased risk of hearing loss. Duration of ART and CD4 count were not associated with hearing loss. Hearing loss increased risk of school non-attendance and poor emotional and academic function |

| In-text Citation | Author, year | Setting | Study design | Population and sample size | Objective | Findings |
|---|---|---|---|---|---|---|
| **(50)** | Olusanya, 2014 | Global | Literature review | Not stated | To highlight global trends of hearing loss from 1985, and to map a plan of action to tackle hearing loss | Developing nations, particularly in Africa and Asia need to be alerted to the enlarging prevalence of hearing loss, and provide interventions that tackle the challenge |
| **(51)** | Torre, 2015 | Cape Town, South Africa | Cross-sectional analysis | Sixty-one PHIV+ children, 24 HIV-uninfected children | To gather data on HIV-uninfected and PHIV+ children in Cape Town | Hearing loss was higher for PHIV+ children at 21.6%, but not significantly different to that of HIV- children who had an 8.3% prevalence of hearing loss. More unilateral hearing loss was seen in PHIV+ adolescents |
| **(52)** | Niskar, 1998 | The United States of America | A population-based cross-sectional Survey (The Third National Health and Nutrition Examination Survey (NHANES III)) between 1988 and 1994 | Six thousand one hundred and sixty-six children aged 6 to 19 years of age | To describe the prevalence of hearing loss and identify sociodemographic profiles of children with hearing loss using pure-tone audiometry | Audiometric screenings revealed that 14.9% of participants had high frequency or low-frequency loss at a 16dB threshold. Most hearing loss was unilateral |
| **(53)** | Hontelez, 2016 | South Africa | A population-based cohort study (Wellcome Trust Africa Centre) between 2009 and 2012 | Thirty-two thousand three hundred and nineteen people above 15 years of age | To evaluate the impact of ART scale-up in South Africa | There has been a rapid scale-up of ART in South Africa. This has resulted in improved primary care, and better health outcomes for HIV-infected and HIV-uninfected community members |

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# PART C: MANUSCRIPT[†]

[†] This journal manuscript meets the requirements set out in the Instructions for Authors of *Tropical Medicine & International Health*. These instructions are appended in Part D: Appendices as originally worded on the journal website. Font style has been altered to match the other parts of this dissertation.

# The Prevalence of Hearing Loss in HIV-infected South African Adolescents on Antiretroviral Therapy

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Keywords: Hearing loss, Perinatal HIV Infection, HIV/AIDS, Adolescent, Cape Town.

Target Journal: Tropical Medicine & International Health

(Manuscript word count =3470)†
(Abstract word count = 249)‡

* As per Master of Public Health Dissertation Guidelines, co-authors are not listed on the journal ready manuscript. The contribution of the supervisor and collaborators is listed in the acknowledgments section of this dissertation.
† Manuscript body word limit for *Tropical Medicine & International Health* is 3500 words.
‡ Abstract body word limit for *Tropical Medicine & International Health* is 250 words.

## Abstract

### Objective

To investigate hearing loss among perinatally HIV-infected (PHIV+) adolescents on antiretroviral therapy (ART), and HIV-non-infected (HIV-) adolescents in Cape Town, South Africa.

### Methods

A cross-sectional analysis was carried out to describe the prevalence, nature and predictors (demographic, past medical history, clinical findings) of hearing loss in adolescents between 9 and 14 years of age. Screening pure-tone air-conduction (AC) thresholds above 30 decibels (dB) were considered to be indicative of debilitating hearing loss. Statistical analysis included univariate analysis and multivariate logistic regression.

### Results

The cross-sectional analysis included data from 540 participants; consisting 273 males (51%), 267 females, 432 PHIV+ and 108 HIV-, with a median age of 12 years. Hearing impairment was observed in 19% of all the adolescents in the study. Multivariate analysis showed the following predictors for any hearing loss: an unmarried primary caregiver (odds ratio (OR) 0.59; 95% confidence interval (CI), 0.39;0.91, p = 0.015), being female (OR 1.67; 95% CI, 1.12;2.51; p = 0.013) and reports of being troubled by ear pain or discharge in the last month (OR 2.54; 95% CI, 1.55;4.17; p = <0.001) after adjustment. Univariate analysis showed an association between hearing loss and a longer duration on ART among PHIV+ adolescents (OR 1.80, 95%CI 1.17;2.75, p = 0.007).

**Conclusion**

The prevalence of hearing loss appears to be comparable between PHIV+ and HIV- adolescents in Cape Town. In low resource settings, a history of ear pain or discharge within the last month may be used as a screening tool for a hearing assessment, and guide referral for formal hearing tests.

**Key words**: Hearing loss, Perinatal HIV Infection, HIV/AIDS, Adolescent, Cape Town.

## 1. Introduction

The global prevalence of hearing loss has risen by almost 30% in the last 10 years, now accounting for over 1.3 billion people, and ranking as the second most prevalent impairment and contributor to years lost due to disability (YLD) worldwide (1). In Sub-Saharan Africa it is estimated that a quarter of the population suffers from hearing impairment (2). Children make up about 10% of people who live with hearing impairment worldwide, the majority living in low and middle income countries (3).

Preliminary data suggest that significantly higher rates of hearing impairment are reported in people living with HIV in comparison to their HIV-uninfected (HIV-) counterparts (4-6). However, there exists conflicting evidence that shows hearing loss rates to be similar in HIV-infected (HIV+) and HIV- populations (7). Ninety percent of all HIV+ children reside in Sub-Saharan Africa (8), with South Africa being one of the six countries contributing to half of the global burden of adolescents living with HIV (9). Notably, half of all children living with HIV were accessing ART by 2015 (8). In the era of ART, HIV+ individuals are living longer, as evidenced by demographic health surveys across Africa (10). This produces a unique group of individuals, including adolescents, who have been chronically infected with HIV and exposed to ART over a long period of time (10-12). Hearing loss in this population has been attributed to a number of possible causes: drug toxicity, opportunistic infections or the HIV virus itself (13). Other risk factors, such as sociodemographic factors, have been identified as potential contributors to hearing loss (14). These require further research, particularly amongst PHIV+ adolescents (2, 5, 7).

Children and adolescents with hearing impairments experience learning difficulties and, by extension, struggle with academic performance and social integration (3, 15). The identification of hearing loss in adolescents enables appropriate interventions and therefore

limits such adverse outcomes (3, 13). There is an urgent need to provide updated means of screening and identifying issues such as hearing loss in HIV-infected populations (12, 13, 15, 16). The objective of this study was to describe the prevalence of hearing loss among PHIV+ on ART and HIV- adolescents, as well as to investigate predictors for the hearing loss, using secondary data from AC testing.

## 2. Methods

### 2.1. Setting

This cross-sectional analysis was carried out within the Cape Town Adolescent Antiretroviral Cohort (CTAAC) using secondary data. The overall methodology and research aims of the CTAAC have been previously described (17). Participants were adolescents receiving ART in public sector care recruited from hospitals and primary care clinics and hospitals around Cape Town.

### 2.2. Participants

Study enrolment ran from July 2013 to February 2015 at the RCWMCH. Cross-sectional analysis was confined to all participants enrolled in the CTAAC who possessed consistent AC testing results (see Appendix C1 Showing Flow Diagram Showing Participant Selection for Analysis). One hundred and eight HIV uninfected and 432 HIV infected adolescents on ART between the ages of 9 and 14 were included in the analysis.

### 2.3. Measurements

Data for this analysis were derived from Case Record Forms (CRF) containing baseline visit information (demographic assessment and medical history), laboratory parameters (HIV disease markers) and clinical examination findings (external ear otoscopy).

*Past Medical History and Demographic Assessment*

Trained counsellors ran interviews and administered questionnaires to participants and their caregivers in a private setting at the CRU. The detailed medical histories of all eligible participants were recorded, including disease symptoms, HIV related history (ART initiation, regimens) and opportunistic infections such as tuberculosis (TB). Demographic information was also collected for each participant. This included their household and family structure, caregiver's social history, living arrangements, educational history, reception of social grants and other questions relevant to socioeconomic status. Trained research assistants verified each participant's record by abstracting their clinical history from the medical history interview and the medical record documentation from service provider sites and referral facilities.

*Physical examination*

Each participant underwent a structured clinical assessment, which included a structured physical examination by a trained senior study nurse, with extensive supervision from senior members of the clinical staff. The adolescent's weight and height were measured as a component of the routine examination. Calculation of Body Mass Index (BMI) was obtained by dividing weight (in kilograms) by the square of height (in meters).

*Laboratory Parameters*

Venepunctures were done to collect blood samples for TB screening, HIV viral load testing and CD4 count. Abbott Molecular Real Time HIV-1 assay was used for the HIV viral load testing. This was done at the National Health Laboratory Services laboratory.

*Pure-Tone Air-Conduction (AC) Testing*

AC testing was carried out as a screening hearing test, and the minimum response level (MRL) was recorded in both the right and left ears at speech-frequency ranges of 250Hz, 500Hz, 1000Hz, 2000Hz and 4000Hz. Pure-tone average (PTA) was calculated per ear from the MRL at conventional frequencies of 500Hz, 1000Hz, 2000Hz (regarded as the main

speech frequencies). Ear-specific PTA was used to define hearing loss. In accordance with the World Health Organisation (WHO) grades of hearing impairment, a threshold of greater than 30dB was considered as debilitating hearing loss (18). Ear-specific PTA was used to compare right and left ear for each participant and to classify better ear PTA and worse ear PTA. Hearing loss was defined as any hearing loss (in either the right or left or both ears), and unilateral hearing loss was defined as better ear PTA < 30dB HL and worse ear PTA ≥ 30dB HL. The GSI 18 (Grason-Stadler, United States patent (19)), single channel screening audiometer was operated according to a standard, defined protocol by a trained physician or medical officer. The AC testing took place in a quiet examination room, at the CRU (typically at the baseline visit or first possible opportunity). The quiet examination room was shown to provide results comparable to those with noise levels of 50-60dBSPL as recommended. This was shown by comparing participant result in EchoRoom to the quiet examination room. Participants with ear-specific PTA ≥30dB were referred for further audiologic assessment.

## 2.4. Analysis

AC testing results were averaged at 500, 1000 and 2000Hz to produce a PTA for the MRL. Binary outcome variables of interest included unilateral hearing loss (vs no hearing loss), bilateral hearing loss (vs no hearing loss) and any hearing loss (vs no hearing loss) based on a MRL threshold of > 30dB. Predictor variables included baseline demographic information, past medical history, examination findings and socioeconomic factors. In PHIV+, other variables considered included viral load, CD4 count (absolute and percentage), ART regimen, age at ART initiation and duration of ART.

The prevalence of hearing loss was calculated. Bivariate variables were compared between the two participant groups (PHIV+ and HIV-) using the appropriate statistical test (chi-squared test or Fisher's exact test). The Wilcoxon rank-sum test for two independent groups was used to compare continuous variables. Hearing loss (none, unilateral, bilateral) was

compared between continuous baseline variables using the ANOVA or Kruskal-Wallis test. Initial multivariable logistic models included all covariates with a $p < 0.20$ level of significance in univariate logistic models. Logistic regression models were used to assess the impact of variables that were associated ($p < 0.05$) with hearing loss (any, unilateral or bilateral). Confounders were adjusted for by inclusion in the final logistic regression models. All calculations were carried out with STATA 13 (STATA for Windows, version 13, STATAcorpLP; College Station, TX)

## 3. Ethics

Ethics committee approval was granted for the cross-sectional analysis by the University of Cape Town, Faculty of Health Sciences Human Research Ethics Committee (HREC), reference number 088/2017. Informed consent/assent was granted for the parent study in accordance with the 2012 Human Sciences Research Council (HSRC) guidelines for research with minors (20). Adolescents who were found to have hearing loss were referred for further audiological assessment.

## 4. Results

The cross-sectional analysis included data from a total of 540 participants (see Appendix C2). Just over half (n=273, 51%) were male, with a median BMI of 17.3 (IQR16.1-19.3) and a median age of 12 years (IQR10.6-13.3.) The majority of participants were black, Xhosa-speaking adolescent students. Most households were recipients of social grants and run by a single parent.

## 4.1. Baseline Characteristics Stratified by HIV Status

Demographic and clinical features of the 540 participants stratified by HIV status (432 PHIV+ (80%) and 108 HIV- (20%)) are presented in Table C1. There were no coloured HIV- participants whilst 37 (9%) of the PHIV+ were coloured. Only PHIV+ participants had abnormal external ear examination findings suggestive of otitis externa. Three participants had no response for current school status, and so were considered as attending school. Less than half of all caregivers were married, and the majority had not achieved tertiary education.

PHIV+ and HIV- participants were similar by age, gender and school attendance, but differed significantly by BMI, language spoken at home, relationship to primary caregiver, primary caregiver marital status and educational level. PHIV+ adolescents had a lower median BMI of 17.1 compared to HIV- adolescents who had a median BMI of 18.6. The PHIV+ adolescent group had twice the proportion of their own parents as primary caregivers, compared to HIV- adolescents. HIV- adolescents were more likely to come from a primarily Xhosa-speaking household. A third of PHIV+ participants (n=149) had a history of previous TB diagnosis, whilst only 2 (2%) HIV- participants had a history of TB, $p<0.001$.

The majority of PHIV+ 160 (38%) had commenced ART before 2 years of age and three quarters (n=330) were virally suppressed (viral load of ≤50cells/ml), and immunocompetent with a CD4 count ≥500cell/$mm^3$. Sixty-one percent (n=259) of PHIV+ participants were on a Highly Active Antiretroviral Therapy (HAART) regimen without a Protease Inhibitor (PI).

## 4.2. Hearing Loss

Appendix C2 shows that the prevalence of any hearing loss among all participants was 19% (n=105). Fifteen percent of hearing loss was unilateral (n=82) and 11% was bilateral (n=61). Stratified by HIV status, the prevalence of hearing loss was 72% (n=312), 16% (n=69), 12%

(n=51) for none, unilateral and bilateral hearing loss in PHIV+, and 79% (n=85), 12% (n =13), 9% (n =10) in HIV-, p=0.392.

The prevalence of hearing loss was similar amongst PHIV+ and HIV- adolescents, at 20% (n=88) and 16% (n=17) respectively, p=0.277. The median PTA of all ears was 20 (IQR15.0-27.5) and 23 (IQR15.0-27.5) for PHIV+ and HIV- respectively, p=0.961. Unilateral hearing loss was 16% (n=69) and 12% (n=13) for PHIV+ and HIV- adolescents. Despite PHIV+ having a higher percentage of unilateral and bilateral hearing loss than HIV- adolescents, the difference was not significant, p=0.392. Similarly, the median hearing thresholds were also alike for right, left, best and worst ear in PIV+ and HIV- participants. The prevalence of right ear hearing loss was 19% (n=81) in PHIV+, 14% (n=15) in HIV-, p=0.237. Left ear hearing loss was 21% (n=90) in PHIV+, 17% (n=18) in HIV-, p=0.333. The median PTA for the best ear was 17dB (IQR11.7-23.3) for PHIV+, and 18dB (11.7-22.5) for HIV- (p = 0.961). Median PTA for the worst ear was 23dB (18.3-33.3) in PHIV+, and 25dB (16.7-30.0) in HIV- (p=0.971).

Table C2 shows PTA (average of 500Hz, 1000Hz, 2000Hz) and MRL for each frequency (250 Hz, 500Hz, 1000Hz, 2000Hz and 4000Hz) by side of ear, best and worst ear stratified by HIV status. The median MRL values for thresholds at 250Hz, 500Hz, 1000Hz, 2000Hz and 4000Hz did not differ significantly between PHIV+ and HIV- participants, although thresholds had a vast range between -10dB and 85dB.

Figures C1.a and C1.b graphically represent trends in hearing thresholds according to frequencies that were recorded in Table C2. Figures C1.a and C1.b show a gradual decrease of MRL as frequency increases in right, left, best and worst ears for both PHIV+ and HIV-, therefore indicating a general improvement of hearing at higher frequencies. However, HIV- showed trends of better hearing at low frequencies (below 2000Hz) compared to PHIV+.

Table C3 shows the proportions of participants who experienced no hearing loss in contrast to those who had unilateral or bilateral hearing loss. The median PTA was worst for those with bilateral hearing loss, compared to those with unilateral hearing loss, with a 12dB sound threshold difference, p<0.001. There was a difference in the proportions of unilateral, bilateral and no hearing loss between participants who had or had not experienced ear pain or discharge in the past month, p<0.001. Among PHIV+, later commencement on ART was more common among those with no hearing loss (5 years IQR2.1-7.8), compared to those with unilateral (3.4 years IQR1.3-6.8) and bilateral hearing loss (3 years IQR1.7-5.2), p=0.009. In accordance with these findings, those with no hearing loss had a shorter duration (7 years IQR4.3-9.3) on ART than participants with unilateral (8 IQR5.4-9.3) and bilateral hearing loss (8 IQR7.1-9.1), p=0.024.

### 4.3. Predictors of Hearing Loss

Table C4 shows the adjusted multivariate model for any hearing loss. Among all participants, females (OR=1.67, 95%CI=1.12; 2.51, p=0.013) and those who reported having been troubled by ear pain or discharge in the past month (OR=2.54, 95%CI=1.55; 4.17, p=<0.001) were more likely to experience hearing loss. Participants with single caregivers (OR=0.59, 95%CI=0.39; 0.91, p=0.015) had a lower risk of experiencing any hearing loss.

HIV infection was not found to be a predictor for any hearing loss according to the univariate analysis, p=0.174. A past history of TB was not identified as a risk factor for hearing loss among all participants, and neither was it a predictor for hearing loss among PHIV+ adolescents.

***Table C1*** *Demographic Variables Stratified by HIV- Infection Status of Study Participants*

| Baseline variable | | Participants (*n = 540*) HIV Infected (n=432) | | HIV Uninfected (n=108) | | P value |
|---|---|---|---|---|---|---|
| **Demographics** | | | | | | |
| **Sex–no. (%)** | Male | 225 | (52) | 48 | (44) | |
| | Female | 207 | (48) | 60 | (56) | 0.156 |
| **Median age-yr. (IQR)** | | 12.0 | (10.6-13.3) | 11.7 | (10.1-13.5) | 0.425 |
| **Age Category –no. (%)** | <12 years | 220 | (51) | 56 | (52) | |
| | ≥12 years | 212 | (49) | 52 | (48) | |
| **Median BMI- (IQR)** | | 17.1 | (15.9-18.9) | 18.6 | (16.7-21.4) | <0.001 |
| **BMI category –no. (%)** | <18.5 | 304 | (70) | 52 | (48) | |
| | ≥18.5 | 128 | (30) | 56 | (52) | |
| **Race–no. (%)** | Black | 395 | (91) | 108 | (100) | |
| | Coloured | 37 | (9) | 0 | (0) | |
| **Socioeconomic Factors** | | | | | | |
| **Primary language spoken at home–no. (%)** | IsiXhosa | 371 | (86) | 104 | (96) | |
| | Afrikaans/English/Sesotho/Other | 61 | (14) | 4 | (4) | |
| **Current[+] caregiver–no. (%)** | Parent | 269 | (62) | 34 | (31) | |
| | Not parent | 163 | (38) | 74 | (69) | |
| **Caregiver marital status–no. (%)** | Married | 134 | (31) | 45 | (42) | |
| | Divorced/Single/Other | 298 | (69) | 63 | (58) | |
| **Caregiver level of education–no. (%)** | Below tertiary | 385 | (90) | 84 | (83) | |
| | Above Tertiary | 43 | (10) | 17 | (17) | |
| **Dependence on social grant–no. (%)** | Yes | 366 | (86) | 81 | (78) | |
| | No | 62 | (14) | 23 | (22) | |
| **Currently still in school–no. (%)[++]** | Yes | 425 | (98) | 108 | (100) | |
| | No | 7 | (2) | 0 | (0) | |
| **Medical History** | | | | | | |
| **Previous diagnosis of TB–no. (%)** | Yes | 149 | (34) | 2 | (2) | |
| | No | 283 | (66) | 106 | (98) | |
| **Experienced ear discharge/ear pain - no. (%)** | Yes | 75 | (17) | 12 | (11) | |
| | No | 357 | (83) | 96 | (89) | |
| **Physical Examination** | | | | | | |
| **External ear examination left an1d right ear** | Both ears normal | 415 | (96) | 108 | (100) | |
| | Sign of otitis externa | 17 | (4) | 0 | (0) | 0.031 |
| **Audiometry -Pure Tone Air Conduction Testing(AC)** | | | | | | |
| **Prevalence of hearing loss(PTA>30dB)– no. (%)‡** | 105(19) | | | | | |
| **Presence of hearing loss(PTA>30dB)– no. (%)** | None | 344 | (80) | 91 | (84) | |
| | Any (bilateral or unilateral) | 88 | (20) | 17 | (16) | 0.277 |
| **Presence of hearing loss(PTA>30dB)– no. (%)** | None | 312 | (72) | 85 | (79) | |
| | Unilateral | 69 | (16) | 13 | (12) | |
| | Bilateral (Better ear >30dB) | 51 | (12) | 10 | (9) | 0.392 |
| **Median PTA of all ears-dB (IQR)** | | 20 | (15.0-27.5) | 23 | (15.0-27.5) | 0.961 |
| **PTA by side of ear– no. (%)** | Right ear PTA ≤ 30 dB | 351 | (81) | 93 | (86) | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Right ear PTA >30 dB | 81 | (19) | 15 | (14) | 0.237 |
| | Left ear PTA ≤ 30 dB | 342 | (79) | 90 | (83) | |
| | Left ear PTA >30 dB | 90 | (21) | 18 | (17) | 0.333 |
| **Median PTA by side of ear -dB (IQR)** | Right | 20 | (13.3-26.7) | 22.5 | (15.0-28.3) | |
| | Left | 20 | (15.0-28.3) | 20 | (13.3-26.7) | |
| **Median PTA by best and worst ear- dB (IQR)** | Best ear | 17 | (11.7-23.3) | 18 | (11.7-22.5) | |
| | Worst ear | 23 | (18.3-33.3) | 25 | (16.7-30.0) | |
| **HIV Related Information** | | | | | | |
| **Median CD4 percentage- % (IQR)** | | 30 | (24.5-34.4) | | | |
| **CD4 percentage – no. (%)** | <25% | 115 | (27) | | | |
| | ≥25% | 317 | (73) | | | |
| **Median absolute CD4 count-cells/ mm$^3$ (IQR)** | | 727 | (578.0-972.0) | | | |
| **Absolute CD4 count– no. (%)** | <200 cells/mm$^3$ | 12 | (3) | | | |
| | ≥200 - <500 cells/mm$^3$ | 55 | (13) | | | |
| | ≥500 cells/mm$^3$ | 365 | (84) | | | |
| **Median viral load- copies per millilitre (IQR)** | | 40 | (40.0-40.0) | | | |
| **Viral load– no. (%)** | ≤50 copies per millilitre | 330 | (76) | | | |
| | >50 - <1000 copies per millilitre | 47 | (11) | | | |
| | ≥1000 copies per millilitre | 55 | (13) | | | |
| **Median age at ART Initiation-yrs. (IQR)** | | 4 | (2.0-7.5) | | | |
| **Age at ART Initiation-- no. (%)** | 0-2 | 160 | (38) | | | |
| | 3-5 | 112 | (26) | | | |
| | 6-14 | 151 | (36) | | | |
| **Median duration on ART- yrs. (IQR)** | | 8 | (4.7-9.2) | | | |
| **Duration on ART – no (%)** | < 8 | 229 | (53) | | | |
| | ≥ 8 | 203 | (47) | | | |
| **ART regimen** | 2 X NRTI + NNRTI | 259 | (61) | | | |
| | 2xNRTI + PI | 157 | (37) | | | |
| | Other* | 8 | (2) | | | |

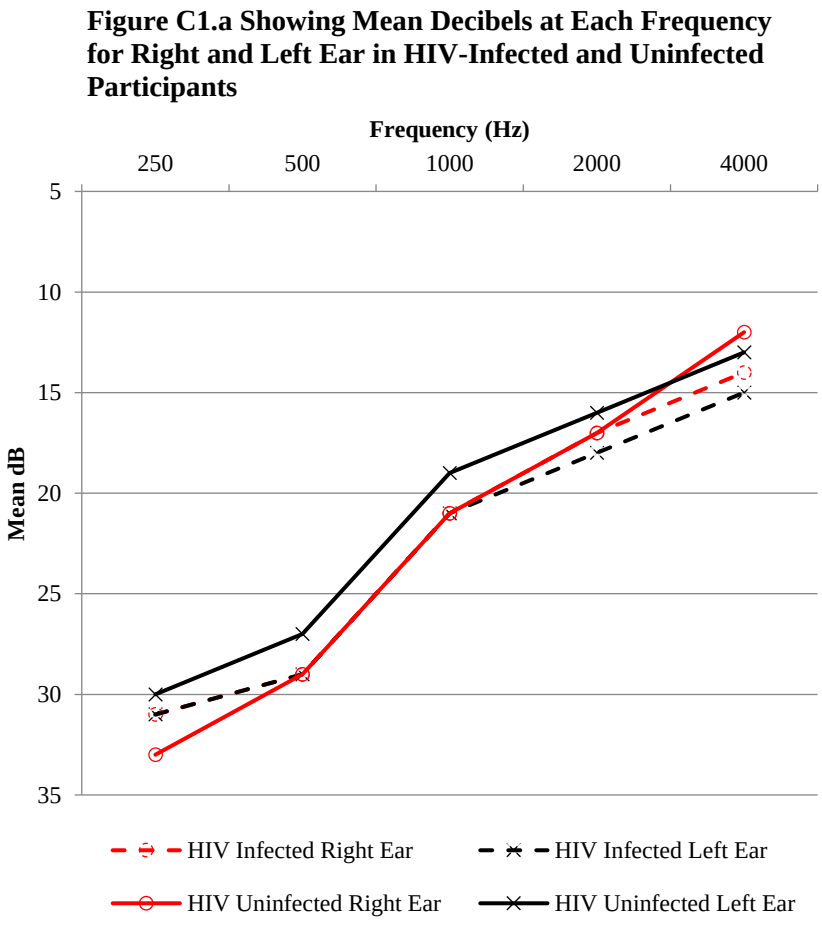
*IQR =interquartile range; no.= number; yr. =years; CI = confidence interval; n = number; BMI = body mass index; TB= Tuberculosis; PTA = average of minimum response levels/pure tone air conduction threshold at 500Hz, 1000Hz, 2000Hz; ART= antiretroviral therapy; ‡In both PHIV+ and HIV- adolescents; †Ear discharge or pain in the last four weeks; *Other ARV regimen = 5 participants lamivudine, 2 participants Lamivudine-Darunavir-Raltegravir-Ritonavir, 1 participant Abacavir-Zidoudine-Lamivudne, NRTI = nucleoside reverse-transcriptase inhibitor; NNRTI= non-nucleoside reverse-transcriptase inhibitor ; PI = protease inhibitor; ART = antiretroviral therapy.*

***Table C2*** *Showing Baseline Pure Tone Air Conduction Threshold Results for Right, Left, Best and Worst Ear Decibels by HIV-Infection Status*

| | Participants (n = 540) | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HIV Infected (n=432) | | | | HIV Uninfected (n=108) | | | | | HIV Infected (n=432) | | | | | | HIV Uninfected (n=108) | | | | |
| **Frequency *(Hz)*** | **Mean dB *(SD)*** | | **Median dB *Min, max*** | | **Mean dB *(SD)*** | | **Median dB *Min, max*** | | **P Value** | **dB>30 *n (%)*** | | **Mean dB *(SD)*** | | **Median dB *Min, max*** | | **dB>30 *n (%)*** | | **Mean dB *(SD)*** | | **Median dB *Min, max*** | | **P Value** |
| | | | | | | | | | | | | | | | | | | | | | |
| | **BEST EAR** | | | | | | | | | **RIGHT EAR** | | | | | | | | | | | |
| | 27 | (11) | 25 | 0,60 | 28 | (11) | 30 | 0,55 | 0.359 | 182 | (42) | 31 | (12) | 30 | 0,70 | 56 | (52) | 33 | (11) | 35 | 5,55 | 0.060 |
| | 25 | (10) | 25 | -5,60 | 25 | (10) | 25 | 0,50 | 0.838 | 134 | (31) | 29 | (12) | 25 | 5,75 | 41 | (38) | 29 | (10) | 30 | 5,50 | 0.202 |
| | 17 | (11) | 15 | -5,65 | 16 | (10) | 15 | 0,50 | 0.907 | 63 | (15) | 21 | (13) | 20 | -5,75 | 13 | (12) | 21 | (10) | 20 | 0,55 | 0.206 |
| | 13 | (11) | 10 | -5,65 | 13 | (10) | 10 | -5,60 | 0.780 | 57 | (13) | 17 | (13) | 15 | -5,75 | 9 | (8) | 17 | (11) | 15 | -5,60 | 0.328 |
| | 10 | (11) | 8 | -10,60 | 8 | (9) | 5 | -10,35 | 0.349 | 54 | (13) | 14 | (13) | 10 | -10,70 | 5 | (5) | 12 | (11) | 10 | -10,50 | 0.196 |
| **PTA** | 19 | (10) | 17 | 3,60 | 18 | (9) | 18 | 2,47 | 0.961 | 81 | (19) | 23 | (12) | 20 | 3,72 | 15 | (14) | 22 | (9) | 23 | 2,50 | 0.211 |
| | **WORST EAR** | | | | | | | | | **LEFT EAR** | | | | | | | | | | | |
| | 35 | (13) | 35 | 5,70 | 35 | (11) | 35 | 5,60 | 0.813 | 200 | (46) | 31 | (13) | 30 | 0,70 | 47 | (44) | 30 | (11) | 30 | 0,60 | 0.436 |
| | 33 | (13) | 30 | 5,75 | 32 | (9) | 30 | 10,50 | 0.770 | 149 | (34) | 29 | (12) | 28 | -5,75 | 32 | (30) | 27 | (10) | 25 | 0,50 | 0.276 |
| | 25 | (14) | 20 | 5,75 | 24 | (11) | 25 | 5,55 | 0.938 | 71 | (16) | 21 | (13) | 20 | 0,70 | 13 | (12) | 19 | (11) | 20 | 0,50 | 0.258 |
| | 22 | (15) | 20 | -5,85 | 21 | (12) | 20 | 0,75 | 0.870 | 60 | (14) | 18 | (14) | 15 | -5,85 | 14 | (13) | 16 | (13) | 13 | -5,75 | 0.208 |
| **4000** | 19 | (15) | 15 | -10,80 | 16 | (13) | 15 | -10,70 | 0.199 | 55 | (13) | 15 | (15) | 10 | -10,80 | 8 | (7) | 13 | (13) | 10 | -10,70 | 0.391 |
| **PTA** | 27 | (13) | 23 | 7,73 | 25 | (9) | 25 | 7,50 | 0.971 | 90 | (21) | 23 | (12) | 20 | 3,73 | 18 | (17) | 21 | (10) | 20 | 3,48 | 0.300 |

*Decibels=Db; standard deviation = SD; min = minimum; max = maximum; n= number, PTA= minimum response level = pure tone average at 500Hz, 1000Hz, 2000Hz*

***Figure C1.a and C1.b*** *Mean Decibels at Each Frequency for Right and Left Ear (1.a.) and Best and Worst Ear (1.b.) in HIV-Infected and HIV-Uninfected Participants*



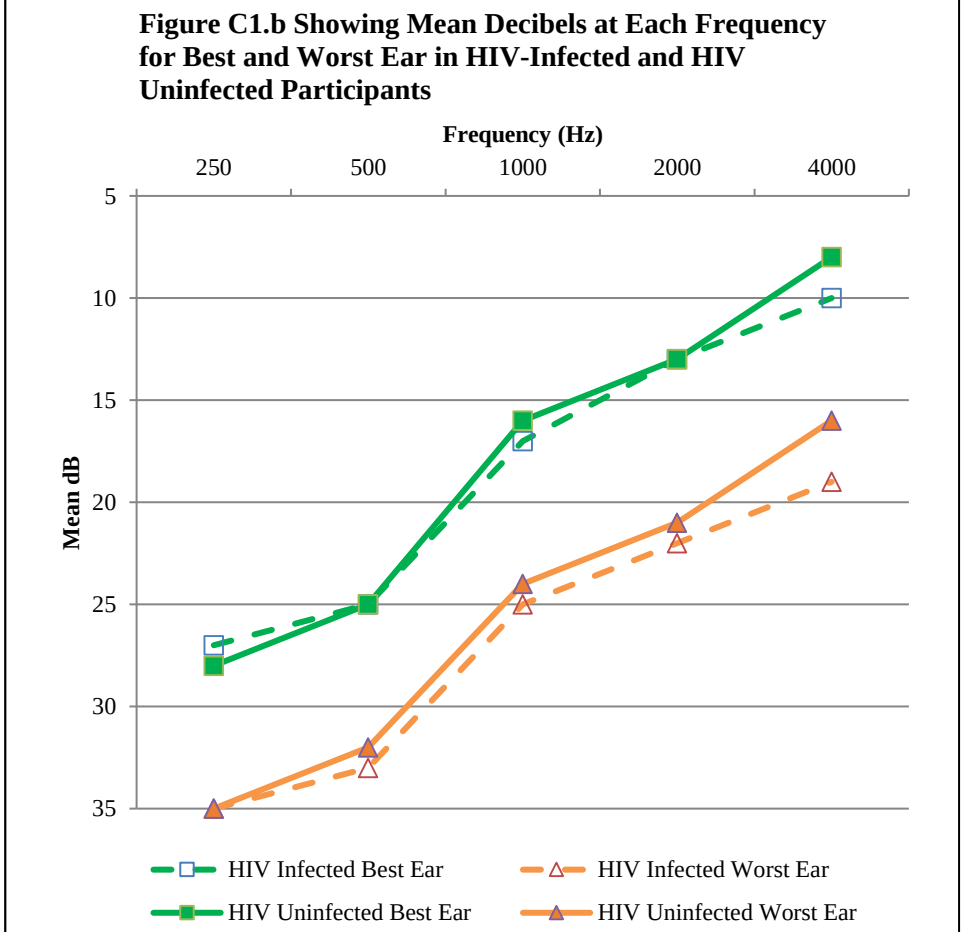



***Table C3*** *Showing Characteristics of 540 Participants by Hearing Loss*

| Baseline variable | | All Participants (*n = 540*) | | | | | | P value |
|---|---|---|---|---|---|---|---|---|
| | | **No Hearing Loss (n=397)** | | **Unilateral Hearing Loss (n=82)** | | **Bilateral Hearing Loss (n=61)** | | |
| **Demographics** | | | | | | | | |
| Sex–no. (%) | Male | 213 | (54) | 35 | (43) | 25 | (41) | |
| | Female | 184 | (46) | 47 | (57) | 36 | (59) | |
| Median age-yr. (IQR) | | 12 | (10.7-13.4) | 12 | (10.3-13.2) | 12 | (10.3-13.0) | 0.186 |
| Age Category –no. (%) | <12 years | 196 | (49) | 44 | (54) | 36 | (59) | |
| | ≥12 years | 201 | (51) | 38 | (46) | 25 | (41) | |
| Median BMI- (IQR) | | 17 | (16.1-19.3) | 17 | (15.6-19.2) | 17 | (15.8-19.2) | 0.423 |
| BMI category –no. (%) | <18.5 | 259 | (65) | 54 | (66) | 43 | (70) | |
| | ≥18.5 | 138 | (35) | 28 | (34) | 18 | (30) | |
| Race–no. (%) | Black | 367 | (92) | 76 | (93) | 60 | (98) | |
| | Coloured | 30 | (8) | 6 | (7) | 1 | (2) | |
| **Socioeconomic Factors** | | | | | | | | |
| Primary language spoken at home–no. (%) | IsiXhosa | 348 | (88) | 71 | (87) | 56 | (92) | |
| | Afrikaans/English /Sesotho/Other | 49 | (12) | 11 | (13) | 5 | (8) | |
| Current caregiver–no. (%) | Parent | 226 | (57) | 43 | (52) | 34 | (56) | |
| | Not parent | 171 | (43) | 39 | (48) | 27 | (44) | |
| Caregiver marital status–no. (%) | Married | 122 | (31) | 36 | (44) | 21 | (34) | |
| | Divorced/Single/Other | 275 | (69) | 46 | (56) | 40 | (66) | |
| Caregiver level of education–no. (%) | Below tertiary | 339 | (87) | 75 | (94) | 55 | (92) | |
| | Above Tertiary | 50 | (13) | 5 | (6) | 5 | (8) | |
| Dependence on social grant–no. (%) | Yes | 331 | (85) | 69 | (85) | 47 | (78) | |
| | No | 60 | (15) | 12 | (15) | 13 | (22) | |
| Currently still in school–no. (%)[++] | Yes | 391 | (98) | 82 | (100) | 60 | (98) | |
| | No | 6 | (2) | 0 | (0) | 1 | (2) | |
| **Medical History** | | | | | | | | |
| Previous diagnosis of TB–no. (%) | Yes | 104 | (26) | 26 | (32) | 21 | (34) | |
| | No | 293 | (74) | 56 | (68) | 40 | (66) | |
| Experienced ear discharge/ear pain- no. (%)† | | | | | | | | |
| | Yes | 50 | (13) | 24 | (30) | 13 | (21) | |
| | No | 347 | (87) | 58 | (71) | 48 | (79) | |
| Participant sero-status | HIV-infected | 312 | (79) | 69 | (84) | 51 | (84) | |
| | HIV-uninfected | 85 | (21) | 13 | (16) | 10 | (16) | 0.392 |
| **Physical Examination** | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| External ear examination left and right ear | Both ears normal | 387 | (97) | 79 | (96) | 57 | (93) | |
| | Signs of otitis externa | 10 | (3) | 3 | (4) | 4 | (7) | 0.189 |
| **Audiometry-Pure Tone Air Conduction Testing(AC)** | | | | | | | | |
| Median PTA of all ears-dB (IQR) | | 18 | (13.3-21.7) | 31 | (27.5-34.2) | 43 | (36.7-48.3) | <0.001 |

| | | HIV Infected Participants (n=432) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **No Hearing Loss (n=312)** | | **Unilateral Hearing Loss (n=69)** | | **Bilateral Hearing Loss (n=51)** | | **P value** |
| **HIV Related Information** | | | | | | | | |
| Median CD4 percentage- %(IQR) | | 30 | (24.0-34.2) | 31 | (25.2-33.8) | 30 | (25.9-37.4) | 0.357 |
| CD4 percentage- no. (%) | <25% | 87 | (28) | 17 | (25) | 11 | (22) | |
| | ≥25% | 225 | (72) | 52 | (75) | 40 | (78) | |
| Median CD4 count- cells/mm$^3$ (IQR) | | 714 | (555.0-933.0) | 733 | (615.0-1093.0) | 773 | (602.0-1031.0) | 0.123 |
| CD4 count- no. (%) | <200 cells/mm$^3$ | 10 | (3) | 1 | (1) | 1 | (2) | |
| | ≥200 - <500 cells/mm$^3$ | 44 | (14) | 5 | (7) | 6 | (12) | |
| | ≥500 cells/mm$^3$ | 258 | (83) | 63 | (91) | 44 | (86) | 0.491 |
| Median viral load- copies/millilitre (IQR) | | 40 | (40.0-40.0) | 40 | (40.0-40.0) | 40 | (40.0-40.0) | 0.386 |
| Viral load- no. (%) | ≤50 copies per millilitre | 234 | (75) | 56 | (81) | 40 | (78) | |
| | >50 - <1000 copies per millilitre | 36 | (12) | 8 | (12) | 3 | (6) | |
| | ≥1000 copies per millilitre | 42 | (13) | 5 | (7) | 8 | (16) | 0.441 |
| Median age at ART Initiation-yrs. (IQR) | | 5 | (2.1-7.8) | 3.4 | (1.3-6.8) | 3 | (1.7-5.2) | 0.009 |
| Age at ARV Initiation-- no. (%) | 0-2 | 106 | (35) | 28 | (42) | 26 | (52) | |
| | 3-5 | 83 | (27) | 16 | (24) | 13 | (26) | |
| | 6-14 | 117 | (38) | 23 | (34) | 11 | (22) | 0.126 |
| Median duration on ART- yrs. (IQR) | | 7 | (4.3-9.3) | 8 | (5.4-9.3) | 8 | (7.1-9.1) | 0.083 |
| Duration on ART – no (%) | < 8 | 178 | (57) | 30 | (43) | 21 | (41) | |
| | ≥8 | 134 | (43) | 39 | (57) | 30 | (59) | 0.024 |
| ART regimen – no (%) | 2 X NRTI + NNRTI | 196 | (64) | 39 | (58) | 24 | (48) | |
| | 2xNRTI + PI | 107 | (35) | 27 | (40) | 23 | (46) | |
| | Other * | 4 | (1) | 1 | (1) | 3 | (6) | 0.073 |

*IQR =interquartile range; no.= number; yr. =years; CI = confidence interval; n = number; BMI = body mass index; TB= Tuberculosis; PTA = average of minimum response levels/pure tone air conduction threshold at 500Hz, 1000Hz, 2000Hz; ART= antiretroviral therapy;* †Ear discharge or pain in the last four weeks, **Other ARV regimen = 5 participants lamivudine, 2 participants Lamivudine-Darunavir-Raltegravir-Ritonavir, 1 participant Abacavir-Zidoudine-Lamivudne, NRTI = nucleoside reverse-transcriptase inhibitor; NNRTI= non-nucleoside reverse-transcriptase inhibitor ; PI = protease inhibitor; ART = antiretroviral therapy*

***Table C4*** *Univariate and Adjusted Associations (Logistic Regression Models) Among all participants and HIV- Infected Participants for Binary Outcome of Any Hearing Loss*

**All Participants (HIV Infected and HIV Uninfected, n= 540)**

| | Univariate Regression Model | | | | Adjusted Regression Model | | | |
|---|---|---|---|---|---|---|---|---|
| **Variable** | N | Odds Ratio | 95% Confidence Interval | P Value | N | Odds Ratio | 95% Confidence Interval | P Value |
| **Body Mass Index ≥18.5** | 539 | 0.89 | (0.59; 1.33) | 0.575 | - | - | - | - |
| **Age ≥12 yr.** | 540 | 0.76 | (0.52; 1.12) | 0.178 | 529 | 0.80 | (0.54;1.20) | 0.286 |
| **Female** | 540 | 1.60 | (1.09; 2.36) | 0.017 | 529 | 1.67 | (1.12;2.51) | 0.013 |
| **Coloured** | 540 | 0.63 | (0.27; 1.47) | 0.284 | - | - | - | - |
| **Not IsiXhosa speaking** | 540 | 0.89 | (0.49; 1.63) | 0.716 | - | - | - | - |
| **Participants has had TB** | 540 | 1.37 | (0.91;2.09) | 0.128 | 529 | 1.22 | (0.77;1.92) | 0.396 |
| **Participant currently in school** | 540 | 2.18 | (0.26; 18.26) | 0.473 | - | - | - | - |
| **Primary caregiver is parent** | 540 | 0.88 | (0.60; 1.30) | 0.525 | - | - | - | - |
| **Caregiver no tertiary education** | 529 | 1.92 | (0.94; 3.89) | 0.072 | 529 | 1.83 | (0.88;3.80) | 0.103 |
| **Caregiver not married** | 540 | 0.67 | (0.45 1.00) * | 0.047 | 529 | 0.59 | (0.39;0.91) | 0.015 |
| **Household has no grant** | 532 | 1.19 | (0.71; 1.98) | 0.508 | - | - | - | - |
| **Troubled by ear pain/discharge†** | 540 | 2.42 | (1.50; 3.90) | <0.001 | 529 | 2.54 | (1.55;4.17) | <0.001 |
| **Any otitis externa/other** | 540 | 1.99 | (0.74; 5.34) | 0.171 | 529 | 2.09 | (0.75;2.35) | 0.159 |
| **HIV infected§** | 540 | 1.42 | (0.86; 2.36) | 0.174 | 529 | 1.33 | (0.06;0.37) | 0.318 |

**Restricted to HIV Infected Participants (n=432)**

| | Univariate Regression Model | | | | Adjusted Regression Model | | | |
|---|---|---|---|---|---|---|---|---|
| **Variable** | N | Odds Ratio | 95% Confidence Interval | P Value | N | Odds Ratio | 95% Confidence Interval | P Value |
| **% CD4 ≥25** | 432 | 1.27 | (0.78;2.07) | 0.338 | - | - | - | - |
| **Duration of ART ≥ 8 yr.** | 432 | 1.80 | (1.17;2.75) | 0.007 | 417 | 1.91 | (0.92;3.97) | 0.083 |
| **CD4 Count (vs<200 cells/mm$^3$)** | 432 | | | | - | | | |
| **≥200 - <500 cells/mm$^3$** | | 1.25 | (0.24;6.55) | 0.792 | - | - | - | - |
| **≥500 cells/mm$^3$** | | 2.07 | (0.45;9.62) | 0.352 | - | - | - | - |
| **Viral load (vs ≤ 50 cells/ml)** | 432 | | | | - | - | - | - |
| **>50 - <1000 copies per millilitre** | | 0.74 | (0.36;1.52) | 0.420 | - | - | - | - |
| **≥1000 copies per millilitre** | | 0.75 | (0.39;1.47) | 0.407 | - | - | - | - |
| **Age at ART initiation (vs 0 -2 yr.)** | 423 | | | | 417 | | | |
| **3-5 yr.** | | 0.69 | (0.40;1.17) | 0.167 | | 0.76 | (0.38;1.51) | 0.437 |
| **6-14 yr.** | | 0.57 | (0.34;0.94) | 0.029 | | 1.18 | (0.46;3.04) | 0.731 |
| **ARV regimen (vs 2XNRTI +NNRTI)** | 424 | | | | 417 | | | |
| **2xNRTI + PI** | | 1.45 | (0.94;2.26) | 0.095 | | 1.31 | (0.78;2.19) | 0.295 |
| **Other regimen** | | 3.11 | (0.76;12.80) | 0.116 | | 2.09 | (0.41;10.11) | 0.383 |
| **Body Mass Index ≥18.5** | 432 | 0.92 | (0.57;1.46) | 0.714 | - | | | |
| **Age ≥12 yr.** | 432 | 0.73 | (0.48;1.11) | 0.139 | 417 | 0.76 | (0.44;1.31) | 0.327 |
| **Female** | 432 | 1.48 | (0.97;2.26) | 0.068 | 417 | 1.62 | (1.01;2.61) | 0.045 |
| **Coloured** | 432 | 0.58 | (0.25;1.36) | 0.213 | 417 | 0.64 | (0.25;1.65) | 0.354 |
| **Not IsiXhosa speaking** | 432 | 0.91 | (0.49;1.69) | 0.771 | - | | | |
| **Participants has had TB** | 432 | 1.33 | (0.86;2.05) | 0.205 | 417 | 1.38 | (0.85;2.23) | 0.196 |
| **Participant currently in school** | 432 | 3.53 | (0.44;28.20) | 0.233 | 417 | 11.50 | (1.16;113.90) | 0.037 |
| **Primary caregiver is parent** | 432 | 0.88 | (0.57;1.35) | 0.546 | - | - | - | - |
| **Caregiver no tertiary education** | 428 | 2.11 | (0.91;4.88) | 0.081 | 417 | 3.04 | (1.18;7.81) | 0.021 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Caregiver not married** | 432 | 0.54 | (0.35;0.84) | 0.007 | 417 | 0.49 | (0.29;0.80) | 0.004 |
| **Household has no grant** | 428 | 1.50 | (0.85;2.66) | 0.160 | 417 | 2.10 | (1.07;4.12) | 0.030 |
| **Troubled by ear pain/discharge** | 432 | 2.80 | (1.67;4.69) | <0.001 | 417 | 3.48 | (1.96;6.18) | <0.001 |
| **Any otitis externa/other** | 432 | 1.87 | (0.70;5.03) | 0.215 | 417 | 2.71 | (0.94;7.86) | 0.066 |

***upper limit raw figure = 0.9954423 §HIV infection was adjusted for despite not meeting the 0.2 level of significance as it was a variable of particular interest in this study,* †Ear discharge or pain in the last four weeks.**

## 5. Discussion

The adverse outcomes of unidentified hearing loss in adolescents are well established (3, 13, 15). We assessed the prevalence and predictors of hearing loss among HIV- and PHIV+ adolescents on ART in Cape Town, South Africa. The prevalence of hearing loss was high regardless of HIV sero-status. Being female, having a married caregiver and a history of earache or ear discharge in the past month were positive predictors of hearing loss among all participants. Among PHIV+, an additional predictor of hearing loss was having a married caregiver without tertiary education. A longer period of time on ART, with an earlier age of inception, were associated with hearing loss in the univariate analysis, but the relationship disappeared in the adjusted model.

The prevalence of hearing loss in PHIV+ adolescents in this analysis was 20% (n=88), which was similar to earlier reports among the HIV-infected adolescents in the Pediatric HIV/AIDS Cohort Study (PHACS) (5), but slightly lower than that of PHIV+ adolescents in Malawi, where almost a quarter of the population was found to have hearing impairments (15). This discrepancy may be best explained by the lower threshold used in the study done in Malawi compared to our study (20dB versus 30dB), allowing for milder cases of hearing loss to be included in the Malawian statistics.

By contrast, the prevalence of hearing loss in our analysis was comparable between PHIV+ and HIV- participants, whilst it was significantly lower among the HIV- participants included as a comparison group in PHACS (5). The most plausible reason for the comparative prevalence of hearing loss between PHIV+ and HIV- adolescents in our study is that the two groups were very similar, as they came from the same source population and thus it probably had the same risk factors for hearing loss, but this is beyound this study scope.

The PHIV+ participants had been on ART for a significant period of time, and were relatively well controlled, which may have reduced their risk of hearing loss from causes such as chronic otitis media. They therefore experienced the same rates of hearing loss as their HIV- counterparts. Another possibility, albeit speculative, is that a proportion of the adolescents in this cross-sectional analysis were HIV-uninfected but HIV-exposed, therefore increasing their chances of hearing loss. This exposure may cause the prevalence of hearing loss among the HIV- group to be comparable to PHIV+ adolescents. It has been shown that HIV-exposed but HIV-uninfected adolescents have a higher risk of hearing loss (5). It is noteworthy that healthy populations of North American children have been found to have hearing loss rates of less than 10% (21) compared to the HIV- South African participants who have a hearing loss rate of 16%.

In keeping with previous research, we found that a history of ear discharge was associated with hearing loss in PHIV+ children (15). This pattern is consistent with findings from other studies of PHIV+ adolescents in Sub-Saharan Africa, in which frequent ear infection has been shown to cause hearing loss. Similarly, as seen in the PHACS, our analysis showed that caregivers who attained lower levels of education were a predictor of hearing loss in adolescents (5).

In the univariate analysis, a longer period of time on ART was associated with hearing impairment in PHIV+ participants. However, this association did not remain consistent after an adjustment for confounders. However, previous studies have identified the use of Nucleoside Reverse-Transcriptase Inhibitor (NRTI) (22-24) and Protease Inhibitor (PI) (25) as risk factors for hearing loss. In addition, unlike other investigators who identified being male and having a single parent caregiver as sociodemographic risk factors for hearing loss (14), we found that being female and having a married caregiver are risk factors. A possible

explanation could be that the underlying population structure differs between the two study locations (China versus South Africa.)

Our study is limited by our reliance on AC testing and our lack of Bone Conduction (BC) testing. Whilst AC testing represents conducted sounds through the full auditory system (inner, middle, external ear, and auditory nervous system), BC testing represents sound thresholds of the auditory nervous system inner ear. Using AC and BC testing in combination allows for a differentiation between external and middle ear pathology, versus the cochlea and central auditory pathway (26). However, the AC testing conducted in this study was intended to function as a screening procedure rather than a diagnostic test, and nevertheless worked to identify participants with hearing impairments. It also enabled the referral of participants who required further audiological evaluation.

A further limitation of this analysis is that the study only investigated the current ART regimen as a predictor of hearing loss. This did not, however, preclude the possibility that past ART regimens may have been risk factors for hearing loss. The use of NRTI and PI (25) have been associated with mitochondrial damage in PHIV+ adolescents, and thus hearing loss (22, 23). We focused on ART exposure at large and did not explore individual agents. Additionally, patients who underwent AC testing whilst experiencing otitis media may have affected hearing test results.

In health care facilities, routine assessments should include taking a history of ear problems in adolescents. In particular, low resource communities providing services for PHIV+ adolescents without access to AC testing should use a history of ear pain or discharge in the last month as a means of early detection of hearing loss. Ear pain or discharge should function as a criterion for referral for further audiological assessment. Caregivers have not proved reliable in identifying hearing loss in the past (15), but adolescents reporting

symptoms of pain or discharge have functioned as reliable predictors of hearing loss among PHIV+ adolescents in our study.

## 6. Conclusion

Despite the limitations of this analysis, there is sufficient evidence that continued investigation of hearing loss among adolescents remains imperative, especially in populations who possess risk factors for hearing loss.

## 7. Conflict of Interest

None


## 8. Funding

None

## 9. Acknowledgements

We thank the CTAAC for supplying data for the secondary analysis, and for providing technical support. We thank Dr Sana Mahatab and Dr Nana Akua Asafu-Asejei for their direction. We thank Professor Heather Zar for her input. We thank Dr Shazia Peer and Silva Kuschke for reading over the manuscript and providing critique relevant to the topic.

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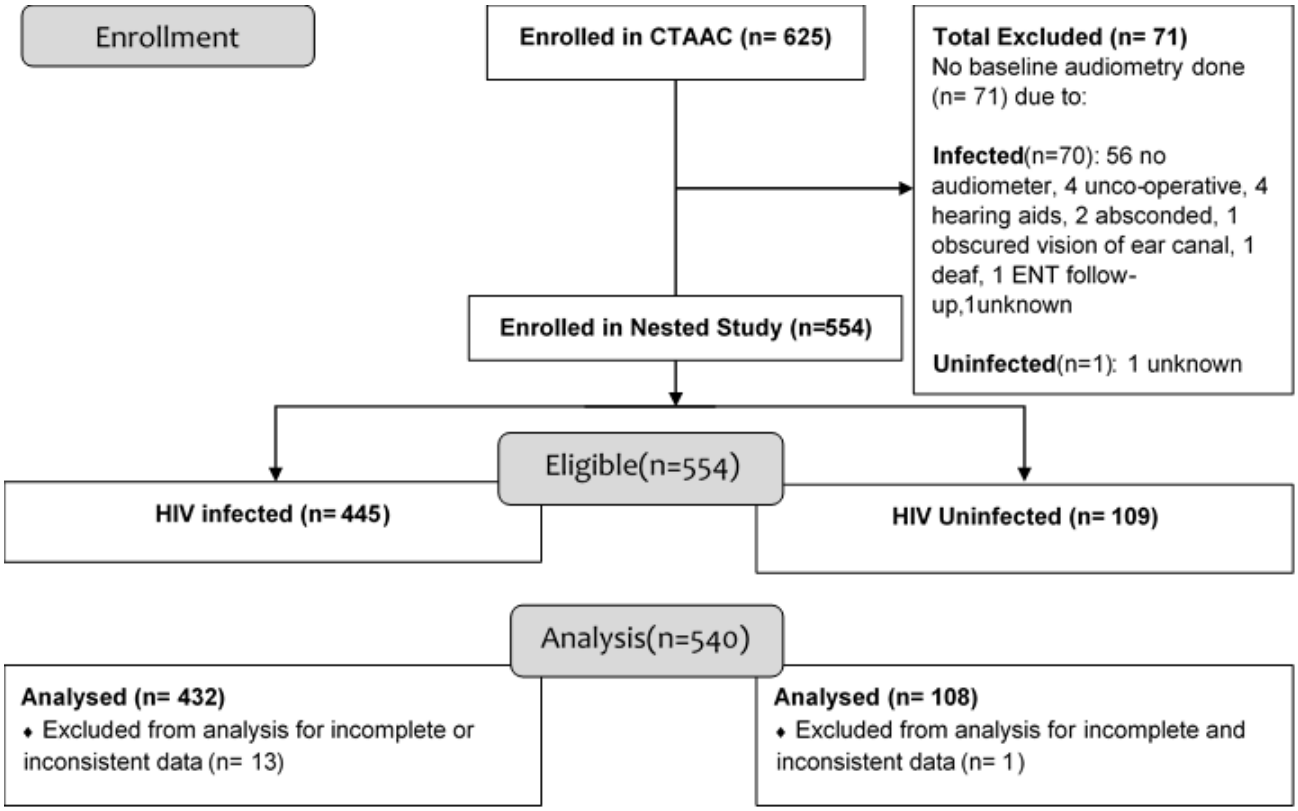
# PART D: APPENDICES

## Appendices for Journal Manuscript

**Appendix C1** Flow Diagram Showing Participant Selection for Analysis




Appendix C1 is a flow diagram that illustrates the selection of participants who were analysed in this study. This flow diagram structure is adapted from the CONSORT Guidelines, despite its traditional use as a tool for randomised trial for two groups, it was useful as we analysed two groups (HIV-uninfected and perinatally HIV-infected).

Appendix C2 shows the baseline characteristics of participants who were included in the cross-sectional analysis. It serves as a descriptive table.

**Appendix C2** Demographic and Clinical Features of all 540 Participants

| Baseline Variable | | n= 540 | |
|---|---|---|---|
| **Demographics** | | | |
| **Sex–no. (%)** | Male | 273 | (51) |
| | Female | 267 | (49) |
| **HIV Status-np. (%)** | Uninfected | 108 | (20) |
| | Perinatally infected | 432 | (80) |
| **Median age-yr. (IQR)** | | 12.0 | (10.6-13.3) |
| **Age Category –no. (%)** | <12 years | 276 | (51) |
| | ≥12 years | 264 | (49) |
| **Median BMI- (IQR)[§]** | | 17.3 | (16.1-19.3) |
| **BMI category –no. (%)** | <18.5 | 356 | (66) |
| | ≥18.5 | 184 | (34) |
| **Race–no. (%)** | Black | 503 | (93) |
| | Coloured | 37 | (7) |
| **Socioeconomic Factors** | | | |
| **Primary language spoken at home–no. (%)** | IsiXhosa | 475 | (88) |
| | Afrikaans/English/Sesotho/Other | 65 | (12) |
| **Current[+] caregiver–no. (%)** | Parent | 303 | (56) |
| | Not parent | 237 | (44) |
| **Caregiver marital status–no. (%)** | Married | 179 | (33) |
| | Divorced/Single/Other | 361 | (67) |
| **Caregiver level of education–no. (%)** | Below tertiary | 469 | (89) |
| | Above Tertiary | 60 | (11) |
| **Dependence on social grant–no. (%)** | Yes | 447 | (84) |
| | No | 85 | (16) |
| **Currently still in school–no. (%)[++]** | Yes | 530 | (98) |
| | No | 10 | (2) |
| **Medical History** | | | |
| **Previous diagnosis of TB–no. (%)** | Yes | 151 | (28) |
| | No | 389 | (72) |
| **Experienced ear discharge or ear pain in the last one month-no. (%)** | Yes | 87 | (16) |
| | No | 453 | (84) |
| **Physical Examination** | | | |
| **External ear examination left and right ear** | Both ears normal | 523 | (97) |
| | Otitis externa/other finding in either or both ears | 17 | (3) |
| **Audiometry -Pure Tone Air Conduction Testing(AC)** | | | |
| **Prevalence of hearing loss (PTA>30Db)– no. (%)** | | 105 | (19) |
| **Presence of hearing loss(PTA>30dB)– no. (%)** | None | 435 | (81) |
| | Any (bilateral or unilateral) | 105 | (19) |
| **Presence of hearing loss(PTA>30dB)– no. (%)** | None | 397 | (74) |
| | Unilateral | 82 | (15) |
| | Bilateral (Better ear >30Db) | 61 | (11) |
| **Median PTA of all ears-dB (IQR)** | | 20.4 | (15.0-27.5) |
| **PTA by side of ear– no. (%)** | Right ear PTA ≤ 30 dB | 444 | (82) |
| | Right ear PTA >30 dB | 96 | (18) |
| | Left ear PTA ≤ 30 dB | 432 | (80) |
| | Left ear PTA >30 dB | 108 | (20) |
| **Median PTA by side of ear -dB (IQR)** | Right | 20.0 | (13.3-27.5) |
| | Left | 20.0 | (15.0-26.7) |
| **Median PTA by best and worst ear- dB (IQR)** | Best ear | 16.7 | (11.7-23.3) |
| | Worst ear | 23.3 | (18.3-33.3) |

*§1 participant missing body mass index*

Appendix C3 and C4 show results for the logistic regression models built for predicting the binary outcome for unilateral hearing loss (unilateral hearing loss not present vs unilateral hearing loss present) and bilateral hearing loss (bilateral hearing loss not present vs bilateral hearing loss present), respectively. Using STATA 13 (STATA for Windows, version 13, STATAcorpLP; College Station, TX), logistic regression models

were used to assess the impact of variables that were associated ($p < 0.05$) with hearing loss (any, unilateral or bilateral). Variables included in the final, multivariate model were those with a p-value$< 0.2$ in the initial univariate analysis as shown. Confounders were adjusted for by inclusion in the final logistic regression models.

**Appendix C3** Univariate and Adjusted Associations (Logistic Regression Models) Among all Participants and HIV Infected Participants for Binary Outcome of Unilateral Hearing Loss

**All Participants (HIV Infected and HIV Uninfected, n= 479)**

| | Univariate Regression Model | | | | Adjusted Regression Model | | | |
|---|---|---|---|---|---|---|---|---|
| **Variable** | N | Odds Ratio | 95% Confidence Interval | P Value | N | Odds Ratio | 95% Confidence Interval | P Value |
| **Body Mass Index ≥18.5** | 479 | 0.97 | (0.59;1.61) | 0.915 | - | - | - | - |
| **Age ≥12 yr.** | 479 | 0.84 | (0.52;1.36) | 0.480 | - | - | - | - |
| **Female** | 479 | 1.55 | (0.96;2.51) | 0.072 | 469 | 1.63 | (0.99;2.69) | 0.056 |
| **Coloured** | 479 | 0.97 | (0.38;2.40) | 0.940 | - | - | - | - |
| **Not IsiXhosa speaking** | 479 | 1.10 | (0.55;2.22) | 0.790 | - | - | - | - |
| **Participants has had TB** | 479 | 1.31 | (0.78;2.19) | 0.308 | - | - | - | - |
| **Participant currently in school**[+] | 470 | | | | - | | | |
| **Primary caregiver is parent** | 479 | 0.83 | (0.52;1.34) | 0.456 | - | - | - | - |
| **Caregiver no tertiary education** | 469 | 2.21 | (0.85;5.74) | 0.102 | 469 | 2.19 | (0.83;5.79) | 0.114 |
| **Caregiver not married** | 479 | 0.57 | (0.35;0.92) | 0.022 | 469 | 0.52 | (0.31;0.86) | 0.011 |
| **Household has no grant** | 472 | 0.96 | (0.49;1.88) | 0.904 | - | - | - | - |
| **Troubled by ear pain/discharge** | 479 | 2.87 | (1.64;5.03) | <0.001 | 469 | 3.05 | (1.71;5.43) | <0.001 |
| **Any otitis externa/other** | 479 | 1.47 | (0.40;5.46) | 0.565 | - | - | - | - |
| **HIV infected** | 479 | 1.44 | (0.76;2.74) | 0.258 | - | - | - | - |

**Restricted to HIV Infected Participants (n=381)**

| | Univariate Regression Model | | | | Adjusted Regression Model | | | |
|---|---|---|---|---|---|---|---|---|
| **Variable** | N | Odds Ratio | 95% Confidence Interval | P Value | N | Odds Ratio | 95% Confidence Interval | P Value |
| **% CD4≥25** | 381 | 1.18 | (0.65;2.16) | 0.584 | - | - | - | - |
| **Duration of ART ≥ 8 yr.** | 381 | 1.73 | (1.02;2.92) | 0.042 | 381 | 1.66 | (0.98;2.82) | 0.061 |
| **CD4 Count (vs<200 cells/mm$^3$)** | 381 | | | | - | | | |
| **≥200 - <500 cells/mm$^3$** | | 1.13 | (0.12;10.82) | 0.912 | | - | - | - |
| **≥500 cells/mm$^3$** | | 2.44 | (0.31;19.42) | 0.399 | | - | - | - |
| **Viral load (vs ≤ 50 cells/ml)** | 381 | | | | 381 | | | |
| **>50 - <1000 copies per millilitre** | | 0.93 | (0.41;2.11) | 0.859 | | 0.97 | (0.42;2.20) | 0.933 |
| **≥1000 copies per millilitre** | | 0.50 | (0.19;1.31) | 0.159 | | 0.55 | (0.21;1.45) | 0.226 |
| **Age at ART initiation (vs 0 -2 yr.)** | 373 | | | | - | | | |
| **3-5 yr.** | | 0.73 | (0.37;1.44) | 0.370 | | - | - | - |
| **6-14 yr.** | | 0.74 | (0.40;1.37) | 0.343 | | - | - | - |
| **ART regimen (vs 2XNRTI +NNRTI)** | 374 | | | | - | | | |
| **2xNRTI + PI** | | 1.27 | (0.74;2.19) | 0.392 | | - | - | - |
| **Other regimen** | | 1.26 | (0.14;11.55) | 0.840 | | - | - | - |

[+]*Not able to model due to small cell size*

**Appendix C4** Univariate and Adjusted Associations (Logistic Regression Models) Among all participants and HIV Infected Participants for Binary Outcome of Bilateral Hearing Loss

**All Participants (HIV Infected and HIV Uninfected, n= 458)**

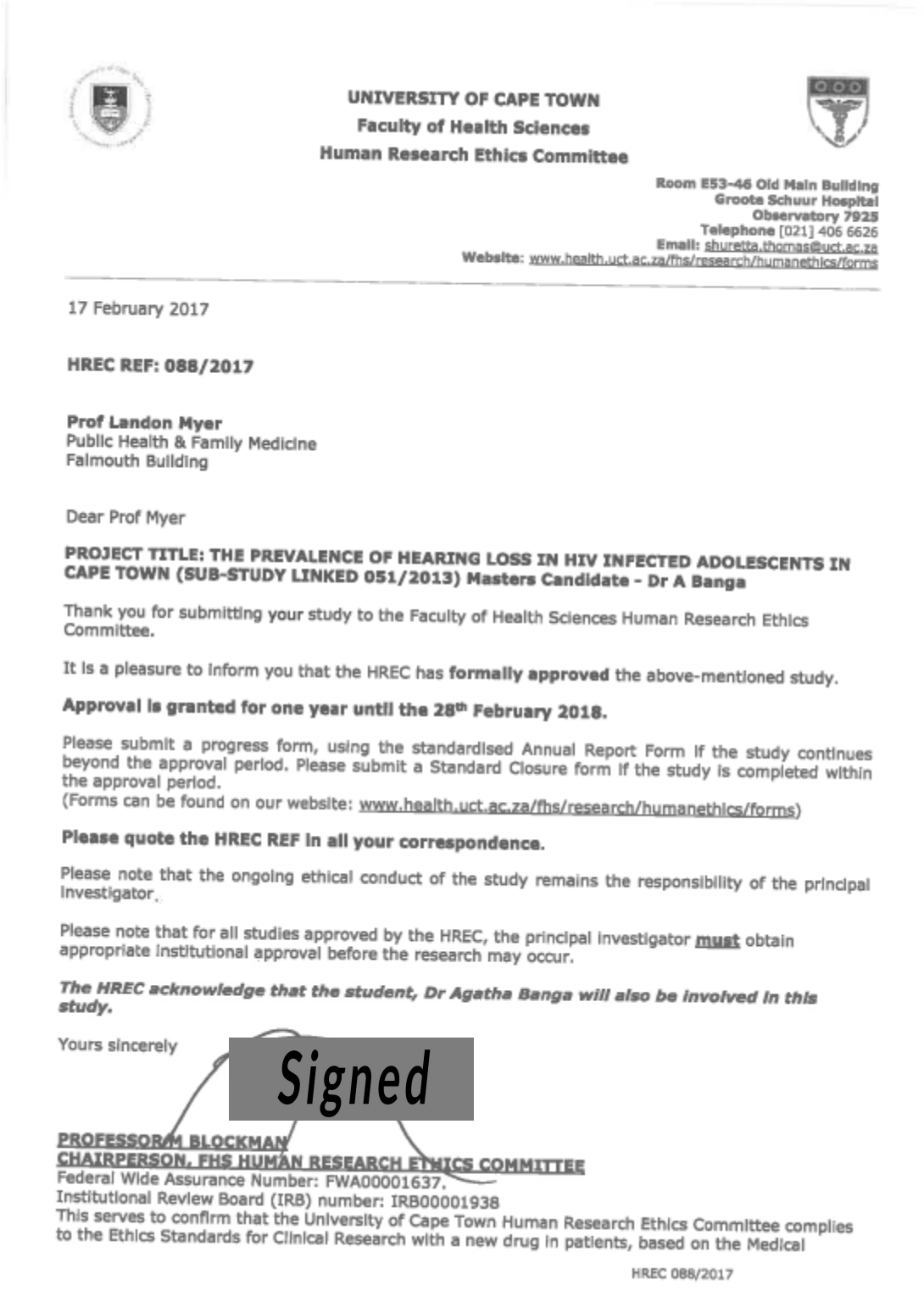
| | Univariate Regression Model | | | | Adjusted Regression Model | | | |
|---|---|---|---|---|---|---|---|---|
| **Variable** | N | Odds Ratio | 95% Confidence Interval | P Value | N | Odds Ratio | 95% Confidence Interval | P Value |
| **Body Mass Index ≥18.5** | 458 | 0.79 | (0.44;1.41) | 0.421 | - | - | - | - |
| **Age ≥12 yr.** | 458 | 0.68 | (0.39;1.17) | 0.162 | 451 | 0.68 | (0.39;1.20) | 0.186 |
| **Female** | 458 | 1.67 | (0.96;2.88) | 0.067 | 451 | 1.61 | (0.92;2.82) | 0.098 |
| **Coloured** | 458 | 0.20 | (0.03;1.52) | 0.121 | 451 | 0.21 | (0.03;1.57) | 0.128 |
| **Not IsiXhosa speaking** | 458 | 0.63 | (0.24;1.66) | 0.354 | - | - | - | - |
| **Participants has had TB** | 458 | 1.48 | (0.83;2.62) | 0.181 | 451 | 1.21 | (0.65;2.24) | 0.547 |
| **Participant currently in school** | 458 | 1.39 | (0.17;11.18) | 0.756 | - | - | - | - |
| **Primary caregiver is parent** | 458 | 0.95 | (0.55;1.64) | 0.861 | - | - | - | - |
| **Caregiver no tertiary education** | 449 | 1.62 | (0.62;4.25) | 0.324 | - | - | - | - |
| **Caregiver not married** | 458 | 0.85 | (0.48;1.49) | 0.562 | - | - | - | - |
| **Household has no grant** | 451 | 1.52 | (0.78;2.99) | 0.218 | 451 | 1.56 | (0.78;3.13) | 0.208 |
| **Troubled by ear pain/discharge** | 458 | 1.88 | (0.95;3.71) | 0.069 | 451 | 1.85 | (0.91;3.72) | 0.087 |
| **Any otitis externa/other** | 458 | 2.72 | (0.82;8.95) | 0.101 | 451 | 2.78 | (0.80;9.59) | 0.106 |
| **HIV infected** | 458 | 1.39 | (0.68;2.85) | 0.370 | 451 | 1.58 | (0.71;3.53) | 0.266 |

**Restricted to HIV Infected Participants (n=363)**

| | Univariate Regression Model | | | | Adjusted Regression Model | | | |
|---|---|---|---|---|---|---|---|---|
| **Variable** | N | Odds Ratio | 95% Confidence Interval | P Value | N | Odds Ratio | 95% Confidence Interval | P Value |
| **% CD4≥25** | 363 | 1.41 | (0.69;2.86) | 0.348 | - | - | - | - |
| **Duration of ART ≥ 8 yr.** | 363 | 1.90 | (1.04;3.46) | 0.037 | 356 | 1.15 | (0.48;2.74) | 0.749 |
| **CD4 Count (vs<200 cells/mm$^3$)** | 363 | | | | - | | | |
| **≥200 - <500 cells/mm$^3$** | | 1.36 | (0.15;12.6) | 0.785 | | - | - | - |
| **≥500 cells/mm$^3$** | | 1.71 | (0.19;13.7) | 0.615 | | - | - | - |
| **Viral load (vs ≤ 50 cells/ml)** | 363 | | | | - | | | |
| **>50 - <1000 copies per millilitre** | | 0.49 | (0.14;1.66) | 0.250 | | - | - | - |
| **≥1000 copies per millilitre** | | 1.11 | (0.49;2.55) | 0.798 | | - | - | - |
| **Age at ART initiation (vs 0 -2 yr.)** | 356 | | | | 356 | | | |
| **3-5 yr.** | | 0.64 | (0.31;1.32) | 0.225 | | 0.81 | (0.35;1.86) | 0.615 |
| **6-14 yr.** | | 0.38 | (0.18;0.81) | 0.012 | | 0.55 | (0.18;1.66) | 0.287 |
| **ART regimen (vs 2XNRTI +NNRTI)** | 357 | | | | 356 | | | |
| **2xNRTI + PI** | | 1.76 | (0.95;3.26) | 0.075 | | 1.44 | (0.74;2.80) | 0.285 |
| **Other regimen** | | 6.13 | (1.29;29.03) | 0.022 | | 4.08 | (0.80;20.71) | 0.090 |

***Initial multivariable models included all covariates with p<0.20 in univariate models.; HIV status included in adjusted analysis despite p value not reaching level of significance because HIV status was of particular interest.***

**Appendix C5** Letter of Study Approval



UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
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Observatory 7925
Telephone [021] 406 6626
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

17 February 2017

**HREC REF: 088/2017**

**Prof Landon Myer**
Public Health & Family Medicine
Falmouth Building

Dear Prof Myer

**PROJECT TITLE: THE PREVALENCE OF HEARING LOSS IN HIV INFECTED ADOLESCENTS IN CAPE TOWN (SUB-STUDY LINKED 051/2013) Masters Candidate - Dr A Banga**

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

**Approval is granted for one year until the 28th February 2018.**

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

**Please quote the HREC REF in all your correspondence.**

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval before the research may occur.

***The HREC acknowledge that the student, Dr Agatha Banga will also be involved in this study.***

Yours sincerely

Signed

**PROFESSOR M BLOCKMAN**
**CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE**
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical

HREC 088/2017

Appendix C5 shows the ethics approval letter issued by the Faculty of Health Sciences Human Research Ethics Committee for this cross-sectional analysis. The approval number is 088/2017.

Appendix C7 shows Tropical Medicine & International Health author guidelines for submitting articles. The manuscript should is duly accompanied by Appendix C6.

**Appendix C6** Author Statement

**Manuscript title**: The Prevalence of Hearing Loss in HIV-infected South African Adolescents on Antiretroviral Therapy.

I have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND

I have drafted the work or revised it critically for important intellectual content; AND

I have approved the final version to be published; AND

I agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

All persons who have made substantial contributions to the work reported in the manuscript, including those who provided editing and writing assistance but who are not authors, are named in the Acknowledgments section of the manuscript and have given their written permission to be named. If the manuscript does not include Acknowledgments, it is because the authors have not received substantial contributions from nonauthors.

Attachment: Yes, No

Signed by candidate

Signature Removed

**Author signature**

Agatha Tafadzwa Banga 13 March, 2017

**Printed name Date signed/appended**

**Appendix C7** Author Guidelines

From: (http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)13653156/homepage/ForAuthors.html)

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Editors H. van Asten, T. Junghanss, T. Marchant, C.G. Meyer and P. Van der Stuyft

Impact Factor: 2.519

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