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TITLE

Incidence of aminoglycoside-induced hearing loss in HIV positive and HIV negative multidrug-resistant tuberculosis patients.

University Of Cape Town

Master of Medicine (MMED) in Otorhinolaryngology

INDEX

This research project addressed 2 related questions:

The first question relates to prevalence of the 6 known aminoglycoside-induced deafness mutations in the *MT-RNR1* gene in a cohort of MDR-TB patients. This data has been published and has been cited in sections where referred to in the text. This data has been included as it forms part of the genetics arm of the study.

This dissertation addresses the second question which relates to the incidence of aminoglycoside–induced hearing loss in a cohort of patients with MDR–TB. The results of this study showed that HIV positive patients on antiretroviral drugs were more likely to develop hearing loss than HIV negative patients. Guidelines for the medical fraternity on monitoring for hearing loss in MDR-TB are also addressed. This data has not been published.

Part A: Research Protocol

PROJECT TITLE

Incidence of aminoglycoside-induced hearing loss in HIV positive and HIV negative multidrug-resistant tuberculosis patients.

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Introduction

Aminoglycosides are clinically important drugs that are used in the treatment of aerobic gram-negative bacterial infections and chronic infections such as tuberculosis (TB) caused by resistant mycobacteria strains.[1] The ototoxic effects of aminoglycoside antibiotics are well documented.

Aminoglycoside drugs exert their antibacterial effects by binding to the 16S rRNA in the 30S subunit of the bacterial ribosome and cause mistranslation or premature termination of protein synthesis.[2,3] The following mechanism for aminoglycoside ototoxicity in humans has been proposed: the aminoglycosides bind to the mitochondrial 12S rRNA, which shares structural similarity with its bacterial counterpart.[2,3] This binding inhibits mitochondrial protein synthesis causing a decline in adenosine triphosphate (ATP) production. This in turn, leads to an increase in the levels of toxic reactive oxygen species (a by-product of respiration), causing damage to mitochondrial and cellular proteins, lipids and nucleic acids.[2] This results in apoptosis of the cochlear outer hair cells and receptor cells of the vestibular organ, leading to hearing and vestibular impairment.[2]

Aminoglycosides can damage the cochlea in a dose-dependent fashion, but there are also patients who have a genetic predisposition to aminoglycoside ototoxicity due to mutations in the mitochondrial 12S rRNA, who may suffer an idiosyncratic reaction (after a single dose).[4] There are six known mutations in the *MT-RNR1* gene which predispose patients to aminoglycoside ototoxicity: A1555G, C1494T, T1095C, 961delT+C(n), A827G and T1291C. All 12S rRNA molecules harbouring the A1555G or C1494T mutations (and possibly the T1095C and the 961 delT+ C mutations), either take up a secondary structure that more closely resembles the bacterial 16S rRNA, or creates a new binding site that results in greater aminoglycoside binding.[5,6,7,8]

Due to its low cost and efficacy, aminoglycosides remain widely used in developing countries and therefore aminoglycoside ototoxicity remains a major problem. Depending on the population studied, aminoglycoside ototoxicity accounts for 3 to 30% of hearing loss.[9] This high prevalence rate may be attributed to genetic susceptibility from mitochondrial mutations in the 12S rRNA gene, the unregulated use of aminoglycoside antibiotics, or other predisposing factors.[9] Risk factors like advanced age , renal dysfunction and concomitant ototoxins are well documented .[4]

The literature suggests that mitochondrial mutations in the 12S rRNA are rare (between 0.09% and 3.96%), depending on the population studied.[10,11] Due to the rarity of these mutations, it may be unlikely that genetic predisposition contributes significantly to the

excessive risk of ototoxicity often seen in developing countries. There may be other environmental factors that cause patients to be at increased risk.

The incidence of TB is highest amongst patients with advanced human immunodeficiency virus (HIV) infection.[12] Patients with advanced HIV are treated with highly active antiretroviral therapy (HAART). In HAART, a combination of antiretroviral drugs is used to suppress viral replication. The recommended drug combination for patients who have not yet been treated for HIV consists of two nucleoside reverse transcriptase inhibitors (NRTIs), together with a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI). Individually these drugs have a range of significant toxicities.[13]

Prior cross-sectional studies and case reports have shown an association between hearing loss and nucleoside reverse transcriptase inhibitors (NRTIs).[14, 15, 16] The mechanism by which NRTIs cause auditory dysfunction includes damage to the mitochondrial DNA.[17]

Mitochondrial anomalies have been linked to a number of causes of hearing loss namely, presbyacusis [18], non-syndromic hearing loss[19], and of course genetic susceptibility to aminoglycoside-induced hearing loss. Since NRTIs cause mitochondrial dysfunction, patients who are taking NRTIs may theoretically be more at risk of hearing loss when exposed to aminoglycosides. If this were to be the case, then a significant number of people are potentially at increased risk of aminoglycoside ototoxicity from concomitant antiretroviral administration; far more so than those from genetic susceptibility.

Brooklyn Chest Hospital in Cape Town, is a dedicated TB hospital where patients with tuberculosis are hospitalised for periods of between 2 to 4 months and receive systemic streptomycin (for retreatment tuberculosis), or kanamycin (for multidrug resistant tuberculosis). Anecdotal evidence suggests that a significant proportion of the patients experience mild to profound hearing loss. It would be important to establish how many of these patients harbour a genetic mutation which would predispose them to aminoglycoside-induced ototoxicity. It would also be important to identify individuals who are at risk of irreversible hearing loss if treated with aminoglycoside antibiotics and to counsel them accordingly.

As part of hospital protocol, all patients are routinely tested for HIV (once consented), unless the HIV status is already known. Pre- and post-test counselling is done by the hospital HIV counsellor and antiretroviral treatment is initiated when appropriate. This allows for comprehensive care.

Hypotheses

- The incidence of aminoglycoside-induced hearing loss is high in the local TB population.
- HIV positive patients are at increased risk of aminoglycoside ototoxicity.

Aims of the study

- To document the incidence and severity of aminoglycoside-induced ototoxicity at Brooklyn Chest Hospital.
- To determine the prevalence of the 6 known aminoglycoside-induced deafness mutations in the *MT-RNR1* gene (A1555G, C1494T, T1095C, T1291C, A827G and 961 indel C) in a cohort of MDR-TB patients.*
- To determine whether HIV positive MDR-TB patients are more likely to develop aminoglycoside-induced hearing loss than HIV negative MDR-TB patients.
- To provide clinical guidelines to the medical fraternity on the use of aminoglycoside antibiotics with regard to the side-effect of ototoxicity.

Inclusion Criteria

- Patients with multidrug-resistant pulmonary tuberculosis who received streptomycin, kanamycin, or amikacin.
- Aminoglycoside treatment started on admission or within three months prior to admission to hospital.
- HIV positive patients on antiretroviral treatment and HIV negative patients.
- Patients with normal hearing on admission (defined as pure tone average $\leq$ 25dB).

Exclusion Criteria

- Patients with sensorineural hearing loss (defined as pure tone average $\geq$ 25dB).
- Patients with conductive hearing loss.
- Patients who started aminoglycoside treatment >3 months before admission. (Patients are often started on aminoglycosides in the community).
- Patients with renal dysfunction.

Material and Method

- Prospective cohort

- Study participants were recruited from Brooklyn Chest Hospital, Cape Town.
- Study was approved by the University of Stellenbosch's Committee for Human Research (study approval number N05/09/165), as well as the University of Cape Town's Research Ethics Committee (study approval number 443/2005).
- Informed written consent was obtained from each individual participating in this study and in the case of minors consent was obtained from their legal guardians.

Genetics Testing*

- Peripheral blood samples were collected in EDTA-coated tubes from each study participant and genetic testing performed to screen for the six known aminoglycoside-induced deafness mutations in the *MT-RNR1* gene (A1555G, C1494T, A827G, T1291C, T1095C and 961 indelC) using the new SNAPshot technique developed by the Tygerberg Genetics Department.*[20, 21]
- Polymerase chain reaction (PCR) primers and SnapShot primers were specially designed that spanned the entire length of the *MT-RNR1* gene.[20]
- The SnapShot technique can detect the 6 known mutations in a single reaction.[20]

As part of the hospital's protocol

- Blood was tested for urea and electrolytes routinely at least once monthly and if deranged, aminoglycosides were stopped and treatment altered.
- Informed consent was obtained from patients for HIV testing (done routinely where HIV status was unknown).
- Pre- and post-test counselling done by the hospital HIV counsellor.

Data collection included

1. Age
2. Gender
3. Ethnic group
4. Specific aminoglycoside therapy
5. Recent exposure to aminoglycoside (within the last 6 months)
6. History of noise exposure
7. Presence of tinnitus
8. HIV status and whether patients were on antiretroviral treatment if HIV positive

Audiology Protocol

- All the audiology tests were undertaken in a sound-treated booth in the audiology department at Brooklyn Chest Hospital.
- Otoscopy, tympanometry, conventional pure tone audiometry were done on admission as a baseline evaluation, and then at weeks 4, 8 and 12.
- A study period of three months was elected as this was the average length of hospitalisation for patients.

Otoscopy

- Heine Minilux otoscope was used for non-diagnostic otoscopic examinations by the audiologist.
- Non-diagnostic otoscopy involved visual inspection of the external auditory meatus and tympanic membrane. If any abnormalities of these structures were observed, appropriate referral was made after the participant's consent to do so.

Tympanometry

- Portable tympanometer was used for tympanometry.
- If abnormalities were detected on tympanometry, appropriate referral was made and the individual excluded from the study.

Conventional Audiometry

- Amplaid 308 audiometer and TDH-39 headphones were used for conventional audiometry.
- Before commencing the test protocol, all tests were explained to the participant in the appropriate language.
- Clear instructions were given before each procedure, and repeated during the test if necessary.
- Pure tone air conduction testing using pure tones from 250 to 8000 Hz at octave intervals was performed.
- Pure tone audiogram testing started at 0 dB HL at 1000 Hz, using an ascending approach.
- The participants had to raise a hand to indicate when the tone was detected.
- The results were recorded in decibel hearing level (dB HL) on an audiogram.

Severity of hearing loss was graded as:

- 0-25 dB HL Within normal limits
- 26-40 dB HL Mild
- 41-55 dB HL Moderate
- 56-70 dB HL Moderate to Severe (or moderately severe)
- 71-90 dB HL Severe
- 91+ dB HL Profound

Analysis of results

- Incidence of aminoglycoside-induced ototoxicity in a cohort of MDR-TB patients.
- Severity of hearing loss.
- Prevalence of known aminoglycoside-induced deafness mutations in the *MT-RNR1* gene [A1555G, C1494T, T1095C, T1291C, A827G, 961delT+C(n)] in a cohort of MDR-TB patients who develop ototoxicity.*[21]
- Incidence of hearing loss in HIV positive MDR-TB patients on HAART.

Possible Conclusions

- The incidence of aminoglycoside-induced ototoxicity in MDR-TB patients is high.
- Genetic predisposition to aminoglycoside-induced hearing loss does not play a major role in our population.
- HIV positive patients with MDR-TB might be at increased risk of ototoxicity.

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Part B: Literature Review

Objectives of the literature review

The objectives of the literature review were to explore studies related to aminoglycoside-induced ototoxicity and to review what has been published thus far in this field.

Context

With MDR-TB, aminoglycosides often have to be administered for a prolonged period of time and the resultant ototoxicity, is permanent.[1] Different studies have reported hearing loss as an adverse drug reaction in patients with MDR-TB ranging from 6-40%.[2,3,4] No data on the incidence of aminoglycoside-induced deafness has ever been documented for this country.

The significant increase in MDR –TB in South Africa has been linked to the HIV epidemic.[5] Scano and Vitoria et al. recently published an article which looks at the management of HIV-infected patients with MDR-TB and highlighted known adverse effects of antituberculous drugs and antiretroviral drugs which may overlap.[6]
The literature is limited in that it does not address HIV positive patients on antiretroviral drugs when looking at ototoxicity in MDR-TB, yet frequently these drugs are given concomitantly. While there is insufficient evidence on the frequency and severity of ototoxicity from antiretroviral drugs and second-line TB drugs when given concomitantly, there is evidence in the literature to suggest that HIV positive patients on antiretroviral drugs may have an increased risk of hearing loss.[7, 8]

Literature search strategy and quality criteria

The information presented in this paper is supported by a Pubmed Medline search using the key words: aminoglycoside antibiotics, HIV, antiretrovirals, ototoxicity, tuberculosis (TB), multidrug resistance, sensorineural hearing loss. Clinical studies and systematic reviews which directly addressed aminoglycoside-ototoxicity were chosen. Several randomised control trials relating to aminoglycoside-induced ototoxicity were identified. Experimental evidence from animal studies has also been included but without expressions relating to randomization. The assessment of quality was based on the design of the study and the levels of clinical evidence. There is very little in the literature relating to antiretroviral drugs and hearing loss. Case studies, case series and cohort studies have been included here as they had clinical significance.

Inclusion criteria for articles were studies relating to:

- The mechanism of aminoglycoside-induced ototoxicity
- The genetics of aminoglycoside-induced ototoxicity
- Hearing loss in MDR-TB patients
- Hearing loss in HIV positive individuals on antiretroviral drugs
- Experimental animal studies where relevant (relating to ototoxicity of antiretroviral drugs)
- Clinical and experimental animal studies relating to otoprotective agents in aminoglycoside ototoxicity

Studies relating to ototoxicity of topically administered aminoglycoside antibiotics were excluded as well as those studies which addressed ototoxicity in neonates and preterm infants.

Summary and interpretation of the literature

Mechanism of aminoglycoside ototoxicity

Aminoglycoside antibiotics were first introduced into clinical practice in the 1940's and proved to be highly effective in the treatment of multidrug-resistant TB, or gram-negative infections, such as those frequently seen in cystic fibrosis.[9] The ototoxic effects of aminoglycosides became evident shortly thereafter, with subsequent histological studies revealing that administration of aminoglycosides could lead to the death of sensory hair cells.[9] Aminoglycosides have variable cochleotoxicity and vestibulotoxicity. Streptomycin and gentamicin are primarily vestibulotoxic; whereas amikacin , neomycin, kanamycin are primarily cochleotoxic.[10] Patients are generally able to physiologically compensate for vestibular damage, but the hearing loss is permanent, therefore cochleotoxicity is considered to be a more serious problem.[10]

Aminoglycosides enter the inner ear fluids shortly after systemic administration. Once in the ear, they are taken up by the cochlear hair cells. Some studies suggest that they are taken up by endocytosis; others suggest that they are taken up by transduction channels.[11, 12, 13] Although they are cleared from the bloodstream within hours, they can remain sequestered within the cells of the inner ear for up to 6 months.[14] Wang et al. demonstrated that the toxicity may persist for up to one year, even after stopping the drug.[15] This is relevant when

looking at ototoxicity in MDR-TB, where patients may be exposed to aminoglycosides for up to 18-24 months.[2]

Aminoglycosides are primarily renally excreted and in cases of renal dysfunction, serum levels of aminoglyoside can increase, placing a patient at risk of ototoxicity.[16] When treating acute infections such as gram-negative sepsis, or where multiple daily doses of the aminoglycoside are administered, serum trough levels are monitored to prevent toxicity.[17] Peloquin et al. in a randomised trial, compared the incidences of toxicities associated with 2 recommended dosing regimens (daily vs. three times per week of intravenous streptomycin, kanamycin, or amikacin) in eighty-seven patients with tuberculosis and non-tuberculous mycobacterial infections. The study showed that the size of the dosage and the frequency of administration were not associated with the incidences of ototoxicity, or nephrotoxicity (determined by elevated serum creatinine levels). Risk of ototoxicity (found in 37% of the patients) was associated with a larger cumulative dose and older age.[18]

The role of mitochondria in aminoglycoside ototoxicity

The generation of highly reactive oxygen molecules is thought to play a key role in the initiation of hair cell death via oxidative stress. Aminoglycoside antibiotics exert their antibacterial effects by interacting with the bacterial ribosome. The aminoglycosides bind to highly conserved sequences of bacterial 16S rRNA and thereby interfere with protein synthesis.[19, 20] Human mitochondrial ribosomes share similarities with bacterial ribosomes, and therefore it is proposed that hair cell mitochondria may be an early target of aminoglycosides.[19,20,21] Interference with mitochondrial ribosomes could lead to the disruption of the electron transport chain and the generation of free radicals resulting in apoptosis of cochlea hair cells.[19, 20]

The A1555G mutation has been mapped to a locus within the mitochondrial ribosomal RNA.[21] Individuals who have this mutation are predisposed to sudden hearing loss after just a single exposure to aminoglycosides. This mutation is also a cause of non-syndromic sensorineural deafness.[21] The discovery of this mutation enabled scientists to understand the role of mitochondria in ototoxicity**.** [21] The A1555G mutation transforms human mitochondrial ribosomal RNA into a configuration that resembles ribosomal RNA of gram–negative bacteria, therefore carriers of this mutation are more susceptible to ototoxicity when exposed to aminoglycosides.[21] There are 6 known mitochondrial mutations which have been linked to aminoglycoside-induced hearing loss: A1555G, C1494T, T1095C, 961delT+C(n), T1291C and A827G, with A1555G being the commonest.[22]

Genetic susceptibility: How many are at risk in the South African population?

The increased exposure of aminoglycosides in our population raises the question: should we be screening for these mutations before administering aminoglycoside antibiotics?

Having data on the prevalence of aminoglycoside use in our population is important as it identifies how many patients are at risk. The overall population frequency of these mutations also bears on the question of whether to screen for these mutations. Data from these types of studies will support discussion on the feasibility of genetic screening in our population. The Tygerberg Hospital genetics department have developed a cost effective method for the simultaneous detection of the six known aminoglycoside-induced mutations.[22,23] This test facilitates the detection of susceptible individuals prior to the start of their aminoglycoside therapy. Knowing an individual is more susceptible to hearing loss before commencing aminoglycoside treatment allows for genetic counselling as well as informed consent. The frequency of the A1555G mutation varies quite significantly between populations (0.09%-0.5%).[24,25,26] Bardien and Human, conducted a study to determine the frequency of the six known mitochondrial mutations (A1555G, C1494T, T1095C, 961 delT+C(n) and A827G) in 439 South African control samples.[23] They found that the frequency of the A1555G mutation in the South African Black control group was 0,9% and the A827G mutation was present in 1,1% of theAfrikaner control group.[22,23] The authors concluded that the high frequency of the A1555G mutation is of concern given the high incidence of MDR-TB in this population group and additional large population studies are needed to refine its occurrence in the South African population.

HIV, mitochondrial toxicity of antiretroviral treatment and hearing loss

An increased incidence of hearing loss among HIV positive patients has been documented in a number of studies.[27,28,29] There are a number of reasons that HIV positive patients are more at risk of hearing loss including: otitis media, opportunistic infections of the CNS, such as toxoplasmosis, cytomegalovirus, tuberculosis, and cryptococcosis; malignancies, including Kaposi's sarcoma and lymphoma, HIV-1 infection and treatment with ototoxic drugs.[30,31,32,33,34]

There are a few reports and studies in the literature which document the ototoxicity of antiretroviral drugs.[35,36,37,38,39,40] Five previous case reports have associated NRTIs with hearing loss; hearing loss was attributed to dideoxycytidine for 3 patients,[35,36,37]didanosine (ddI) for 1 patient,[38] and the combination of ddI and zidovudine for 1 patient.[39] Simdon

reported three subjects who experienced ototoxicity with combination antiretroviral treatment (ART). All three patients received combination antiretroviral treatment (ART) with 2–3 nucleoside reverse transcriptase inhibitors (NRTIs) plus a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI). All 3 subjects were also over the age of 45, had prior hearing problems and prior exposure to occupational noise.[8] Marra et al. in a study of 99 HIV-1-infected patients who attended an outpatient clinic in Seattle, found that 29% demonstrated abnormalities on screening audiometry.[40] Similarly, Birchall et al. and Chandrasekhar et al. also reported hearing loss in a third of their patients.[27,29] These investigators noted that the use of NRTIs within the previous 6 months was significantly associated with hearing loss in patients aged >35 years. Several authors attributed the hearing loss seen in HIV positive patients to mitochondrial DNA damage caused by the NRTIs.[8,40,41] Mitochondrial toxicity of the nucleoside analogues has already been confirmed by *in vitro* studies .[41,42]

NRTIs act as chain terminators of mitochondrial deoxyribonucleic acid (DNA).[41] The NRTIs are phosphorylated intracellularly to active triphosphate forms and are then incorporated into new DNA strands synthesized by HIV reverse transcriptase.[41] Medina et al. demonstrated that within a few days of exposure to antiretroviral drugs, the mitochondrial DNA content of cells decreased.[41] The medium- to long-term toxicities of NRTIs are thought to be secondary to the inhibition of mitochondrial DNA polymerase γ, resulting in impaired synthesis of the mitochondrial enzymes that generate adenosine triphosphate by oxidative phosphorylation.[41] Cortopassi established that aging is also associated with mitochondrial DNA mutations in the cochlear hair cells, thereby causing hearing loss.[43] Simdon postulated that if NRTIs cause hearing loss through mitochondrial toxicity, it is possible that they synergize with mitochondrial abnormalities induced by age to facilitate the development of clinically distinguishable hearing problems.[8] This may explain why older patients who are on antiretroviral drugs are more at risk of hearing loss.[8,27,31]

A prospective study by Schouten and Lockhart et al. reported on the audiometric findings on 33 patients who received zidovudine (ZVD) and /or didanosine (DdI). In contrast to previous reports in the literature, they found no significant change in patients' hearing after 32 weeks of antiretroviral treatment.[44]

A recent animal study (CBA/CaJ mice) describe the effects of regular dosing with a common antiretroviral drug combination on outer hair cell (OHC) function using objective audiological tests, namely distortion product otoacoustic emissions (DPOAEs) and auditory brainstem responses (ABRs). Experimental mice were treated daily over a 3 month period

with the nucleoside reverse transcriptase inhibitors (NRTIs), zidovudine (ZDV) and lamivudine (3TC), which was dissolved in their drinking water. The control subjects received untreated water. DPOAE levels and ABR detection thresholds were determined prior to and after 12 weeks of NRTI treatment. The DPOAE and ABR thresholds did not differ between experimental and control groups.[45]

To determine whether exposure to NRTI 's potentiates the adverse effects of loud noise on OHC function, both experimental and control mice were exposed 1 week later, while still on the drug regimen, to a loud noise (10-kHz octave-band noise at 105 dB SPL for 1 h). The mice who received NRTI's for 12 weeks, showed significantly greater permanent reductions in DPOAE levels 2 weeks after the noise exposure than the untreated control animals.[45]

The authors concluded that, while there was no evidence in mice for any adverse effects on cochlear function from NRTIs when administered alone; when combined with another cochlear insult such as a loud noise, NRTIs can act synergistically and adversely affect cochlear hair cell function.[45]

One could postulate that patients on NRTIs would be more susceptible to aminoglycoside-induced hearing loss due to possible synergistic effects (as both aminoglycosides and NRTIs cause damage to mitochondrial DNA).

Prospective studies are needed to confirm whether NRTIs are associated with hearing loss and to identify the NRTIs most likely to induce ototoxicity and which patients are most at risk.

Identification of gaps or needs for further research

Development of new antituberculous drugs which are highly active, low cost and have reduced toxicity are needed. New protocols are needed for efficacious clinical control of TB patients using ordinary antimycobacterial drugs thus preventing MDR-TB and aminoglycoside use. Unfortunately research in this area has been very slow. No new drugs except rifabutin and rifapentine have been marketed for TB (mainly in the US) during the 40 years after release of rifampicin.[46]

During the past decade, scientists have made enormous progress in understanding the biochemical events that lead to ototoxic hearing loss. As previously mentioned, compelling evidence from animal models suggests that reactive oxygen species are part of the initial mechanisms that trigger apoptotic and necrotic cell death in the inner ear.[47] Animal studies have shown that antioxidants can protect against aminoglycoside-induced hearing loss in

animals and, more importantly, they do so without compromising drug serum levels or antibacterial efficacy.[47]

Trials on protection from aminoglycoside ototoxicity on human subjects have only recently been reported. Based on the finding that salicylate afforded protection in animals, Chen and Huan et al. tested the efficacy of aspirin (acetyl salicylate) in a randomized double-blind placebo-controlled study in patients receiving gentamicin for acute infections. The authors demonstrated that there was a significant difference in the frequency of hearing loss between the placebo group and the aspirin group. Fourteen of 106 patients (13%) developed hearing loss in the placebo group while only 3/89 (3%) were affected in the aspirin group ($p = 0.013$). Aspirin did not influence gentamicin serum levels or the course of therapy.[48] These results indicate that therapeutic protection from aminoglycoside ototoxicity may be extrapolated from animal models to the clinic. Furthermore, medications as common as aspirin can significantly attenuate the risk of gentamicin-induced hearing loss. Its efficacy in chronic aminoglycoside exposure has not been investigated in clinical studies.

Similarly, D-methionine has been shown in animal studies (Wistar rats) to be protective against hearing loss caused by aminoglycoside without interfering with the antimicrobial efficacy of the antibiotic.[49] While this drug has been shown to be very promising in animal studies; and is as effective in its oral form, it is not yet FDA approved. One of the problems of MDR-TB is the fact that aminoglycosides have to be given for prolonged periods, therefore further studies are need to determine whether D-methionine will be as effective in preventing aminoglycoside-induced hearing loss in humans with MDR-TB.

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Incidence of aminoglycoside-induced hearing loss in HIV positive and HIV negative multidrug-resistant tuberculosis patients

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Abstract

Objectives

Multi-drug resistant tuberculosis (MDR-TB) requiring aminoglycosides is a significant problem in Southern Africa with resultant increased risk of ototoxicity.

The aims of the study were to: document the incidence of ototoxicity in MDR-TB patients with and without HIV, and develop clinical guidelines relating to ototoxicity in such patients.

Design

Prospective cohort study

Setting

Brooklyn Chest Hospital in Cape Town

Subjects

153 HIV positive and HIV negative MDR-TB patients with normal hearing and middle ear status at baseline.

Outcome Measures

Pure tone audiometry was performed monthly for 3 months to screen for hearing loss. HIV status was recorded. Genetic screening was done on 115 patients using the SNaPshot technique to screen for six mutations in the *MT-RNR1* gene: A1555G, C1494T, T1095C, 961delT+C(n), A827G and T1291C.

Results

Fifty-seven percent (87/153) developed high frequency hearing loss over the 3 month period. Of the patients who developed hearing loss, 69% (60/87) were HIV positive and 31% were HIV negative. Of the 115 patients who had genetic screening, none had any known mutations.

Conclusions

Ototoxic hearing loss is common in MDR-TB patients. HIV positive patients have an increased risk of ototoxicity. Objective auditory monitoring should be undertaken for all MDR-TB patients and particularly for at risk groups, such as those that are HIV positive. Auditory rehabilitation for patients developing ototoxicity should be an integral part of the package of care of MDR-TB patients.

Key Words: Aminoglycoside, ototoxicity, Multi-drug resistant TB, HIV, Audiology, MDR-TB, Hearing loss, deafness

Introduction

Multidrug-resistant tuberculosis (MDR-TB), defined as resistance to both isoniazid and rifampicin, is a growing problem globally but even more so in sub-Saharan Africa where HIV contributes to the burden of TB.[1] The World Health Organization (WHO) reported that even in the absence of a nationwide survey, South Africa identified 17,615 MDR-TB isolates over a 4 year period.[2] A survey done by *Medicins Sans Frontiers* showed that Cape Town achieved one of the best cure rates in the country with a 76% cure rate and a 82% completion rate in 2006.[2] Despite this, MDR-TB increased twelve –fold in Khayelitsha (9 cases in 2004, and 109 cases in 2006).[3] This increase has been linked to the HIV epidemic.[3]

WHO guidelines dictate that, depending on susceptibility patterns, residual first-line oral drugs must be appropriately combined with additional second-line drugs comprising injectable drugs (amikacin, kanamycin, capreomycin), fluoroquinolones (ofloxacin, levofloxacin, moxifloxacin, gatifloxacin), old bacteriostatic second-line anti-tuberculosis agents (ethionamide, prothionamide, cycloserine, para-aminosalicylic acid) and anti-tuberculosis agents with uncertain efficacy such as clofazimine, amoxicillin/clavulanate, clarithromycin, linezolid.[4]

Treatment of MDR-TB is challenging even in resource rich settings. Patients have to be treated for at least 18-24 months with second-line TB drugs that have significant side effects e.g. ototoxicity and nephrotoxicity. While nephrotoxicity is reversible, otoxicity is permanent.[5] Consequently, an increasing number of people are at risk of developing aminoglycoside or polypeptide related ototoxicity every year in Southern Africa, and a lack of audiology monitoring facilities and efficacious, cost-effective alternatives increase the risk of ototoxicity.

In the genetics arm of this study it was determined that none of the 115 MDR-TB patients who had genetic screening, had any of the known six mutations which would predispose them to hearing loss.[6,7] Eight of the patients who developed ototoxicity had the entire mitochondrial genome sequenced and this did not reveal any mutations in the *MT-RNR1* gene.[7]

The aims of the study were to 1) document the incidence and audiological profile of aminoglycoside-induced hearing loss in MDR-TB patients, 2) compare the incidence of

aminoglycoside-induced hearing loss between HIV positive and HIV negative patients with MDR-TB, 3) and to develop clinical guidelines on the use of aminoglycoside antibiotics in MDR-TB patients relating to hearing loss.

Methods

The study was approved by the Committee for Human Research at Stellenbosch University (protocol number: N05/09/165) and the Research Ethics Committee at the University of Cape Town (ref:443/2005). MDR-TB patients were recruited and appropriately consented at Brooklyn Chest Hospital in Cape Town, South Africa. Brooklyn Chest Hospital is a dedicated TB hospital where patients are admitted and directly observed therapy (DOT) is instituted. Admission criteria are MDR-TB, extrapulmonary TB or retreatment/treatment failures whom due to general ill-health and/or social circumstances are unable to attend their local clinics for TB treatment. All patients are routinely tested for HIV after consent is taken and pre- and post-test counselling has been done.

Inclusion criteria for the study were that patients receive aminoglycosides as part of their treatment within three months of hospitalisation and had normal hearing on admission. Serum aminoglycoside levels were not recorded. Patients underwent pure tone audiometry (PTA) and tympanometry on admission, then monthly for three months. Pure tone audiograms, including both air and bone conduction threshold measurements between 250 Hz and 8000 Hz, were performed in a sound treated room. The criteria for ototoxic threshold shift from the initial baseline audiogram were (in decibel [db]): (1) $\geq$ 20 dB decrease at any one test frequency, (2) $\geq$ 10 dB decrease at any two adjacent frequencies, or (3) loss of response at three consecutive frequencies where responses were previously obtained. The severity of hearing loss was graded (in decibel hearing level [dB HL]) as: 1) mild: PTA between 26-40 dB HL, moderate : PTA between 41-55 dB HL, moderately severe : PTA between 56-70 dB HL, severe between 71-90 dB HL and profound PTA > 90dB HL. Data recorded included age, gender and ethnic group and a history of noise exposure. The type of aminoglycoside, when it was commenced and whether patients had been on aminoglycosides in the previous 6 months were also recorded; as was HIV status and whether patients were on antiretroviral treatment. Aminoglycoside treatment is frequently commenced by local TB clinics several weeks prior to admission to Brooklyn Chest Hospital. Although it was not possible to perform pre-treatment baseline audiometry on this subgroup of patients who had

already been commenced on aminoglycosides, only patients with normal hearing on admission to the hospital were included in the study. Patients were screened for the six known mitochondrial mutations associated with aminoglycoside-induced hearing loss using the SNapShot technique.[6,7] The SnapShot technique uses polymerase chain reaction (PCR) method to simultaneously detect the six known mutations using a single reaction. This test was developed by the Tygerberg genetics department.[6, 7]

The t-test two sample assuming unequal variances test was used to calculate the difference in the mean ages between the HIV positive and HIV negative patients with hearing loss at a 95% confidence interval. The odds ratio (at 95% confidence interval) was used to calculate the measure of association of HIV and aminoglycoside-induced hearing loss. The Fisher's exact test was used to determine whether there were any significant associations between race, gender and hearing loss and whether there was a significant association between HIV status and the severity of hearing loss.

Results

One hundred and fifty-three MDR-TB patients were included in the study. Median age of patients was 36, with a range of 14 to 70 years. Sixty-six percent were female and 34% were male.

All patients were on long-term treatment plans with second-line injectables (at least 6 months with an aminoglycoside or polypeptide or 4 months after sputum culture conversion, i.e. 2 sputum cultures negative one month apart). Ninety-six percent had received kanamycin, 3 % streptomycin and 1% capreomycin. Eighty-five percent (74/87) of patients who developed hearing loss had acquired MDR-TB and had been on streptomycin in the preceding 6 months. Fifteen percent (13/87) of patients had primary MDR-TB (i.e. no previous history of TB or anti-tuberculosis treatment for <1 month).

Five subjects were treated as retreatment cases initally and therefore were on streptomycin, but then changed to kanamycin once the sputum culture and drug susceptibility test results were available and confirmed MDR-TB. The two subjects on capreomycin were included in the study as they were on kanamycin before and were changed to capreomycin (a polypeptide, also with ototoxicity as side effect) towards the end of the study.

Fifty-six percent of patients were HIV positive (86/153), 42% (65/153) were HIV negative; 2 subjects refused HIV testing. All HIV positive patients were on highly active antiretroviral therapy (HAART) which consists of a combination of two nucleoside reverse transcriptase

inhibitors (NRTI), together with a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI).

Eighty-seven patients (57%) developed high frequency hearing loss following admission to hospital. Association of hearing loss with ethnicity, gender and HIV status is summarized in table 1.

HIV positive patients (60 of 87) were more likely to develop hearing loss than HIV negative patients (27 of 67; odds ratio 3.42, 95% confidence interval 1.66-7.09; $p<0.0001$).

The mean age of the patients with HIV and sensorineural hearing loss (SNHL) was 34 years (standard deviation [SD] 9,1 years) and patients who were HIV negative had a mean age of 40 years (SD13,7). There was no significant difference in the mean ages of the two groups. Figure 1 shows the age distribution of the HIV positive and negative patients who developed hearing loss.

Of the HIV positive patients, 23% developed mild to moderate hearing loss and 46% had severe to profound hearing loss. Of patients who were HIV negative, 10% had mild to moderate hearing loss and 30% had moderate to profound hearing loss (table 2). There was no association between HIV status and the severity of hearing loss.

One hundred and fifteen patients consented to genetic testing and none of these patients had any of the known mitochondrial mutations.[6,7] Thirty-eight of the patients that developed hearing loss refused genetic testing therefore the presence of mutations in these patients is unknown. Of the patients who developed hearing loss (n=87), 49 had genetic testing and the hearing loss in these patients were not due to known mutations conferring genetic susceptibility.

In all patients the hearing loss involved the higher frequencies (4000-8000 Hz) and in 6% this progressed to involve lower frequencies (500, 1000, 2000 and 3000 Hz). Fifteen percent (13/87) of patients developed asymmetrical hearing loss, and 13% (11/87) of patients with hearing loss complained of tinnitus.

Discussion

Pathophysiology of ototoxicity

The major target for aminoglycoside ototoxicity is the sensory neuroepithelium of the inner ear.[8] Outer hair cells of the cochlea are more susceptible than inner hair cells. Loss of cochlear hair cells results in secondary degeneration of the auditory nerve.[8] Labyrinthine

injury is usually gradual, progressive, symmetrically bilateral and permanent.[8] As in our study, asymmetrical losses have been reported.[9] Ototoxic injury and associated hearing loss may progress for weeks following cessation of aminoglycoside treatment, due to the long half–life of aminoglycosides in cochlear tissue.[10] The basal region of the cochlea is more susceptible to injury than the apical region, and is referred to as the 'cochleotopic' gradient of susceptibility. This cochleotopic gradient of susceptibility is expressed as high frequency hearing loss, which extends to include progressively lower frequencies as the cochlear damage becomes more extensive.[11] This is consistent with our finding that hearing loss, as documented by pure tone audiometry, involved higher frequencies (4000 to 8000 Hz), in 57% of MDR-TB patients using a single parenteral second-line aminoglycoside. In all of patients the hearing loss involved higher frequencies and in 6% of patients this progressed to involve lower frequencies (500, 1000, 2000 and 3000 Hz), thus affecting speech comprehension.

Diagnosis and monitoring of ototoxicity

Ototoxicity is diagnosed by comparing the patient's initial baseline audiogram, ideally obtained prior to ototoxic drug administration, to subsequent audiograms. Detecting changes in pure tone hearing thresholds using serial audiograms is considered a good indicator of ototoxic hearing loss, particularly when ultra-high frequency thresholds are included.[12, 13] Ideally, patients receiving ototoxic antibiotics should be monitored by undergoing audiological evaluations 1–2 times per week following initial baseline evaluation.[5, 12, 13] Otoacoustic emissions (OAEs) are more sensitive at detecting auditory dysfunction than high frequency pure-tone audiometry and has also been employed to monitor ototoxicity.[14] While 57% of this study's participants developed hearing loss, limitations of this study are that this figure could be larger if a more sensitive monitoring method was used i.e. high frequency audiometry or OAE, and may also increase if patients are followed until after completion of the aminoglycoside therapy. Monitoring for hearing loss should take place for the entire duration of treatment. Due to the long half life of aminoglycoside in cochlear tissues, patients should be monitored up to about 6 months following completion of MDR therapy.[15] For the purpose of this study pure tone audiometry was performed monthly for three months. However, once patients are discharged from Brooklyn Chest Hospital they are often unable to report back monthly to the hospital for audiometric evaluation, and because there is a paucity of audiological services at peripheral clinics where patients are referred to, monitoring for hearing loss following discharge from hospital is in reality non-existent.

Genetic and other risk factors

Although ototoxicity usually develops gradually, sudden profound sensorineural loss may follow a short duration of treatment or even a single dose of aminoglycoside. This may be associated with a genetic susceptibility caused by the 1555A to G substitution on the mitochondrial 12SrRNA.[16] If genetic susceptibility to aminoglycoside-induced ototoxicity is suspected on the basis of family history, this can be confirmed by genetic testing. One hundred and fifteen patients were screened for the six known mitochondrial mutations using the SNapShot technique.[6,7] None of these patients had the mitochondrial mutations.[6,7] Further studies are warranted to determine whether the South African population harbors novel mitochondrial mutations. Other risk factors for ototoxicity include cumulative drug dose, duration of treatment, bacteraemia, renal or liver failure, as well as concomitant administration of drugs like loop diuretics which have a synergistic ototoxicity effect.[17]

Clinical pointers to ototoxicity

The relationship between auditory symptoms and ototoxicity with aminoglycoside antibiotics has not been investigated. In this study only 13% of patients with hearing loss reported tinnitus. It is however advisable to note any new auditory symptoms as they may be a pointer to ototoxicity.

Incidence of ototoxicity

Fifty-seven percent of the patients in our study developed high frequency hearing loss following admission to hospital. In a study which examined side effects associated with MDR-TB treatment, Torun et al. reported that side effects occurred in 182 of 263 patients (69%) and that treatment had to be modified in 55% of cases.[18] Ototoxicity was the most common side effect and was noted in 41,8% and occurred at a mean of 4,7 $\pm$1,7 months of treatment. This frequent and early occurrence of ototoxicity may be due to extended exposure to aminoglycosides. Forty percent of these patients previously had been exposed to streptomycin. [18] This is similar to our study where 85% of patients had previously been exposed to streptomycin, and is consistent with findings of Moore et al. who showed an association between ototoxicity and cumulative dose of aminoglycoside.[19]

HIV and ototoxicity

It is estimated that 8% of new TB cases are attributable to HIV co-infection.[20] None of the studies of aminoglycoside-induced ototoxicity in MDR-TB has included significant numbers

of HIV patients.[17, 18] Fifty–six percent of patients in our study were HIV positive. Our study showed that HIV positive patients had a four times greater risk of developing ototoxic hearing loss than HIV negative patients.

Strategies to reduce ototoxicity

Very little is known about the co-administration of antiretroviral drugs and second-line TB treatment, particularly with regard to ototoxicity. In a recent WHO report about drug interaction and drug toxicity among patients receiving both antiretroviral drugs and second-line TB regimens, it was recommended that, for side effects such as peripheral neuropathy and psychiatric complications, the offending drug should be stopped, but only if it did not compromise cure.[21] Some side effects like renal failure and pancreatitis are life threatening but also reversible on stopping the offending agent. Aminoglycoside-induced ototoxicity is however not reversible on stopping the drugs. There are studies where aminoglycosides were stopped and the treatment regimen was altered when patients developed ototoxicity, without compromising cure.[17, 18] These studies were however conducted in HIV negative MDR-TB patients who were hospitalised and had side effects monitored for the entire duration of their treatment.[17, 18] The treatment regimen should therefore be reassessed and adapted if possible to minimize further toxicity. There is however, a trend towards poorer outcomes and significant mortality in the first few months in HIV positive patients with MDR-TB.[22, 23] Consequently, aminoglycosides are generally continued in HIV positive patients co-infected with MDR-TB despite the risk of developing hearing loss.

Situation in South Africa

In the Western Cape the majority of MDR-TB patients are admitted to MDR-TB hospitals like Brooklyn Chest Hospital. The hospital has an audiology service where patients are screened for hearing loss. Waiting lists for admission to the hospital are about 4-6 weeks, and this is likely to increase due to the increase in MDR-TB cases. In certain areas of South Africa, patients may wait for several months before an MDR-TB hospital bed is available.[24] Parenteral aminoglycosides are therefore commenced in the community where audiology screening for hearing loss generally is not done. In a recent report on audiological services in the Western Cape, Swart reported that the serious lack of audiological testing facilities, hearing aids and audiological rehabilitation is compromising MDR-TB care for patients who have become deaf as a consequence of ototoxic drug therapy.[25]

Conclusions

This is the 1st report that demonstrates an association between HIV and ototoxicity in MDR-TB patients. Further study is required to elucidate the mechanism of this increased risk of ototoxicity in HIV positive patients.

This study also provides alarming evidence of how commonly aminoglycoside-induced hearing loss occurs in MDR-TB patients, a problem that is likely to increase given the high prevalence of HIV in South Africa. It highlights the importance, when treating MDR-TB patients, of including ototoxicity as part of the informed consent process prior to commencing aminoglycoside therapy, of careful audiological monitoring prior to and during therapy, and of the need to provide hearing aids and audiological rehabilitation for patients that become significantly handicapped due to deafness. Due to the long waiting lists to access TB hospitals, the need to decentralise audiological services to TB clinics is also self-evident.

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Table 1 Frequency of hearing loss: total number and according to ethnic groups, gender and HIV status

Characteristic	Total number in study (% of total)	Hearing loss (% of total in characteristic group)
Total number of patients	153	87 (57)
Ethnic group Black Coloured Cape Malay Caucasian	 78 (51) 70 (46) 3 2	 46 (59) 38 (54) 2 (67) 1(50) Exact p-value= 0.8
Gender Female Male	 101 (66%) 52 (34)	 55 (63) 32 (37) Exact p-value= 0.5
HIV status HIV positive* HIV negative	 86 (56) 67 (44)	 60 (70) 27 (40) Exact p-value= 0.001

* Hearing loss occurred significantly more in HIV positive compared to HIV negative patient; odds ratio 3.42, 95% confidence interval 1.66-7.09; $p<0.0001$

Table 2. Severity of hearing loss in HIV positive and negative subjects

HIV Status	Severity of Hearing loss	
	* Mild-Moderate	** Severe- Profound
Positive	20	40
Negative	7	20

*Mild to moderate : PTA between 26-70 dBHL

**Severe to profound : PTA > 71dB HL

Exact p-value= 0.6

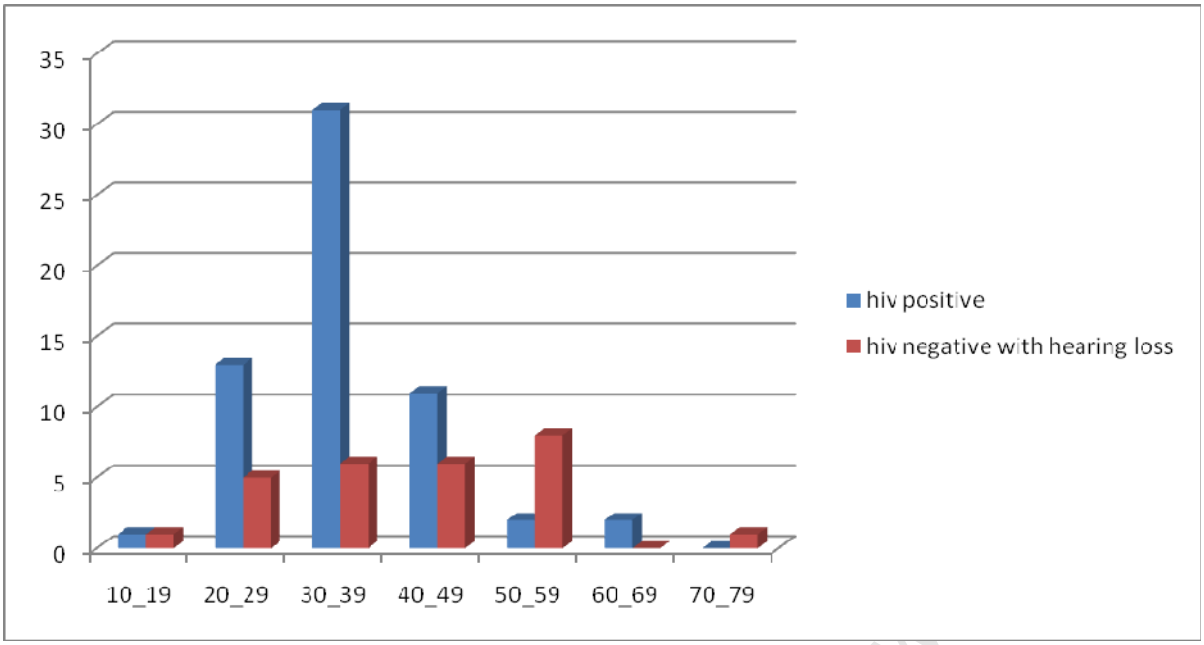

Figure 1. Graph showing age distribution of HIV positive and negative patients with ototoxic hearing loss

BROOKLYN CHEST HOSPITAL

AUDIOLOGY DEPARTMENT

TEL: 021 508 7429

CASE HISTORY: PATIENTS NO: AUDIO NO:

- HEARING LOSS NOTED
- EAR INFECTION
- TINNITUS
- PAIN
- FAMILY HISTORY OF HEARING LOSS
- NOISE EXPOSURE
- MDR-TB
- KANAMYCIN
- STREPTOMYCIN
- HIV

COMMENTS:

ASSESSMENTS RESULTS AND MANAGEMENT

DATE:

OTOSCOPY: R TYMPS: R

L L

AUDIOGRAM:

DATE:

OTOSCOPY: R TYMPS: R

L L

AUDIOGRAM:

DATE:

OTOSCOPY: R TYMPS: R

L L

Patient no.	Age	Folder no	Prev amino	Gender	Ethnicity	RVD+/-	Drug	Lab no.	H/Loss	Symmetry	Severity	Tinnitus
1	52	16333239	Y	Female	Caucasian	Neg	Kana	6112	N			N
2	38	89207286	Y	Female	Coloured	Pos	Kana	6113	Y	Asym	Severe	N
3	31	16643942	Y	Female	Somalian	Pos	Kana	6114	N			N
4	44	16253205	Y	Female	Coloured	Pos	Kana	6115	N			N
5	70	16484610	N	Male	Black	Neg	Kana	6116	Y	Asym	Severe	Y
6	37	16486953	Y	Male	Black	Pos	Kana	6117	Y	Sym	Severe	N
7	48	81195620	Y	Male	Coloured	Neg	Kana	6174	Y	Sym	Moderate	N
8	31	15246630	Y	Male	Black	Pos	Kana	6175	N			N
9	57	58446535	Y	Female	Coloured	Neg	Kana	6184	N			N
10	35	16902652	Y	Female	Black	Pos	Kana	6185	N			N
11	42	17034950	N	Female	Coloured	Neg	kana	6186	Y	Sym	Moderate	N
12	41	156624	Y	Female	Black	Pos	Kana	6187	N			N
13	44	69324200	Y	Male	Coloured	unknown	Kana	6196	N			N
14	19	43503002	Y	Male	Coloured	Neg	Kana	67.03	N			N
15	25	75053785	Y	Female	Coloured	Neg	Kana	68.18	Y	Sym	Mild	N
16	37	28460780	Y	Male	Black	Pos	Kana	68.19	N			N
17	37	28314813	N	Female	Coloured	Neg	Kana	68.2	Y	Sym	Profound	N
18	57	78937422	Y	Male	Coloured	Neg	Kana	68.23	Y	Sym	Moderate	Y
19	33	53180063	Y	Female	Coloured	Neg	Kana	F_SAM	N			N
20	22	25758483	Y	Female	Coloured	Neg	Kana	68.4	N			N
21	37	28487247	Y	Female	Black	Pos	Kana	68.42	N			N
22	70	12021143	Y	Male	Coloured	Neg	Kana	68.41	N			N
23	25	58320912	Y	Male	Coloured	Neg	Kana	68.43	Y	Sym	Mild	N
24	51	52915287	Y	Male	Cape Malay	Neg	Kana	68.55	Y	Sym	Moderate	Y
25	40	24751406	Y	Male	Coloured	Neg	Kana	68.56	Y	Sym	Severe	N
26	22	16764375	Y	Male	Black	Neg	Kana	68.62	N			N
27	25	29260171	Y	Female	Black	Pos	Kana	68.63	N			N
28	27	17793951	Y	Female	Cape Malay	Neg	Kana	68.75	N			N
29	30	84862549	N	Female	Black	Pos	Kana	68.76	Y	Sym	Severe	N
30	31	26857516	Y	Female	Black	Pos	Kana	68.77	N			N
31	33	13605779	Y	Female	Black	Pos	Kana	68.78	N			N
32	32	19624139	N	Female	Black	Pos	Kana	B_SON	Y	Sym	Severe	N
33	29	29150133	N	Female	Black	Neg	Kana	P_SIC	Y	Sym	Mild	Y
34	54	23591084	Y	Male	Caucasian	Neg	Strep	69.06	Y	Sym	Profound	Y
35	31	30037121	Y	Male	Black	Neg	Kana	69.07	N			N
36	27	29814753	Y	Male	Black	Neg	Kana	69.08	N			N
37	39	17362666	Y	Female	Coloured	Pos	Kana	69.09	N			N
38	39	86196094	Y	Male	Coloured	Neg	Kana	69.1	N			Y
39	24	65243156	Y	Female	Black	Neg	Kana	69.48	N			N
40	26	20378303	N	Male	Black	Pos	Kana	69.49	Y	Sym	Severe	N
41	23	76562214	Y	Male	Coloured	Neg	Kana	69.5	N			N
42	29	29941242	Y	Male	Coloured	Neg	Kana	69.51	N			N
43	58	24752701	N	Male	Coloured	Neg	Kana	69.52	Y	Sym	Severe	Y
44	27	62758032	Y	Male	Coloured	Neg	Kana	69.69	N			N
45	29	13771688	N	Female	Black	Pos	Kana	69.7	Y	Sym	Moderate	N
46	31	56674807	Y	Female	Coloured	Pos	Kana	69.71	N			N
47	39	79363990	Y	Male	Coloured	Neg	Kana	69.87	Y	Sym		N
48	44	57168296	Y	Male	Coloured	Neg	Kana	69.88	Y	Sym	Severe	N
49	20	30054134	Y	Female	Coloured	Neg	Kana	69.89	Y	Sym	Severe	Y
50	41	29595469	Y	Female	Coloured	Neg	Kana	69.9	N			N

Patient no.	Age	Folder no	Prev amino	Gender	Ethnicity	RVD+/-	Drug	Lab no.	H/Loss	Symmetry	Severity	Tinnitus
51	37	17510421	N	Female	Coloured	Pos	Kana	69.91	Y	Sym	Mild	N
52	37	20675930	Y	Male	Black	Pos	Strep	70.06	N			N
53	41	30226765	Y	Male	Coloured	Neg	Kana	70.07	N			N
54	32	56534019	Y	Female	Cape Malay	Neg	Kana	70.08	Y	Sym	Severe	N
55	31	30869507	Y	Female	Black	Pos	Kana	70.66	N			N
56	25	66948951	Y	Female	Black	unknown	Kana	70.67	N			N
57	33	30879746	Y	Male	Black	Neg	Kana	70.68	Y	Asym	Severe	N
58	55	28558674	Y	Female	Coloured	Neg	Kana	70.69	Y	Asym	Severe	N
59	40	77064087	Y	Female	Coloured	Neg	Kana	71.01	N			N
60	50	20026613	Y	Female	Black	Neg	Kana	71.02	N			N
61	22	16917783	Y	Female	Black	Pos	Kana	71.03	Y	Sym	Mild	N
62	42	70244900	Y	Male	Coloured	Neg	Kana	71.04	Y	Sym	Severe	N
63	26	31595341	Y	Male	Black	Neg	Strep	71.61	N			N
64	29	23765878	Y	Female	Coloured	Pos	Kana	71.62	Y	Sym	Moderate	N
65	38	31229610	Y	Male	Black	Pos	Strep	71.63	Y	Asym	Mild	Y
66	24	70037189	Y	Male	Coloured	Pos	Kana	71.64	Y	Asym	Moderate	N
67	38	18935635	Y	Male	Black	Pos	Kana	71.65	Y	Sym	Moderate	N
68	41	32615817	Y	Female	Coloured	Pos	Kana	71.78	Y	Sym	Mild	Y
69	21	69497519	Y	Male	Coloured	Neg	Kana	72.14	N			N
70	45	32617151	Y	Female	Coloured	Neg	Kana	72.15	Y	Sym	Severe	N
71	26	70124292	N	Female	Coloured	Neg	Strep	72.16	Y	Sym	Profound	Y
72	38	29434164	Y	Female	Black	Pos	Kana	72.17	Y	Sym	Severe	N
73	28	32736530	Y	Female	Coloured	Neg	Kana	72.18	Y	Sym	Severe	Y
74	30	59654517	Y	Male	Coloured	Neg	Kana	72.19	N			N
75	35	88298567	Y	Female	Coloured	Neg	Kana	72.27	N			N
76	31	33645045	N	Female	Black	Neg	Kana	72.28	Y	Asym	Severe	N
77	32	19266204	Y	Female	Black	Pos	Capr	72.42	Y	Asym	Moderate	Y
78	48	30648810	Y	Male	Black	Pos	Kana	72.66	Y	Asym	Mild	N
79	47	52882917	Y	Female	Black	Pos	Kana	72.67	Y	Asym	Moderate	Y
80	31	55893721	Y	Female	Coloured	Pos	Kana	72.71	Y	Sym	Moderate	N
81	32	34364182	Y	Male	Black	Neg	Kana	72.72	Y	Sym	Profound	N
82	43	19349174	Y	Female	Black	Pos	Kana	72.85	Y	Sym	Moderate	N
83	21	20044178	Y	Female	Coloured	Neg	Kana	72.86	N			N
84	34	19547538	Y	Male	Black	Pos	Kana	73.24	Y	Sym	Profound	N
85	44	34678003	Y	Female	Coloured	Neg	Kana	73.47	N			N
86	25	71399430	Y	Male	Coloured	Neg	Kana	73.48	N			N
87	36	60063781	Y	Male	Black	Pos	Capr	73.49	N			N
88	30	13680426	Y	Female	Black	Pos	Kana	73.5	N			N
89	19	80777030	Y	Male	Coloured	Neg	Kana	73.74	N			N
90	30	41578790	N	Female	Black	Pos	Kana	73.83	Y	Sym	Profound	N
91	19	32526048	Y	Female	Coloured	Neg	Strep	73.84	N			N
92	35	16401499	Y	Female	Black	Pos	Kana	73.85	N			N
93	32	28066231	Y	Male	Black	Pos	Kana	73.86	Y	Asym	Mild	N
94	27	18011791	N	Female	Black	Pos	Kana	74.07	Y	Asym	Mild	N
95	54	69370567	Y	Female	Coloured	Pos	Kana	74.08	Y	Asym	Severe	N
96	18	37667680	Y	Female	Black	Pos	Kana	74.09	N			N
97	51	69018687	Y	Female	Coloured	Neg	Kana	74.1	Y	Sym	Severe	N
98	21	16936403	Y	Female	Coloured	Neg	Kana	75.3	Y	Asym	Mild	N
99	28	38690541	Y	Female	Coloured	Pos	Kana	75.31	N			N
100	51	74980319	Y	Female	Coloured	Neg	Kana	76.28	Y	Sym	Profound	N
101	40	70515416	Y	Female	Coloured	Pos	Kana	76.29	Y	Asym	Moderate	Y

Patient no.	Age	Folder no	Prev amino	Gender	Ethnicity	RVD+/-	Drug	Lab no.	H/Loss	Symmetry	Severity	Tinnitus
102	27	45273349	N	Female	Black	Pos	Kana	81.32	Y	Sym	Severe	N
103	23	78898632	Y	Female	Coloured	Pos	Kana	81.71	N			N
104	14	46272654	Y	Female	Coloured	Neg	Amik	81.91	Y	Asym	Severe	N
105	20	15459753	Y	Female	Black	Pos	Kana	81.92	N			N
106	20	46113668	Y	Female	Black	Neg	Amik	81.93	N			N
107	43	38734596	Y	Female	Black	Pos	Kana	82.83	N			N
108	26	40091282	Y	Female	Black	Pos	Kana	83.65	Y	Sym	Profound	N
109	19	46074472	Y	Female	Black	Neg	Kana	83.66	N			N
110	38	33249012	Y	Female	Coloured	Neg	Kana	83.94	N			Y
111	25	22411607	Y	Female	Black	Pos	Kana	83.95	Y	Sym	Profound	N
112	33	56549058	Y	Female	Coloured	Neg	Kana	83.96	N			N
113	34	41974205	Y	Female	Black	Pos	Kana	84.69	Y	Sym	Severe	N
114	18	1.04E+08	Y	Female	Coloured	Pos	Kana	84.93	Y	Sym	Moderate	N
115	41	54545215	Y	Female	Coloured	Neg	Kana	80.99	N			N
116	28	62413554	Y	Male	Black	Neg	Kana		N			N
117	37	82668062	Y	Female	Coloured	Pos	Kana		Y	Asym	Moderate	N
118	33	20822052	Y	Male	Black	Pos	Kana		Y	Sym	Profound	N
119	34	19842756	Y	Male	Black	Pos	Kana		Y	Sym	Severe	N
120	32	43808914	Y	Male	Black	Pos	Kana		Y	Sym	Profound	N
121	40	19106160	N	Male	Black	Pos	Kana		Y	Sym	Severe	N
122	23	45288230	Y	Female	Coloured	Neg	Kana		N			N
123	31	88849922	Y	male	Black	Pos	Kana		Y	Sym	Profound	N
124	56	26993303	Y	Female	Black	Pos	Kana		N			N
125	40	23050750	Y	Male	Black	NEG	Kana		N			N
126	31	44621555	N	Female	Coloured	Pos	Kana		Y	Sym	Mild	N
127	22	71163133	Y	Male	Black	Neg	Kana		N			N
128	36	30912576	Y	Female	Coloured	Pos	Kana		Y	Asym	Severe	N
129	35	70122445	Y	Female	Coloured	Pos	Kana		N	Sym	Mild	N
130	23	35449933	Y	Male	Black	Pos	Kana		Y	Sym	Severe	N
131	48	46001665	N	Female	Black	Pos	Kana		Y	Sym	Mild	N
132	31	88849922	Y	Male	Black	Pos	Kana		Y	Sym	Severe	N
133	41	8444062	Y	Female	Black	Pos	Kana		N			N
134	45	10448492	N	Female	Black	Pos	Kana		Y	Sym	Profound	N
135	14	88630348	Y	Female	Coloured	Neg	Kana		N			N
136	70	38342317	Y	Male	Black	Pos	Kana		Y	Sym	Profound	N
137	35	23581847	Y	Female	Coloured	Pos	Kana		Y	Sym		N
138	52	39818455	Y	Female	Black	Pos	Kana		Y	Asym	Profound	N
139	34	29165420	Y	Female	Black	Pos	Kana		Y	Sym	Severe	N
140	21	26423566	Y	Female	Black	Neg	Kana		Y	Sym	Profound	N
141	34	82668062	N	Male	Black	Pos	Kana		Y	Asym	Severe	N
142	22	39343793	Y	Female	Coloured	Pos	Kana		Y	Sym	Severe	N
143	21	46625786	Y	Female	Black	Pos	Kana		Y	Asym	Severe	N
144	15	46155404	N	Female	Coloured	Pos	Kana		Y	Sym	Profound	N
145	63	48517031	Y	Female	Black	Pos	Kana		Y	Sym	Severe	N
146	38	43752724	Y	Male	Black	Pos	Kana		Y	Sym	Severe	N
147	24	86786324	Y	Male	Black	Pos	Kana		Y	Asym	Severe	N
148	14	1.04E+08	Y	Female	Black	Neg	Kana		N			N
149	50	35924034	N	Female	Coloured	Pos	Kana		Y	Sym	Profound	N
150	38	12744587	Y	Male	Coloured	Pos	Kana		Y	Sym	Severe	N
151	22	20561551	Y	Female	Black	Pos	Kana		Y	Asym	Severe	N
152	53	24567820	Y	Female	Coloured	Pos	Kana		Y	Sym	Profound	N
153	33	34577287	N	Female	Black	Pos	Kana		N			N

Enquiries : Dr B Patel
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E-mail : bpatel@pgwc.gov.za
Reference : Research
Date : 11 October 2006

Departement van Gesondheid
Department of Health
ISebe lezeMoilo

Dr Zubair Doolarkhan & Co-investigators

Dear Dr Doolarkhan

RESEARCH: OTOTOXICITY STUDY

Your recent letter to the hospital refers.

You are hereby granted permission to proceed with your research.

Please note the following:-

a) Your research may not interfere with normal patient care.
b) Hospital staff may not be asked to assist in the research.
c) No hospital consumables and stationery may be used.
d) Please introduce yourself to the person in charge of an area before commencing.

I would like to wish you every success with your project.

Yours truly

DR B PATEL
For CHIEF EXECUTIVE OFFICER

BP/em 11/10/06

Groote Schuur Hospital
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UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
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05 June 2007

REC REF: 443/2005

Dr Z Doolarkhan
Dept of Orthorhinolaryngology
GSH

Dear Dr Doolarkhan

PROJECT TITLE: GENETICS OF AMINOGLYCOSIDE OTOTOXICITY

Thank you for submitting your study to the Research Ethics Committee for review.

I have pleasure in informing you that the Ethics Committee has **formally approved** the above mentioned study. We also note the approval letters from Brooklyn Chest, Groote Schuur and New Somerset Hospitals. Please ensure that you comply with requirements specified by the respective hospitals.

This serves to confirm that the University of Cape Town Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

The Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

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

Please quote the REC. REF in all your correspondence.

Yours sincerely

PROF M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

lemjedi

UNIVERSITEIT·STELLENBOSCH·UNIVERSITY
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19 June 2008

Dr S Bardien
Division of Molecular Biology and Human Genetics
Dept of Biomedical Science

Dear Dr Bardien

RESEARCH PROJECT: **"AN INVESTIGATION OF THE ROLE PATHOGENIC AND DISEASE PREDISPOSING GENETIC FACTORS IN MITOCHONDRIAL DNA IN DIFFERENT FORMS OF HEARING IMPAIRMENT"**
PROJECT NUMBER : N05/09/165

At a meeting of the Committee for Human Research that was held on 11 June 2008 the progress report for the abovementioned project has been approved and the study has been granted an extension **for a period of one year from this date**.

Renewal Date: 11 June 2008 **Expiry Date:** 11 June 2009

Please remember to submit progress reports in goodtime for annual renewal in the standard CHR format.

For standard CHR forms and documents please visit:
www.sun.ac.za/knowledgepartner/committees_CHR.htm

Yours faithfully

MERTRUDE DAVIDS
RESEARCH DEVELOPMENT AND SUPPORT (TYGERBERG)
Tel: +27 21 938 9207 / E-mail: mertrude@sun.ac.za

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17 February 2010 **MAILED**

Dr S Bardien
Dep Biomedical Sciences
Molecular Biology and Human Genetics

Dear Dr Bardien

"An investigation of the role of pathogenic and disease predisposing genetic factors in mitochondrial DNA in different forms of hearing impairment."

ETHICS REFERENCE NO: N05/09/165

RE : AMENDMENT

Your letter dated 11 February 2010 refers.

The Chairperson of the Health Research Ethics Committee approved the amended documentation in accordance with the authority given to him by the Committee.

Please note: This request is acceptable in this context so long as publication cannot identify sub groups especially if numbers are small.

Yours faithfully

MRS EL ROHLAND
RESEARCH DEVELOPMENT AND SUPPORT
Tel: 021 938 9677 / E-mail: elr@sun.ac.za
Fax: 021 931 3352

11 February 2010

To: **Dr Lyn Horn**
Health Research Ethics Committee
Faculty of Health Sciences
Stellenbosch University

Dear Dr Horn

re: amendment to project: **An investigation of the role of pathogenic and disease predisposing genetic factors in mitochondrial DNA in different forms of hearing impairment.** Ref no. **N05/09/165**

Your letter dated 8 February refers. My apologies if it was not clear in my letter dated 3 February 2010 that the HIV-status of patients only (the cases) and not controls has been recorded.

The purpose of my letter was to determine whether we can make this amendment to the study and use the information on the HIV-status to see if it is one of the factors that may impact on drug-induced hearing loss.

As requested, please find the 'Patient Informed consent form' attached.

Yours sincerely

Dr. Soraya Bardien
University of Stellenbosch
Molecular Biology and Human Genetics
Fourth Floor, Fisan Building
Faculty of Health Sciences
Tel: +27 -21-938-9681
email: sbardien@sun.ac.za

PARTICIPANT INFORMATION AND INFORMED CONSENT FORM

TITLE OF RESEARCH PROJECT:

An investigation of the role of pathogenic and disease predisposing genetic factors in mitochondrial DNA in different forms of hearing impairment.

ETHICS REFERENCE NUMBER: N05/09/165 (US)

INVESTIGATORS: Dr Soraya Bardien[1], Prof Greetje de Jong[1], Prof Simon Schaaf[1], Mrs Debbie Lombard[1], Dr Tashneem Harris[2], Prof Johan Fagan[2], Ms Lucretia Petersen[2], Ms Sadika Parker[1&2]

ADDRESSES:
[1] University of Stellenbosch, Tygerberg,
[2] University of Cape Town/ Groote Schuur Hospital, Observatory.

CONTACT NUMBERS: Tel: Prof Greetje de Jong **021-938-4217**
Tel: Mrs Debbie Lombard **082 428 8693**

Dear study participant

We would like to invite you to participate in a research study that involves DNA (genetic) analysis and possible long-term storage of blood or tissue specimens.

- Please take some time to read the information presented here which will explain the details of this project.
- Ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved.
- Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever.
- You are also free to **withdraw from the study** at any point, even if you do agree to take part initially.

This research study has been approved by the ethics **Committees for Human Research at Stellenbosch University and at the University of Cape Town** and it will be conducted according to international and locally accepted ethical guidelines for research, namely the Declaration of Helsinki and the South African Medical Research Council's (MRC) Guidelines on Ethics for Medical and Genetic Research.

What is DNA analysis or genetic research?
Genetic material, also called DNA, is usually obtained from a small blood sample. Occasionally other tissues may be used. DNA consists of numerous genes, strung together in long strands and found in every cell in the human body. Genes are the "blueprint" that determines what we look like and sometimes what kind of diseases we may be susceptible to. Worldwide research in this field is continuously discovering new information that may be of great benefit to future generations and also that may benefit people today, who suffer from particular diseases or conditions.

What is this research study all about?
In most of your cells there are tiny structures called *mitochondria* that are responsible for producing energy needed for various processes in the cell. It has been shown that defects in the genes in the mitochondria can cause hearing loss. Either these defects result in hearing loss on their own or sometimes they need other factors such as certain types of antibiotics to result in hearing loss. This research project will investigate

whether defects in mitochondrial genes account for a significant proportion of individuals with hearing loss in the South African population.

Why have you been invited to participate?
We would like to establish whether the genes in your mitochondria contain any defects that has resulted in hearing impairment or may predispose you/ your child to hearing loss when certain antibiotics are taken.

What procedures will be involved?
We will need to take a small sample of blood (10-20 ml which is equivalent to 2–4 teaspoons) from one of your veins, usually in the arm. We will also need to test your hearing using a standard procedure.

Are there any risks involved in genetic research?
You may experience minor pain and/or bruising at the site where blood is taken.
Some insurance companies may mistakenly assume that taking part in genetic research indicates a higher risk for disease. Thus no information about you or your family will be shared with such companies.

Are there benefits to taking part in this study & will you get told your results?
This research project is important as it may reveal some of the genetic factors involved in hearing impairment in South African populations. The results from this study may have important implications for your family members. Your personal results will be made known to your clinician **only if they indicate** that you may have a genetic predisposition for developing hearing loss and therefore may need genetic counseling by a trained genetic counselor.

How long and where will your blood be stored?
Your blood and DNA will be stored for about 5-10 years in the Division of Medical Biochemistry at the University of Stellenbosch, Faculty of Health Sciences.

Is there a chance that it will be used for other research?
Your blood will only be used for genetic research that is directly related to studies on hearing impairment. Also if the researchers wish to use your stored blood for **additional research in this field** they will be required to apply for permission to do so from the Human Research Ethics Committees at Stellenbosch University and the University of Cape Town.

If you do not wish your blood specimen to be stored after this research study is completed you will have an opportunity to request that it be discarded when you sign the consent form.

How will your confidentiality be protected?
Your identity will be kept confidential and the research staff will use a numbered code for your DNA sample. Access to your personal information will be limited to authorised scientists and clinicians only. Any scientific publications, lectures or reports resulting from the study will not identify you by name.

Will you or the researchers benefit financially from this research?
You will not be paid to take part in this study and the researchers will not benefit financially by conducting this research.

In the unlikely event that the research leads to the development of a commercial application or patent, you or your family will not receive any profits or royalties. However profits will be reinvested to supporting the cause of further research, which may bring benefits to your family or community in the future.

Thank you for taking part in this research project!

Signed at (*place*) ..... on (*date*) .....

.....
Signature of participant/ legal guardian

.....
Signature of witness

Declaration by investigator

I *(name)* ..... declare that:

- I explained the information in this document to .....
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research as discussed above.
- I did/did not use a translator. *(If a translator is used then the translator must sign the declaration below.*

Signed at (*place*) ..... on (*date*) .....

.....
Signature of investigator

.....
Signature of witness

Declaration By Translator

I *(name)* .....declare that:

- I assisted the investigator (*name*) .....

  to explain the information in this document to (*name of participant*)

  ..... using the

  language medium of Afrikaans/Xhosa.

- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.

Patient consent form

PARTICIPANT INFORMATION AND INFORMED CONSENT FORM FOR RESEARCH INVOLVING GENETIC STUDIES

TITLE OF RESEARCH PROJECT:

Genetics of Aminoglycoside Ototoxicity.

Reference Number: 443/2005

Principal Investigators: Dr Tashneem Harris ; Lucretia Petersen; Dr Soraya Bardien

Address: Department of Otolaryngology, H53 old main building, Groote Schuur Hospital,Observatory, 7925

Contact Number: 021 406 6420

Dear study participant/ legal guardian of the study participant ***(delete where not applicable)***

We would like to invite you/ your child to participate in a research study that involves DNA (genetic) analysis and possible long-term storage of blood or tissue specimens.

- Please take some time to read the information presented here which will explain the details of this project.
- Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved.
- Also, your/ your child's participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you/ your child negatively in any way whatsoever.
- You/ your child are also free to **withdraw from the study** at any point, even if you do agree to take part initially.

This research study has been approved by the **Faculty of Health Sciences Research Ethics Committee of the University of Cape Town.**

What is DNA analysis or genetic research?

Genetic material, also called DNA, is usually obtained from a small blood sample. Occasionally other tissues may be used. DNA consists of numerous genes, strung together in long strands and found in every cell in the human body. Genes are the "blueprint" that determines what we look like and sometimes what kind of diseases we may be susceptible to. Worldwide research in this field is continuously discovering new information that may be of great benefit to future generations and also that may benefit people today, who suffer from particular diseases or conditions.

What does this particular research study involve?
In most of your cells there are tiny structures called *mitochondria* that are responsible for producing energy needed for various processes in the cell. It has been shown that defects in the genes in the mitochondria can cause hearing loss. Either these defects result in hearing loss on their own or sometimes they need other factors such as certain types of antibiotics to result in hearing loss. This research project will investigate whether defects in mitochondrial genes account for a significant proportion of individuals with hearing loss in the South African population.

Why have you or your child been invited to participate?
We would like to establish whether the genes in your/ your child's mitochondria contain any defects that have resulted in hearing impairment or may predispose you/ your child to hearing loss when certain antibiotics are taken.

What procedures will be involved in this research?
We will need to take a small sample of blood (10-20 ml which is equivalent to 2–4 teaspoons) from one of your / your child's veins, usually in the arm. We may also need to test your / your child's hearing using a standard procedure.

Are there any risks involved in genetic research?
You/ your child may experience minor pain and/or bruising at the site where blood is taken. Some insurance companies may mistakenly assume that taking part in genetic research indicates a higher risk for disease. Thus no information about you or your family will be shared with such companies.

Are there any benefits to your taking part in this study and will you get told your results?
This research project is important as it may reveal some of the genetic factors involved in hearing impairment in South African populations. The results from this study may have important implications for your family members. Your/ your child's personal results will be made known to your clinician **only if they indicate** that you/ your child may have a genetic predisposition for developing hearing loss and therefore may need genetic counseling by a trained genetic counselor.

How long will your/ your child's blood be stored and where will it be stored?
Your/ your child's blood and DNA will be stored for about 5-10 years in the Division of Medical Biochemistry at the University of Stellenbosch, Faculty of Health Sciences.

If your or your child's blood is to be stored is there a chance that it will be used for other research?
Your/ your child's blood will only be used for genetic research that is directly related to studies on hearing impairment. Also if the researchers wish to use your/ your child's stored blood for **additional research in this field** they will be required to apply for permission to do so from the Human Research Ethics Committee at Stellenbosch University.

If you do not wish your/ your child's blood specimen to be stored after this research study is completed you will have an opportunity to request that it be discarded when you sign the consent form.

How will your confidentiality be protected?
Your/ your child's identity will be kept confidential and the research staff will use a numbered code for your DNA sample. Access to your personal information will be limited to authorised scientists and clinicians only. Any scientific publications, lectures or reports resulting from the study will not identify you by name.

Will you or the researchers benefit financially from this research?
You/ your child will not be paid to take part in this study and the researchers will not benefit financially by conducting this research.

In the unlikely event that the research leads to the development of a commercial application or patent you or your family will not receive any profits or royalties. However profits will be reinvested to supporting the cause of further research, which may bring benefits to your family or community in the future.

Thank you for taking part in this research project!

Declaration by participant or legal guardian (in the case of a minor)

By signing below, I .....
agree/ or give permission for my child to take part in a genetic research study entitled "An investigation of the role of pathogenic and disease predisposing genetic factors in mitochondrial DNA in different forms of hearing impairment"

I declare that:

- ☐ I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- ☐ I have had a chance to ask questions and all my questions have been adequately answered.
- ☐ I understand that taking part in this study is voluntary and I / my child have not been pressurised to take part.

(Please tick the option you choose below

- ☐ I agree that my / my child's blood or tissue sample can be stored, but I can choose to request at any time that my stored sample be destroyed. I have the right to receive confirmation that my request has been carried out.

OR

- ☐ Please destroy my/ my child's blood sample as soon as the current research project has been completed.

SOUTH AFRICAN MEDICAL JOURNAL
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All named authors must give consent to publication. Authorship should be based only on substantial contribution to: (i) conception, design, analysis and interpretation of data; (ii) drafting the article or revising it critically for important intellectual content; (iii) final approval of the version to be published. All three of these conditions must be met (Uniform requirements for manuscripts submitted to biomedical journals; www.icmje.org/index.html).

RESEARCH ETHICS COMMITTEE APPROVAL
Evidence must be provided of Research Ethics Committee approval of the research where relevant.

CONFLICT OF INTEREST
Authors must declare all sources of support for the research and any association with the product or subject that may constitute conflict of interest.

PROTECTION OF PATIENT'S RIGHTS TO PRIVACY
Identifying information should not be published in written descriptions, photographs, and pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) gives informed written consent for publication. Informed consent for this purpose requires that the patient be shown the manuscript to be published. (www.icmje.org)

ETHNIC CLASSIFICATION
Work that is based on or contains reference to ethnic classification must indicate the rationale for this.

MANUSCRIPTS

Short items are more likely to appeal to our readers and therefore to be accepted for publication.

Original articles of 3 000 words or less, with up to 6 tables or illustrations, should normally report observations or research of relevance to clinical medicine. References should preferably be limited to no more than 15.

MANUSCRIPT PREPARATION

Research articles should have a structured abstract not exceeding 250 words (50 for short reports) comprising: Objectives, Design, Setting, Subjects, Outcome measures, Results and Conclusions.

Refer to articles in recent issues for guidance on the presentation of headings and subheadings.

Abbreviations should be spelt out when first used in the text and thereafter used consistently.

Scientific measurements should be expressed in SI units except: blood pressure should be given in mmHg and haemoglobin values in g/dl.

If in doubt, refer to 'uniform requirements' above.

ILLUSTRATIONS

Figures consist of all material that cannot be set in type, such as photographs and line drawings. If any tables or illustrations submitted have been published elsewhere, the author should obtain written consent to republication from the copyright holder and the author(s).

All illustrations, figures etc. must be of high resolution/quality, preferably jpeg or equivalent but not powerpoint, and preferably attached as supplementary files.

REFERENCES

References should be inserted in the text as superior numbers and should be listed at the end of the article in numerical and not in alphabetical order.

Authors are responsible for verification of references from the original sources.

References should be set out in the Vancouver style and approved abbreviations of journal titles used; consult the List of Journals in Index Medicus for these details.

Names and initials of all authors should be given unless there are more than six, in which case the first three names should be given followed by et al. First and last page numbers

should be given.

Journal references should appear thus:

Price NC . Importance of asking about glaucoma. BMJ 1983; 286: 349-350.
Book references should be set out as follows:

Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975: 96-101.
Weinstein L, Swartz MN. Pathogenic properties of invading microorganisms. In: Sodeman WA jun, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974: 457-472.
Manuscripts accepted but not yet published can be included as references followed by (in press).

Unpublished observations and personal communications may be cited in the text, but not in the reference list

PROOFS
Proofs will be forwarded to the author before publication and if not returned within 2 weeks will be regarded as approved. Alterations at this stage are costly and may be charged to the authors.

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As part of the submission process, authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to these guidelines.

1. The submission has not been previously published, nor is it before another journal for consideration (or an explanation has been provided in Comments to the Editor).
2. The submission file is in Microsoft Word or RTF document file format.
3. When available, the URLs to access references online are provided, including those for open access versions of the reference. The URLs are ready to click (e.g., http://pkp.sfu.ca).
4. The text is single-spaced; uses a 12-point font; employs italics, rather than underlining (except with URL addresses). Figures consist of all material that cannot be set in type,

such as photographs and line drawings. If any tables or illustrations submitted have been published elsewhere, the author should obtain written consent to republication from the copyright holder and the author(s). All illustrations, figures etc. must be of high resolution/quality, preferably jpeg or equivalent but not powerpoint, and preferably attached as supplementary files.

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6. Research Ethics Committee approval was obtained for the research (this should be indicated in the text of the paper).
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