A STUDY OF

MITOCHONDRIAL A1555G MUTATION IN

AMINOGLYCOSIDE INDUCED OTOTOXICITY

By

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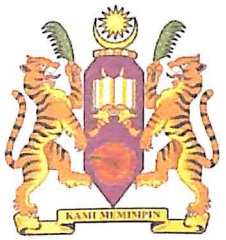

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LIST OF ABBREVIATIONS

AHA.....	American speech, language, hearing association
ATP.....	Adenosine Triphosphate
dB	Decibel
DPOAE.....	Distortion product otoacoustic emission
DNA.....	Deoxyribonucleic acid
Hz.....	Hertz
IHCs.....	Inner hair cells
Khz.....	Kilo Hertz
µl.....	Microlitres
OHCs.....	Outer hair cells
PTA.....	Pure Tone Audiometry
PCR.....	Polymerase Chain Reaction
SNHL.....	Sensorineural Hearing Loss
WHO.....	World Health Organization

DEFINITION OF TERMS

Audiometry.....	Measurement of the hearing threshold for the various frequencies of sound waves
Chromosome.....	A structure containing many genes arranged on a long strand of DNA. Each person has 23 pairs of chromosome, including a pair of sex chromosomes
DNA.....	(deoxyribonucleic acid) The chemical that makes up genes. It is composed of adenine (A), cytosine (C), guanine (G) and thymidine (T).
Gene.....	A unique sequence of DNA that serves as a specific set of instructions in the body
Mitochondria.....	Small structures within cells that provide energy for the cell. Mitochondria have their own DNA, different from the cell's DNA.
Mutation.....	A change in a gene sequence that often disrupts the function of the gene.
Nonsyndromic deafness.....	Hearing loss that occurs in the absence of other syndrome and medical problems

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ABSTRAK DALAM BAHASA MALAYSIA

Tajuk : 'KAJIAN MENGENAI MUTASI MITOKONDRIA A1555G DI DALAM PESAKIT MENGALAMI OTOTOKSIK DISEBABKAN OLEH UBAT AMINOGLIKOSID"

Pengenalan: Ototoksisiti disebabkan oleh ubat-ubatan antibiotik 'aminoglikosid' adalah di antara penyebab-penyebab utama kepada masalah kepekakan perolehan (acquired deafness) yang melibatkan sistem auditori (pendengaran) dan vestibular (keseimbangan). Kebanyakan kecacatan Vestibular dan Audiotoksisiti adalah kekal. Di dalam negara membangun seperti Malaysia, ubat aminoglikosid kerap digunakan untuk rawatan infeksi yang kecil kerana kosnya yang murah dan mudah diperolehi. Kajian yang telah dijalankan di Negara-negara Asia mendapati ubat ini boleh menyebabkan masalah kurang pendengaran kepada 10 hingga 20% pesakit yang menerimanya. (Mutasi mitokondria A1555G - adalah perubahan genetik atau kecacatan pada sesuatu gen yang dikaitkan dengan masalah pendengaran yang diwarisi dan diturunkan daripada ibu kepada bayi semasa di dalam kandungan). Mereka yang membawa genetik yang bermutasi pada mitokondria DNA (mtDNA) A1555G ini pula telah dikenalpasti mempunyai risiko yang lebih tinggi untuk mendapat masalah kurang pendengaran jika mengambil ubat aminoglikosid ini (seperti streptomycin, gentamycin) berbanding mereka yang tidak mempunyai genetik mutasi. Di dalam satu kajian di Jepun, hampir 40% daripada mereka yang mempunyai masalah kurang pendengaran sensorineural disebabkan oleh ubat aminoglikosid ini, adalah mereka yang membawa genetik yang mempunyai mutasi pada mtDNA A1555G. Melalui kajian ini, kami berharap dapat mengesan lebih awal genetik

bermutasi A1555G ini terutama di kalangan bayi baru lahir, supaya dapat mengelakkan pemberian ubat-ubatan aminoglikosid kepada mereka di masa hadapan.

Objektif: Tujuan kajian ini adalah untuk mengenalpasti prevalen genetik mutasi A1555G di dalam pesakit yang ototoksik disebabkan ubat aminoglikosid. Kami juga mengkaji kaitan diantara masalah kurang pendengaran ini dan genetik mutasi A1555G.

Metodologi: Kajian 'cross-sectional' telah dijalankan di klinik otorinolaringologi di HUSM dan juga di Pusat Kesihatan Bandar, Kota Bharu daripada Jun 2007 hingga Mei 2008. Dua puluh dua subjek ototoksik disebabkan ubat aminoglikosid dan dua puluh dua subjek tidak ototoksik selepas didedahkan kepada ubat aminoglikosid telah dipilih untuk menyertai kajian. Ototosik di buktikan oleh ujian 'pure tone audiometry' (PTA) dan 'distortion product otoacoustic emission' (DPOAE). Sampel mukosa 'buccal'(mulut) diambil daripada subjek yang sukarela melalui kaedah sapuan (swab) dan analisis genetik dilakukan.

Keputusan: Di dapati 1 subjek (4.54%) yang membawa genetik mutasi A1555G dikenalpasti mengalami masalah pendengaran selepas mengambil ubat aminoglikosid. Tiada kaitan di antara mtDNA A1555G mutasi dan ototoksik dalam kajian ini (P=0.500).

Kesimpulan: Di dalam kajian ini, kami mendapati seorang subjek yang mempunyai kurang pendengaran disebabkan ubat ototoksik membawa mtDNA mutasi A1555G. Ianya didapati tidak signifikan dari segi statistik. Ini berkemungkinan menunjukkan bahawa prevalen mtDNA mutasi A1555G di kalangan penduduk kita adalah sememangnya rendah. Namun begitu, kajian yang melibatkan lebih banyak sampel dan meliputi kawasan liputan yang lebih luas lagi perlu dijalankan bagi membuktikan keputusan kami ini.

ABSTRACT IN ENGLISH

Title: "A STUDY OF MITOCHONDRIAL A1555G MUTATION IN AMINOGLYCOSIDE INDUCED OTOTOXICITY"

Introduction: Aminoglycoside induced ototoxicity is one of the most common causes of acquired deafness, involving the auditory and vestibular system. The Vestibular and Audiotoxicity is frequently irreversible. In developing countries, eg Malaysia, aminoglycoside are more routinely used even for a minor infection, since it is relatively cheap and easily available. However, studies conducted in Asian country had proved that aminoglycoside caused 10 to 20% of hearing loss. (mitochondrial A1555G Mutation - An alteration or change, as in nature, form, or quality of a gene that maternally inherited gene during fetal life and has an association with hearing loss). People who carry the genetic mutation of mitochondrial DNA (mtDNA) A1555G, has a higher risk of having hearing loss when expose to aminoglycoside drug (eg: streptomycin, gentamycin etc) compare to them who do not carry the mutation gene. A study which was conducted in Japan, found that 40% of the patient who had hearing loss due to this drugs, also carry the mtDNA A1555G mutation. From this study, we hope that we can detect early genetic A1555G mutation, especially in newborn baby, so that prevention can be made early and precaution should be taken before prescribing aminoglycoside drugs.

Objectives: The purpose of this study is to determine the prevalence and association of mtDNA A1555G mutation in subjects with aminoglycoside-induced ototoxicity.

Methodology: A cross sectional study was carried out in Otorhinolaryngology clinic HUSM and PKB, Kota Bharu from June 2007 to May 2008. Twenty two subjects with aminoglycoside induced ototoxicity and twenty two control subjects without ototoxicity

after exposed to aminoglycosides were included in this study. Ototoxicity was confirmed by pure tone audiometry and distortion product otoacoustic emission (DPOAE). All eligible and consented patient was underwent buccal mucosa swab for further genetic analysis for mtDNA A1555G mutation.

Results: There was 1 subject (4.54%) in the aminoglycoside-induced ototoxicity group identified to have the mtDNA A1555G mutation. There was no significant association between mtDNA A1555G mutation and ototoxicity in this study (P = 0.500).

Conclusion: In our study, we found mtDNA A1555G mutation in one of our subject who had hearing loss secondary to ototoxic drug. Though, it was not significant statistically. There is a possibility that the prevalence of this mtDNA mutation is truly very low in our population. However, a further larger study with a bigger sample size and a wider area of coverage need to be done to confirm our finding.

CHAPTER 1

INTRODUCTION

CHAPTER 1: INTRODUCTION

The most common form of sensory impairment all over the world is hearing loss. About 1 in 750 children have a significant prelingual hearing impairment (Fortnum and Davis, 1997). Majority of the cases is multifactorial in origin, in which it is due to the interaction between environmental factors and genetic factors. Most of the environmental factors contributing to hearing loss can be identified, such as, trauma, exposure to loud noise, infection or ototoxicity. However in most instances, the genetic factor was still remaining unclear and may account for about 50% of prelingual hearing loss.

Several papers have suggested the hereditary factor in aminoglycoside-induced hearing loss (Donald and Sellars, 1981, Prazic *et al.*, 1964, Tsuiki and Murai, 1971, Johnsonbaugh *et al.*, 1974, Viljoen *et al.*, 1983). Tsuki and Murai, 1971 reported on 16 families with the hearing of several members were affected after administration of streptomycin. Higashi *et al.* (1989) suggested in his study that most of them were maternally inherited in which indicating mitochondrial inheritance. In 1993, several studies had proven the involvement of inherited mitochondrial DNA mutation of A1555G in patient with aminoglycoside induced hearing loss (Prezant *et al.*, 1993, Fischel-Ghodsian *et al.*, 1993, Hutchin *et al.*, 1993).

1.0 Anatomy and Physiology of Hearing

Normal hearing is dependent on the function of hair cells and the stria vascularis, which maintain the ionic gradients necessary for sound signal transduction (Fischel-Ghodsian, 2005). Both, the hair cells and stria vascularis are highly metabolically active and rich in

mitochondria. They can be easily compromised by a dysfunction in mitochondrial Adenosine Triphosphate (ATP) production as a consequent of mitochondrial deoxyribonucleic acid (mtDNA) mutation. Moreover, the hair cells of the cochlea do not replicate and tend to accumulate mutant mtDNA.

1.0.1. Organ of Corti, Outer Hair cells, Inner Hair cells

The inner ear includes the osseous labyrinth and membranous labyrinth. The osseous labyrinth is the outer layer of inner ear which consists of cochlea for hearing whereas vestibule and semicircular canal is mainly for balance. The membranous labyrinth house in the osseous labyrinth and specialized in three parts of labyrinth to form receptors of sound (organ of corti), static balance (macula) and kinetic balance (crista).

The auditory component of the inner ear is the cochlea. It contains organ of Corti which is the auditory sensory cells or "hair cell". It has highly specialized structures that respond to fluid-borne vibrations in the cochlea with a shearing vector in the hairs of some cochlear hair cells. It contains between 15,000 - 20,000 auditory nerve receptors. Each receptor has its own hair cell; the outer hair cells (OHCs) and the inner hair cells (IHCs) (Figure 1.1). The inner hair cells (IHCs) and outer hair cells (OHCs) both play active roles in the transduction of sound. Each inner hair cell is innervated by 15-20 Type I neurons, while approximately 10 outer hair cells is innervated by a single Type II neuron. Overall, there are about 50,000 neurons from the spiral ganglion innervating the cochlea; 90-95% innervates the IHCs, while the remaining 5-10% innervates the OHCs. The OHCs are more sensitive to ototoxic injury than

the inner hair cells. In mammals, the OHCs are arraalinged in three or four rows that lie more lateral to the modiolus than do the IHCs.

The stria vascularis is located along the outer wall of the scala media (Figure 1.1) and secretes the fluid in scala media or the "endolymph". They responsible for maintaining the endocochlear potential through the action of sodium-potassium-ATP pumps(Weber *et al.*, 2001).

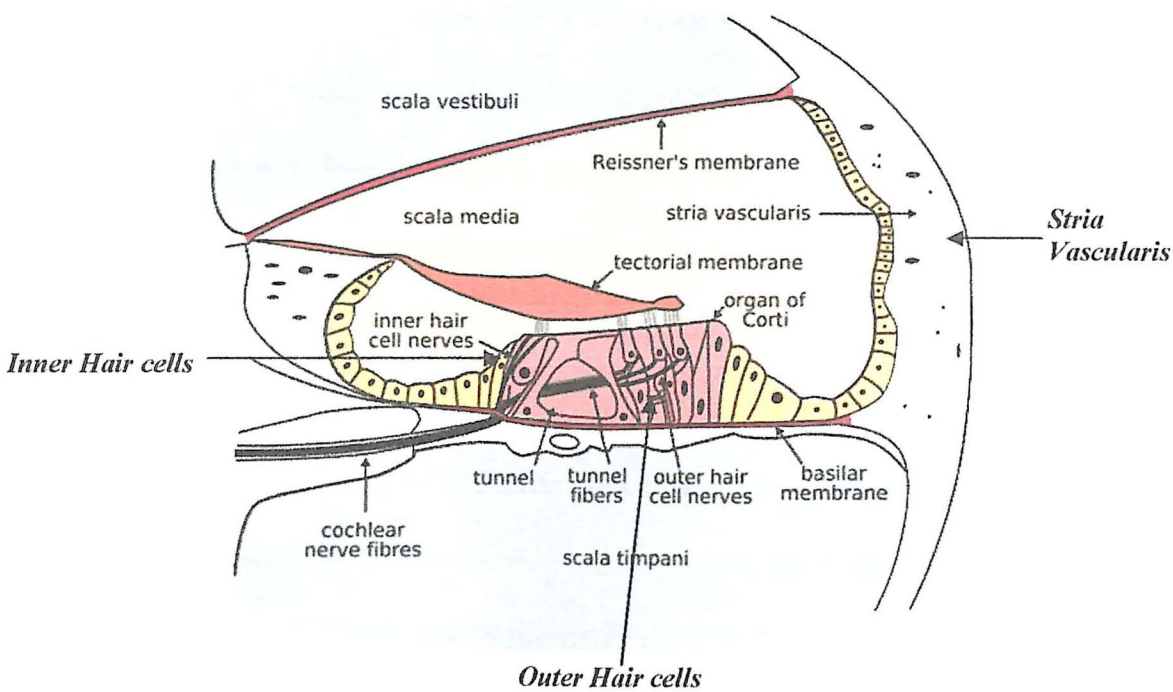

Figure 1.1: Cross section of the cochlea

Stria vascularis located on the right side (lateral). It secretes 'endolymph' into the scala media. (Adapted from http://upload.wikimedia.org/wikipedia/commons/0/0c/Cochlea-crosssection.png)

The fundamental role of the OHCs and the IHCs is to function as sensory receptors. The main function of the IHCs is to transmit sound information via afferent neurons. They transmit by transducing mechanical movements or signals into neural activity. When stimulated, the stereocilia on the IHCs move, causing a flow of electrical current to pass through the hair cells. This electrical current creates an action potentials within the connected afferent neurons.

OHCs are different in that they are actually contribute to the active mechanism of the cochlea. They are receiving mechanical signals or vibrations along the basilar membrane, and transducing this vibrations into electrochemical signals. The stereocilia found on teh OHCs are in contact with the tectorial membrane (Figure 1.2). Therefore, when the basilar membrane moves due to vibrations, the stereocilia bend. The direction in which they bend, dictates the firing rate of the auditory neurons connected to the OHCs. The bending of the stereocilia towards the basal body of the OHCs causes excitation of the hair cell. Thus, create an increase in firing rate of the auditory neurons connected to the hair cell. On the other hand, the bending of the stereocilia away from the basal body of the OHCs causes inhibition of the hair cell. Thus, lead in a decrease in firing rate of the auditory neurons connected to the hair cell. OHCs are unique in that they are able to contract and expand (electromotility). Therefore, in response to the electrical stimulations provided by the efferent nerve supply, they can alter in length, shape and stiffness. These changes influence the response of the basilar membrane to sound.

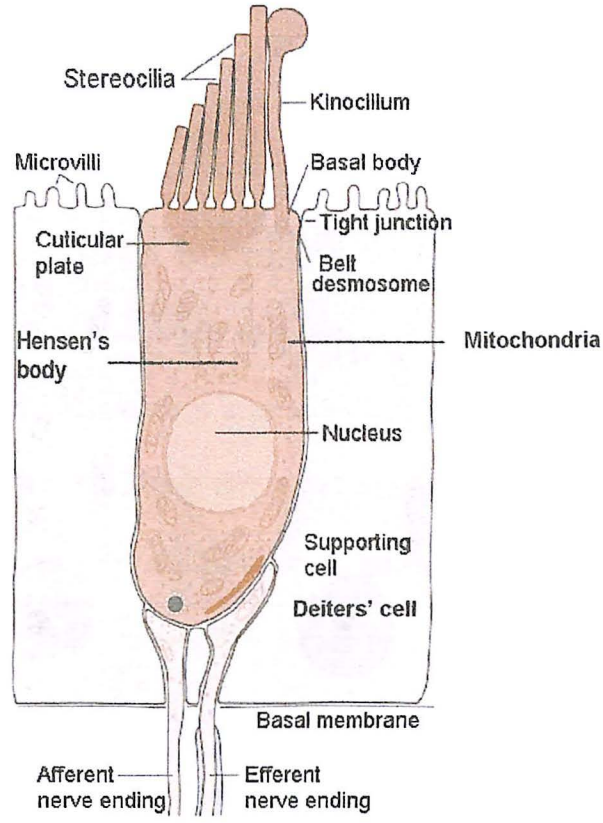

Figure 1.2: An outer hair cell (OHCs).
All hair cells have an eccentric nucleus, numerous mitochondria and basal body with their cuticular plate connected to the adjacent cells via tight junction complexes. Stereocilia of varying heights project from the surface of the cuticular plate. Various types of supporting cells, Deiters' cell, Hensen's body. Afferent and efferent nerve endings also visible on the lower part. (Adapted from http://www.mc.vanderbilt.edu/histology)

It is therefore clear that the OHCs play a major role in the active processes of the cochlea. The main function of the active mechanism is to finely tune the basilar membrane, and provide it with a high sensitivity to quiet sounds. The active mechanism is dependent on the cochlea being in good physiological condition. Though the OHCs are very sensitive organ, they are very susceptible to ototoxic damage.

1.0.2. **Physiology of Hearing**

Hearing is a complex process that consists of many steps. The ear acts to gather acoustic energy and transform it into neural stimulus which is transmitted to the brain for processing. The sound is conducted by the pinna, external auditory canal, tympanic membrane and the ossicles into the inner ear and cochlea. The tympanic membrane provides a large area to collect acoustic energy to transmit to the small area of stapes footplate; with the effective ratio of 14:1. The ossicles act as a lever mechanism which has mechanical advantages of ratio 1.3: 1. This transformer mechanism will increase the forces exerted by the stapes footplate on the oval window and further upon the cochlear fluid, while reducing the amplitude of vibration at the stapes compared to at the tympanic membrane.

In the cochlea, the perilymph (cochlear fluids) is stimulated by the mechanical energy vibrations to form a fluid wave within the cochlea. This energy vibration takes approximately 5 millisecond to travel along the length of the cochlea. As it passes the basilar membrane of the cochlear duct, the fluid wave causes the basilar membrane to move in a wave-like fashion (i.e. up and down). Maximal displacement of the basilar membrane occurs at the base of the cochlea with high frequency sounds and towards the apex with low frequency sounds. This mechanical energy is subsequently transduced to electrical or neural energy via the hair cells of the organ of Corti. The electrical potentials follow accurately the wave form and changes in intensity of the stimulating source. These are known as 'cochlear microphonics' (CM). It is thought that the cochlea is acting as a microphone, hence the term is and this CM is picked up by electrocochleography.

1.1. Ototoxicity

1.1.1. Definition

Ototoxicity is defined as damage to the cochlea or vestibular apparatus due to exposure to toxic source either from the ototoxic drug or other chemicals substances. Ototoxicity can involve either the auditory system causing cochleotoxicity and may present as hearing loss or tinnitus. It can also involve the vestibular system causing vestibulotoxicity and may presented as vertigo or giddiness (Lortholary *et al.*, 1995). The cochleotoxicity and vestibulotoxicity is frequently irreversible. The symptom can manifest during the treatment or after completion of treatment. Usually it affected the high frequencies hearing first, followed by the lower frequencies from mild to profound hearing loss. It is irreversible and always heralded by tinnitus and the patient may initially complain of tinnitus rather than hearing impairment (Scott and Griffiths, 1994).

1.1.2. Types of Ototoxic Drugs - Aminoglycoside

These are several drugs and chemicals that can damage the inner ear and causing sensorineural hearing loss, tinnitus or vertigo (Table 1.1).

Among all the ototoxic causes, aminoglycoside induced ototoxicity is known to be one of the most common causes of acquired sensorineural deafness.

Table 1.1 Types of Ototoxic Drugs and Chemicals

Ototoxic Drugs	
Aminoglycoside antibiotics	***Cytotoxic drugs***
- streptomycin	- cisplatin
- gentamicin	- carboplatin
- neomycin	
- kanamycin	***Chemicals***
- amikacin	- alcohol
- tobramycin	- tobacco
	- marijuana
Analgesics	- carbon monoxide poisoning
- aspirin	
- indomethacin	
- ibuprofen	***Others***
	- erythromycin
Diuretics	- ampicillin
- frusemide	- propanolol
	- propyl thiouracil
	- deferoxamine

Aminoglycoside

Aminoglycoside antibiotics were developed in 1944 to treat Gram-negative bacteria that were not responsive to conventional antibiotics, such as penicillin. In developed country, aminoglycoside drugs are mainly used in the treatment of hospitalized patient with aerobic gram negative bacterial infection only. However in developing countries, such as Malaysia, Indonesia, aminoglycoside are more routinely and widely used even for relatively minor infection, since it is relatively cheaper and easily available.

All aminoglycoside antibiotics display ototoxicity but vary in their preferential damage to the cochlea or vestibule. These including streptomycin, gentamicin and tobramycin that are primarily vestibulotoxic and selectively destroy type 1 cells of crista ampullaris. Streptomycin was the first known to be effective drug