

# Pharmacogenetic screening of A1555G and C1494T mitochondrial mutations and the use of ototoxic drugs among Jordanians

A. YEHYA<sup>1</sup>, B. AL-TRAD<sup>2</sup>, M. BANI-HMOUD<sup>2</sup>, A.M. RABABA'H<sup>3</sup>

<sup>1</sup>Department of Clinical Pharmacy and Pharmacy Practice, Faculty of Pharmacy, Yarmouk University, Irbid, Jordan.

<sup>2</sup>Department of Biological Sciences, Yarmouk University, Irbid, Jordan

<sup>3</sup>Department of Clinical Pharmacy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan

**Abstract.** – **OBJECTIVE:** Hearing loss may impact an individual's psychosocial behaviors and lead to cognitive decline. The goals of this study were to describe the frequency of nonsyndromic hearing loss (NSHL) among Jordanian patients with regular exposure to ototoxic drugs, perform screening for A1555G and C1494T mitochondrial mutations (12S rRNA gene) and identify predictors of hearing loss.

**MATERIALS AND METHODS:** A cross-sectional study was conducted in which medical records were reviewed to record the pattern of ototoxic drug use among participants. The pure tone audiometry (PTA) test was used to assess hearing performance. Direct sequencing was performed following PCR amplification to screen for mitochondrial mutations of interest.

**RESULTS:** One hundred sixty-two patients reported regular use of ototoxic drug(s); sixty-five percent of them suffered from NSHL, mostly of mild-moderate severity. No A1555G or C1494T mutation was detected in any participant. Aspirin (82%) was the most commonly used ototoxic drug, followed by loop diuretics (77%) and aminoglycosides (57%). Advanced age, more comorbidities and more ototoxic drugs taken increased the likelihood of hearing loss ( $p < 0.01$ ).

**CONCLUSIONS:** Hearing loss is prevalent among Jordanian patients treated with ototoxic drugs. Early intervention and management services for this population remain critical needs.

*Key Words:*

Pharmacogenetics, Pharmacology, Mitochondrial mutation, Ototoxic drugs, Hearing loss, Jordan.

cellular dysfunction of the tissue of the inner ear or auditory nerve occurs<sup>1</sup>. Drug-induced ototoxicity can cause temporary or permanent hearing loss depending on several factors and manifests as auditory (cochlear) damage (e.g., tinnitus) and/or vestibular damage (e.g., vertigo)<sup>2</sup>. The tendency to experience hearing impairment after treatment with ototoxic drugs varies considerably, and several factors, including advanced age, prolonged exposure, the use of multiple ototoxic drugs and genetic susceptibility, have been found to increase the risk of drug-induced ototoxicity<sup>3</sup>. Mitochondrial DNA (mtDNA) mutations in the 12S rRNA gene have been identified as associated with SNHL. Specifically, increased prevalence of the A1555G and C1494T mutations in SNHL has been reported in American, European, Asian and Arab populations<sup>4,5</sup>. There are more than 200 pharmacological drugs that induce ototoxicity, and currently, no treatment for ototoxicity is available. Ototoxic drugs damage the hair cells of the inner ear by increasing the formation of reactive oxygen species (ROS). Protective strategies include the development of antioxidant therapies to inhibit apoptosis and to induce neutralization of ROS<sup>6</sup>. Hearing impairment may negatively impact patients' psychosocial behaviors and lead to cognitive decline<sup>7</sup>. Considering that ototoxicity is not considered a life-threatening condition, it can be underestimated and is often overlooked by healthcare professionals. It is important to apply rigorous protocols to evaluate patients' hearing performance and to monitor their use of ototoxic drugs. In this study, the frequency of nonsyndromic hearing loss (NSHL) among Jordanian patients with exposure to ototoxic drugs was

## Introduction

Exposure to ototoxic drugs can be associated with sensorineural hearing loss (SNHL), where

determined, and then screening for the A1555G and C1494T mitochondrial mutations (12S rRNA gene) was conducted. Finally, predictors of hearing loss were identified.

## Materials and Methods

### Study Participants and Setting

The cross-sectional study design included a sample of Jordanian outpatients attending King Abdullah University Hospital (KAUH), Irbid, Jordan, between January and February 2021. Medical records were reviewed to identify patients with regular use of one or more of the following ototoxic drugs: aspirin (an NSAID), cisplatin (a platinum-based chemotherapy drug), aminoglycosides (administered via IV), and loop diuretics<sup>8,9</sup>. Demographics and clinical characteristics of the participants were recorded. All participants were asked to sign an informed consent form. The study protocol was approved by the Institutional Review Board (IRB) (Ref. number 2281-1-13).

### Hearing Loss Assessment

The pure tone audiometry (PTA) test was performed for each participant. The results were interpreted according to the four grades describing hearing impairment (none, mild, moderate, profound) ([Supplementary Table I](#)).

### Analysis of Mitochondrial DNA Mutations

#### DNA Extraction

A venous blood sample (1-1.5 milliliters) was collected from each participant in EDTA-coated tubes and then stored at -20°C. DNA was extracted using the G-spin™ Total DNA Extraction Mini Kit (iNtRON Biotechnology, Inc., Korea) according to the manufacturer's instructions. DNA samples were stored at -20°C for further analysis. DNA quantity and quality were assessed using a Nano Drop spectrophotometer at  $\lambda = 260$  nm and by conducting 0.8% agarose gel electrophoresis (V = 100 volts) for 30 minutes.

#### DNA Amplification and Sequencing

A fragment of 339 bp (to detect MT-RNR1 rs267606617) was amplified directly using polymerase chain reaction (PCR) with the forward primer -5'-GCT CAG CCT ATA TAC CGC CAT CTT CAG CAA-3' and the reverse primer -5'-

TTT CCA GTA CAC TTA CCA TGT TAC GAC TGG-3'. PCR was performed under the following conditions: initial denaturation at 95°C for 7 minutes; 40 cycles of denaturation at 95°C for 1 minute, annealing at 64.1°C for 1 minute and extension at 72°C for 1 minute; and a final extension step at 72°C for 7 minutes. The reaction was stopped after 5 minutes at 4°C. PCR was performed using a total volume of 30  $\mu$ l comprising 2  $\mu$ l of genomic DNA, 0.5  $\mu$ l of each primer, 6  $\mu$ l of 5xFIREPol® Master Mix (Solis Biodyne, Tartu Science Park, Baltic States) and nuclease-free water up to 30  $\mu$ l. The PCR products (339 bp) were visualized using a UV box transilluminator via 1.5% agarose gel electrophoresis and sent for sequencing to Macrogen, Inc. (Korea).

### Statistical Analysis

In addition to degree of hearing loss, demographic and clinical characteristics were described as categories and presented as percentages. Pearson correlation (r) coefficients were calculated to describe the relationships between the variables assessed in the thesis and hearing loss. Data were analyzed using Statistical Package for Social Sciences (SPSS version 21, Chicago, IL, USA).

## Results

### Participants' Characteristics and Hearing Loss Assessment

A total of 162 patients participated in the study. The demographics and clinical characteristics of the participants are shown in Table I. Approximately 42% of the participants were female; 43%

**Table I.** Demographics and clinical characteristics of participants.

Variable	Percent (%)	N
Gender		
Male	58.0	94
Female	41.9	68
Age group		
40-50	43.2	70
51-60	33.9	55
> 60	22.8	37
Comorbidity		
Cancer	25.0%	41
CVD	19.5%	39
DM	8.5%	27
Multiple	47%	55

were between 40 and 50 years old, and 57% were older than 50 years old. Regarding the clinical characteristics, 25% of the participants were cancer patients, 19.5% of them had cardiovascular disease, and 47% had multiple comorbidities. The results of the PTA test showed that 24% of participants had normal hearing, whereas 76% had NSHL, stratified into mild (37.6%), moderate (25.9%), severe (10.4%) and profound (1.8%) cases (Figure 1).

### Screening for Mitochondrial DNA Mutations

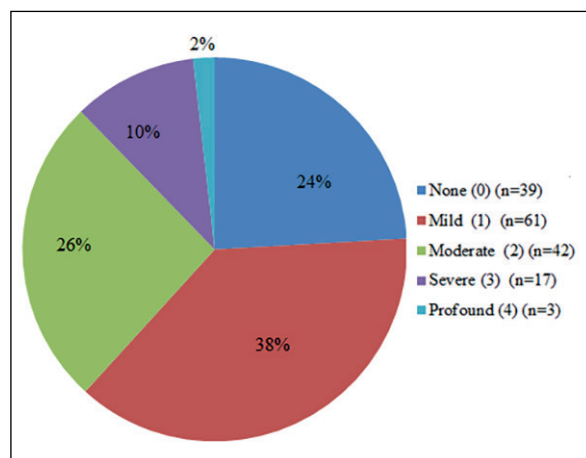
Direct sequencing was performed following PCR amplification of a 339 bp fragment of the 12S rRNA gene to screen reportedly related mitochondrial mutations (Figure 2). The results revealed that none of the participants carried the A1555G or C1494T mutation (Figure 3).

### Usage Pattern of Ototoxic Drugs

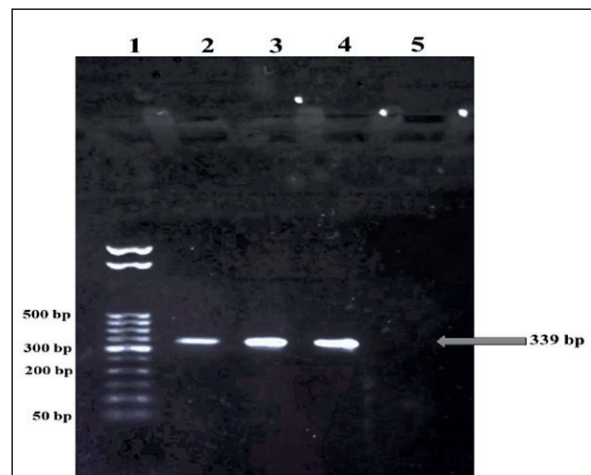
The average number of ototoxic drugs used among participants was 1.85. Concomitant use of three and four ototoxic medications was identified in 37% and 22% of the participants, respectively (Figure 4A). The most common ototoxic medication taken by older adults was aspirin (82%), followed by loop diuretics (77%) and aminoglycosides (57%) (Figure 4B).

### Predictors of Hearing Loss

The Pearson correlation analysis revealed a positive correlation between age and the likelihood of hearing loss ( $p < 0.01$ ). Similarly, participants with multiple diseases were more likely



**Figure 1.** Participants stratified according to the grade of hearing loss.

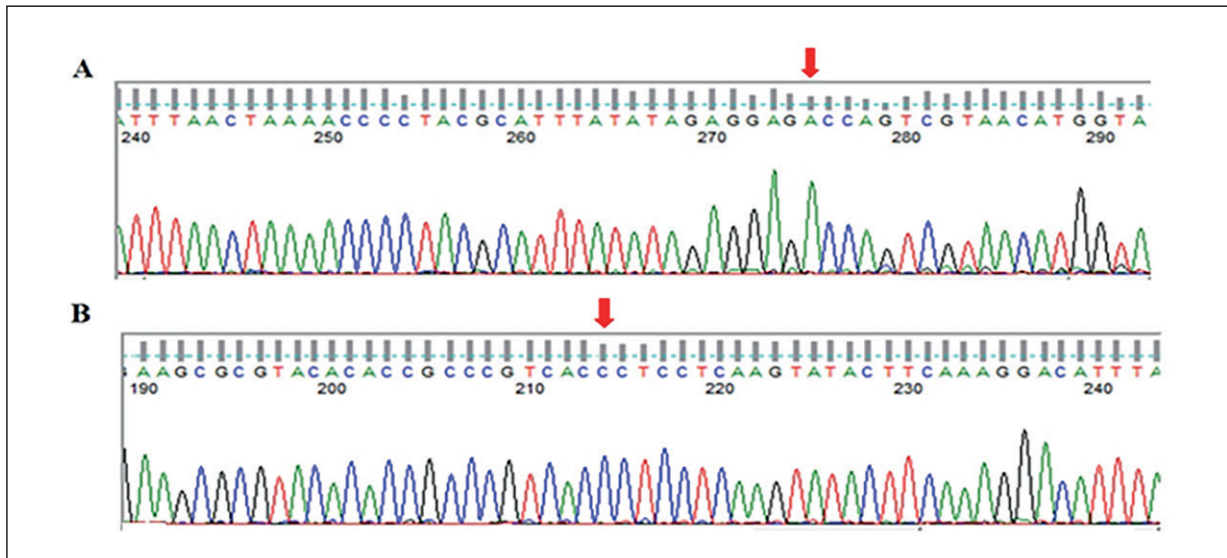


**Figure 2.** Results of PCR analysis. Lane 1 shows the 50 bp DNA ladder. A 339 bp PCR fragment is shown in each of lanes 2-4, and the negative control is shown in lane 5.

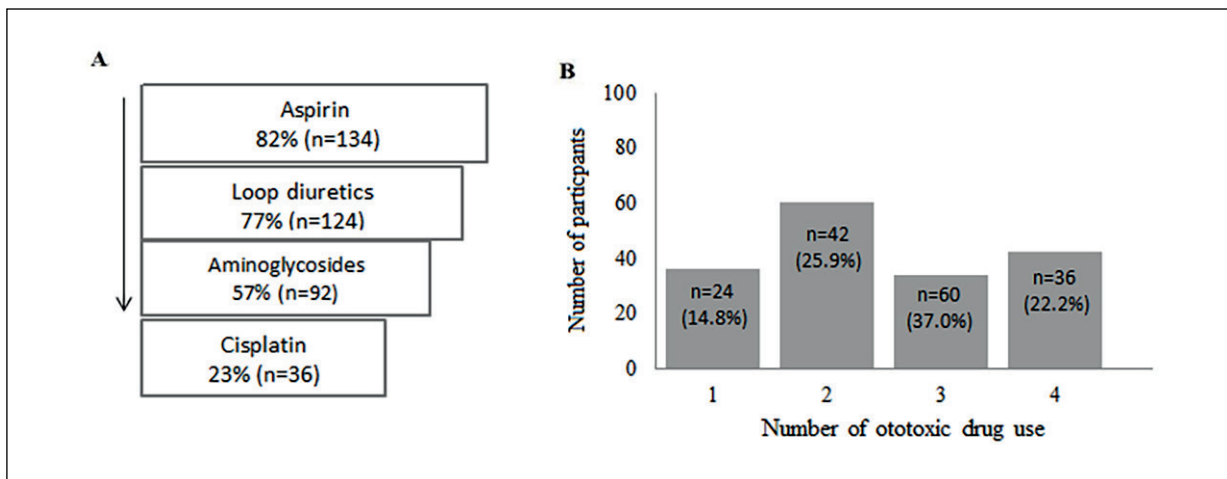
than those without to suffer from hearing loss ( $p < 0.01$ ). Additionally, the tendency to have multiple diseases was higher among female patients; however, only a weak correlation was found between female sex and hearing loss. Furthermore, the number of ototoxic drugs was positively correlated with the number of diseases and the risk of hearing loss ( $p < 0.01$ ) (Figure 5).

## Discussion

Adverse drug reactions (ADRs) are a broad term used to refer to the unwanted potential effects of a drug during treatment. The incidence and severity of ADRs vary among patients depending on multiple factors, including drug class and genetic susceptibility. This study revealed that 75% of the participants with exposure to ototoxic drugs suffered from NSHL, mostly of mild-moderate severity. NSHL can be inherited in an autosomal recessive manner (75-80%) or an autosomal dominant manner (20-25%); in rare instances, NSHL can exhibit an X-linked or mitochondrial pattern of inheritance (1-2%)<sup>10</sup>. In this study, neither the A1555G nor C1494T mtDNA mutation was detected in patients with NSHL or in those with normal hearing. A lack of both mutations was similarly reported among Tunisian<sup>11</sup> and Iranian patients and controls<sup>12</sup>. The absence of the A1555G mutation was reported among Qataris<sup>13</sup>, whereas a frequency of 1.3% was reported among Egyptians<sup>4</sup>. These findings indicate that



**Figure 3.** Representative chromatogram showing the sequence of mitochondrial DNA amplicon. The sequence of the genomic DNA amplicon RNR1 rs267606617 for randomly selected samples. Arrows show the sites of the (A) A1555G mutation and (B) C1494T mutation.



**Figure 4.** Ototoxic drug use among participants. (A) pattern of ototoxic drug use, (B) frequency of ototoxic drug use.

	Gender	Age	No. diseases	No. ototoxic drugs	Hearing loss
Gender	1				
Age	1	1			
No. diseases	-.434**	0.303	1		
No. ototoxic drugs	-0.024	0.872**	0.673**	1	
Hearing loss	-0.266	0.880**	0.868**	0.790**	1

\*\* Correlation is significant at the 0.01 level (2-tailed).

**Figure 5.** Pearson correlation analysis.

the screened mutations might be uncommon in Arab populations. The A1555G mutation has reported frequencies of 0.6% in the United States<sup>14</sup> and 2% in Brazil<sup>15</sup>. Higher frequencies have been reported in Asian countries, namely, 3.96% and 3.45% among Chinese<sup>16</sup> and Japanese patients<sup>17</sup>, respectively.

The most common ototoxic drug used was aspirin, followed by loop diuretics and aminoglycosides. In Jordan, noncommunicable diseases (NCDs) are the leading cause of death; more than one-third of NCDs are cardiovascular diseases, and 14% are cancers<sup>18</sup>. The most recent guidelines for the primary prevention of cardiovascular events recommend aspirin therapy (75–325 mg/d), as it may reduce the relative risk of a first heart attack or stroke, and one-third of Americans 40 years or older take aspirin daily<sup>19,20</sup>. The use of ototoxic drugs should be decided by making a risk/benefit assessment, but this is not straightforward. For example, loop diuretics (e.g., furosemide), which are ototoxic drugs, are considered to be the most potent diuretics and are widely used in the treatment of pulmonary and systemic edema. Thiazide diuretics are not ototoxic, but they are considered less potent than loop diuretics and are ineffective when the glomerular filtration rate falls below approximately 30 ml/min<sup>21</sup>. Moreover, in this study, a positive correlation was found between hearing loss and older age ( $p < 0.01$ ). In addition, our results showed that suffering from multiple comorbidities and being exposed to several ototoxic drugs increased the risk of hearing loss ( $p < 0.01$ ). These results highlight the importance of frequent performance of the hearing assessment test. Most guidelines recommend screening for hearing loss beginning at age 50, assuming a certain level of health and no known hearing problems. Testing should begin at a younger age in individuals at high risk<sup>22</sup>. Various otoprotective agents under development may have action in reducing the toxicity of ototoxic medications, including vitamin E, N-acetyl cysteine and D-methionine<sup>23</sup>. The key role of antioxidant molecules is to detoxify or prevent the formation of free radicals such as ROS<sup>24</sup>. The currently available options to improve hearing ability are the use of hearing aids and cochlear implantation<sup>25,26</sup>. In Jordan, hearing aid devices are covered by medical insurance, and the PTA test is performed free of charge in a number of public hospitals. It is important to fight the stigma associated with hearing loss and encourage frequent hearing testing, as the

majority of patients undergo PTA testing only after referral from a physician. In addition, it is necessary to monitor the consequences associated with untreated hearing loss, which mainly include psychosocial behaviors and cognitive decline<sup>27,28</sup>. The main limitation of this study is the small sample size; future studies with a multicenter design would allow a higher rate of patient enrollment. The main novelties of the present work include the following: First, in this study, both mitochondrial mutations and the pattern of ototoxic drug usage were investigated, which allowed the identification of several predictors of hearing loss among Jordanian patients. Second, this study provides the first overview of ototoxic ADRs among Jordanian patients, which may guide the development of more rigorous protocols for hearing loss performance tests.

## Conclusions

Hearing loss is prevalent among Jordanian patients treated with ototoxic drugs. Early intervention and management services for this population remain critical needs.

---

### Conflict of Interest

The Authors declare that they have no conflict of interests.

---

### Declarations Ethics Approval and Consent to Participate

All participants were asked to sign an informed consent form. The study protocol was approved by the Institutional Review Board (IRB) (Ref. number 2281-1-13) and was conducted in accordance with the Declaration of Helsinki and good clinical practice guidelines.

---

### Acknowledgements

The authors would like to thank all the patients who participated in this study.

---

### Funding

This study was funded by the Scientific Research and Graduate Studies Deanship-Yarmouk University (Jordan) (Ref. no. 201721). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

---

### ORCID ID

Alaa Yehya <https://orcid.org/0000-0001-5267-6406>.

## References

- 1) Yorgason JG, Fayad JN, Kalinec F. Understanding drug ototoxicity: molecular insights for prevention and clinical management. *Expert Opin Drug Saf* 2006; 5: 383-399.
- 2) Ganesan P, Schmiedge J, Manchaiah V, Swapna S, Dhandayutham S, Kothandaraman PP. Ototoxicity: A Challenge in Diagnosis and Treatment. *J Audiol Otol* 2018; 22: 59-68.
- 3) Bisht M, Bist S. Ototoxicity: the hidden menace. *Indian J Otolaryngol Head Neck Surg* 2011; 63: 255-259.
- 4) Fassad MR, Desouky LM, Asal S, Abdalla EM. Screening for the mitochondrial A1555G mutation among Egyptian patients with non-syndromic, sensorineural hearing loss. *Int J Mol Epidemiol Genet* 2014; 5: 200-204.
- 5) Jing W, Zongjie H, Denggang F, Na H, Bin Z, Aifen Z, Xijiang H, Cong Y, Yunping D, Ring HZ, Ring BZ. Mitochondrial mutations associated with aminoglycoside ototoxicity and hearing loss susceptibility identified by meta-analysis. *J Med Genet* 2015; 52: 95-103.
- 6) Huth M, Ricci A, Cheng A. Mechanisms of aminoglycoside ototoxicity and targets of hair cell protection. *Int J Otolaryngol* 2011; 2011: 37861.
- 7) Loughrey DG, Kelly ME, Kelley GA, Brennan S, Lawlor BA. Association of age-related hearing loss with cognitive function, cognitive impairment, and dementia: a systematic review and meta-analysis. *JAMA Otolaryngol Head Neck Surg* 2018; 144: 115-126.
- 8) American academy of audiology American Academy of Audiology's Position Statement and Practice Guidelines on Ototoxicity Monitoring (PDF). "<https://www.audiology.org/publications-resources/document-library/ototoxicity-monitoring>".
- 9) American Ear Hearing and Audiology Ototoxic Medicines. 2021. "<https://www.americanear.com/your-hearing/ototoxic-medicines-list>".
- 10) Ding Y, Leng J, Fan F, Xia B, Xu P. The role of mitochondrial DNA mutations in hearing loss. *Biochem Genet* 2013; 51: 588-602.
- 11) Mkaouar-Rebai E, Tilili A, Masmoudi S, Charfeddine I, Fakhfakh F. New polymorphic mtDNA restriction site in the 12S rRNA gene detected in Tunisian patients with non-syndromic hearing loss. *Biochem Biophys Res Commun* 2008; 369: 849-852.
- 12) Montazer Zohour M, Tabatabaiefar MA, Dehkordi FA, Farrokhi E, Akbari MT, Chaleshtori MH. Large-scale screening of mitochondrial DNA mutations among Iranian patients with prelingual nonsyndromic hearing impairment. *Genet Test Mol Biomarkers* 2012; 16: 271-278.
- 13) Khalifa Alkowiari M, Giroto G, Abdulhadi K, Dipresa S, Siam R, Najjar N, Badii R, Gasparini P. GJB2 and GJB6 genes and the A1555G mitochondrial mutation are only minor causes of non-syndromic hearing loss in the Qatari population. *Int J Audiol* 2012; 51: 181-185.
- 14) Li R, Greinwald JH Jr, Yang L, Choo DI, Wenstrup RJ, Guan MX. Molecular analysis of the mitochondrial 12S rRNA and tRNASer (UCN) genes in paediatric subjects with non-syndromic hearing loss. *J Med Genet* 2004; 41: 615-620.
- 15) Abreu-Silva RS, Lezirovitz K, Braga MC, Spinelli M, Pirana S, Della-Rosa VA, Otto PA, Mingroni-Netto RC. Prevalence of the A1555G (12S rRNA) and tRNA Ser (UCN) mitochondrial mutations in hearing-impaired Brazilian patients. *Braz J Med Biol Res* 2006; 39: 219-226.
- 16) Lu J, Li Z, Zhu Y, Yang A, Li R, Zheng J, Cai Q, Peng G, Zheng W, Tang X, Chen B, Chen J, Liao Z, Yang L, Li Y, You J, Ding Y, Yu H, Wang J, Sun D, Zhao J, Xue L, Wang J, Guan MX. Mitochondrial 12S rRNA variants in 1642 Han Chinese pediatric subjects with aminoglycoside-induced and nonsyndromic hearing loss. *Mitochondrion* 2010; 10: 380-390.
- 17) Usami S, Abe S, Akita J, Namba A, Shinkawa H, Ishii M, Iwasaki S, Hoshino T, Ito J, Doi K, Kubo T, Nakagawa T, Komiyama S, Tono T, Komune S. Prevalence of mitochondrial gene mutations among hearing impaired patients. *J Med Genet* 2000; 37: 38-40.
- 18) Tarawneh M, Nimri O, Arkoob K, Zagher M. Cancer incidence in Jordan 2009. Non-Communicable Diseases Directorate, Jordan Cancer Registry. Ministry of Health 2009.
- 19) Guirguis-Blake JM, Evans CV, Senger CA, O'Connor EA, Whitlock EP. Aspirin for the primary prevention of cardiovascular events: a systematic evidence review for the US Preventive Services Task Force. *Ann Intern Med* 2016; 164: 804-813.
- 20) Gu Q, Dillon CF, Eberhardt MS, Wright JD, Burt VL. Preventive aspirin and other antiplatelet medication use among US adults aged ≥ 40 years: data from the National Health and Nutrition Examination Survey, 2011–2012. *Public Health Rep* 2015; 130: 643-654.
- 21) Sica DA, Carter B, Cushman W, Hamm L. Thiazide and loop diuretics. *J Clin Hypertens (Greenwich)* 2011; 13: 639-643.
- 22) Health engine Ototoxicity. 2008. "[https://healthengine.com.au/info/ototoxicity#Clinical\\_Examination](https://healthengine.com.au/info/ototoxicity#Clinical_Examination)".
- 23) Campbell KC, Le Prell CG. Potential therapeutic agents. Paper presented at: Seminars in Hearing 2011; 32: 281-296.
- 24) Campbell KC. Audiologic monitoring for ototoxicity. Ototoxicity. 1st ed. Hamilton: BC Decker Inc 2004: 153-60.
- 25) Carlson ML. Cochlear implantation in adults. *N Engl J Med* 2020; 382: 1531-1542.
- 26) Dillon H. Hearing Aids: Thieme; 2012.
- 27) Coll KM, Cutler MM, Thobro P, Haas R, Powell S. An exploratory study of psychosocial risk behaviors of adolescents who are deaf or hard of hearing: Comparisons and recommendations. *Am Ann Deaf* 2009; 154: 30-35.
- 28) Wang W-J, Lessard D, Abu H, et al. Hearing loss and cognitive decline among older adults with atrial fibrillation: the SAGE-AF study. *Journal of geriatric cardiology: JGC* 2020; 17: 177-183.