Epidemiological analysis of the association between hearing and barium in humans

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Running title: Barium-mediated hearing loss in humans.

ABSTRACT

Our previous study experimentally showed barium (Ba)-mediated hearing loss in mice. To our knowledge, however, it remains unknown whether Ba affects hearing in humans. This epidemiological study aimed at investigating ototoxicity of Ba in humans. Associations of Ba levels in hair, toenails and urine with hearing levels (1, 4, 8 and 12 kHz) were analyzed in 145 Bangladeshi subjects. Binary logistic regression analysis with adjustment for age, sex, BMI and smoking showed that Ba levels in hair had significant associations with hearing loss at 8 kHz (OR = 4.75; 95% CI: 1.44, 17.68) and 12 kHz (OR = 15.48; 95% CI: 4.04, 79.45). Ba levels in toenails were also associated with hearing loss at 8 kHz (OR = 3.20; 95% CI: 1.35, 7.85) and 12 kHz (OR = 3.63; 95% CI: 1.58, 8.55), whereas there was no correlation between Ba level in urinary samples and hearing. There was a significant correlation between hearing loss and Ba levels in hair and toenails in the model adjusted with arsenic levels as the confounder. In conclusion, this study suggested that Ba levels could be a new risk factor for hearing loss, especially at high frequencies of 8 and 12 kHz, in humans.

Key words: barium; hearing loss; ototoxicity; toenail; hair; ICP-MS.

Introduction

Hearing loss is one of the most common disorders affecting quality of life (QOL)[1]. The number of patients with hearing loss is estimated to be 360 million worldwide, which is equivalent to 5.3% of the world's population. It is estimated that the 360 million patients include 328 million adults and 32 million children[2]. In addition, it is estimated that one third of people over 65 years of age worldwide have age-related hearing loss[2]. Genetic, aging and environmental factors intricately affect the onset of hearing losses, which lower quality of life (QOL)[1]. However, information about the environmental factors affecting hearing and the number of patients is limited.

Barium (Ba) has been shown to be included in raw surface water, tap water supplies and tube wells at concentrations ranging from ≤ 5 to 15 mg/L[3-5]. In addition, seaweed, fish and some foods have been shown to contain Ba[6,7]. In a diet investigation in Canada, Ba levels in nuts including peanuts and peanut butter were shown to be relatively high (2,919.11 μg/kg). Fruits and vegetables contain Ba levels at concentrations ranging from 57.62 to 3,750.03 μg/kg and from 47.99 to 2,282.23 μg/kg, respectively, while Ba levels in meats and fish are relatively low (ranging from 12.06 to 237.57 and from 36.17 to 481.34 μg/kg, respectively). The average Ba intake in humans from diets has been shown to be 8.817 μg/kg/day[8]. An investigation in Canary Islands, Spain showed that Ba levels ranged from 5.210 ± 2.117 mg/kg in nuts to 0.035 ± 0.043 mg/L in water and that total intake of Ba was 0.685 mg/day, mainly from cereals[9]. Thus, diets that we ingest in daily life contain Ba as one of the general elements. However, Ba is not regarded as one of the elements affecting our health.

The possible association between exposure to Ba and health problems in humans was investigated in previous epidemiological studies. Residents living in communities in Illinois, USA where tap water contains high levels of Ba (2~10 mg/L) have been shown to have significantly high mortality rates associated with arteriosclerosis and cardiovascular disease[10-12]. However, the relevance was not analyzed with potential confounders (e.g., population mobility, water softeners, duration of exposure, and actual barium intake). Also, the prevalences of cardiovascular disease in two Illinois communities were compared in a follow-up cross-sectional study. The two communities had similar demographic and socioeconomic characteristics, but Ba levels in drinking water were different. However, no significant differences were found between the two communities in mean systolic or diastolic blood pressure or in history of hypertension, heart disease, stroke or kidney disease for men or women when many of the possible confounding factors not considered in earlier studies were accounted for[13]. In previous studies performed in India and the Kingdom of Saudi Arabia, prevalence rates of cancer and autism have been shown to be associated with Ba levels in biological samples and drinking water[14-16]. However, there is very limited information about a correlation between exposure to Ba and other health problems in humans.

In our experimental study, oral exposure of mice to Ba by drinking water was shown to cause severe hearing loss with degeneration of the organ of Corti in mice. Ba-exposed mice showed high levels of Ba in inner ears compared to the levels in mice not exposed to Ba[17]. However, there is no information about the correlation between Ba levels in biological samples and health problems in humans. In this pilot study, we performed hearing examinations and measurements of Ba levels in human biological samples to determine whether Ba levels in biological samples are associated with hearing loss.

Methods

Study Subjects

The study was performed for 145 subjects in Bangladesh aged from 12 to 55 years (mean ± SD, 29.58 ± 10.92 years) who agreed in written form to participate in hearing examinations. In this study, we did not include subjects who had a previous history of ear diseases and suffered from illness at the time of investigation. We also did not include subjects who had a habit of drinking alcohol or using a portable music player (e.g., MP3 player) with earphones. In addition, another ethnic group or race was not included as subjects in this study. This investigation was performed using a self-reporting questionnaire on smoking, age, clinical history, weight and height of subjects. Body mass index (BMI; mean ± SD = 21.99 ± 3.42) was obtained by using the following formula: weight in kg/height in meter squares. The procedures were explained and informed consent was obtained from all of the subjects. This study was ethically approved by Nagoya University International Bioethics Committee following the regulations of the Japanese government (approval number 2013-0070) and the Faculty of Biological Science, University of Dhaka (Ref. no. 5509/Bio.Sc).

Measurement of auditory thresholds

Auditory thresholds at frequencies of 1, 4, 8 and 12 kHz were measured by pure tone audiometry (PTA). We measured hearing level at an extra-high frequency (12 kHz) because hearing level of the frequency is sensitive to environmental factors including smoking[18,19]. The sound data of PTA were installed into an iPod. The sound stimuli were output by the iPod with an earphone-type headphone (Panasonic RP-HJE150) in a sound-proof room as described previously[19-21]. Sound signals at frequencies of 1, 4, 8 and 12 kHz were output to each subject until the thresholds of sound were identified. Hearing levels of the subjects were all measured by providing an initial stimulus of 5 decibels (dB) followed by a stepwise increase in sound level by 5 dB. Duplicated measurement of hearing was performed in each subject to verify the repeatability of values.

Measurement of barium levels in biological samples

We measured Ba levels in biological samples by the method previously described[17,22,23]. In short, biological samples were put into a 15 ml polypropylene tube with 3 ml of HNO_3 (61%). The sample tubes were incubated at 80˚C for 48 hours and then allowed cool to room temperature for 1 hour. Then, 3 ml of H_2O_2 (30%) was added to each tube and the tubes were incubated at 80˚C for 3 hours. After the samples had been diluted with ultrapure water, the Ba level in each sample was measured by using an inductively coupled plasma mass spectrometer (ICP-MS; 7500cx, Agilent Technologies, Inc.) with a reaction cell for absence of ArCl ion interference. Total Ba levels in urinary samples were corrected by specific gravity expressed as µg/L[24,25].

Statistical analysis

Statistical analyses were performed following the method previously reported[18,26]. No randomization was used and the investigators were not blinded to the group allocation during the experiments or when assessing the outcomes. For univariate analyses, Spearman correlation coefficients were used to determine a significant association between nonparametric variables, since the Shapiro-Wilks normality test showed that Ba levels in biological samples were not normally distributed. The two-tailed Mann-Whitney *U* test (equivalent to the Wilcoxon rank sum test) and Steel-Dwass test were also used for nonparametric data to determine a significant difference of hearing levels between two groups and among three groups, respectively, since hearing levels are discontinuous variables. Steel-Dwass test was performed with set the alpha level to 0.05. Levene's test and Bartlett's test for equality of variances were conducted. Difference with $p < 0.05$ was considered significant, and the actual p value for each test was displayed except for the p values below 0.0001. For multivariate analysis, we categorized subjects into two or three groups according to sex, age, BMI, smoking and Ba concentration in biological samples and compared the average auditory thresholds at frequencies of 1, 4, 8 and 12 kHz. We performed binary logistic regression analysis with auditory thresholds at 1 kHz (> 10 dB)[27], 4 kHz (> 10 dB)[28], 8 kHz (> 25 dB)[29,30] and 12 kHz (> 40 dB)[18] of hearing levels as dependent variables and Ba levels in hair, toenail and urine samples as independent variables. Models were adjusted for age[31], sex[32], smoking[33] and BMI[34] because associations between hearing loss and these factors have been shown. All statistical analyses were performed using JMP Pro (version 11.0.0; SAS Institute Inc., Cary, NC, USA).

Results

Correlation between hearing levels and confounding factors including age, BMI, sex and smoking

Characteristics of subjects and cut-off values of confounders analyzed in this study are shown in Table 1. We set the mean age of the subjects (i.e., 30 years old) as the cut-off value for age. The average auditory thresholds at 1, 4, 8 and 12 kHz in the older group (n = 68) were significantly higher (1 kHz, p = 0.0098; 4, 8 and 12 kHz, p < 0.0001) than those in the younger group (n = 77) (Figure 1A). We also categorized the subjects into three groups based on body mass index (BMI) set by the WHO category (underweight, < 18.5; normal range, 18.5–25; overweight, 25 ≤) as shown in Table 2. There were no significant differences in average auditory thresholds among the three groups (Figure 1B). The average auditory thresholds at 4, 8 and 12 kHz in females (n = 76) were significantly higher (4 kHz; p = 0.0257, 8 kHz; p = 0.0004, 12 kHz; p = 0.0066) than those in males (n = 69) (Figure 1C). We next compared the auditory thresholds in smokers (n = 31) and non-smokers (n = 114) since smoking has been shown to be one of the risk factors for hearing loss[18,19,33]. The average auditory thresholds at 1, 4, 8 and 12 kHz in the smoking group were significantly higher than those in the non-smoking group (1 kHz; p = 0.0057, 4 kHz; p < 0.0001, 8 kHz; p = 0.0002, 12 kHz; p < 0.0001) (Figure 1D). Hearing level on average at extra-high frequency (12 kHz) was more affected compared to those at lower frequencies (Figure 1D).

Correlation between hearing levels and Ba levels in human biological samples

Barium levels (mean ± SD) in hair, toenail and urine samples from all subjects were 3.21 ± 2.87 μg/g, 7.08 ± 5.54 μg/g and 3.87 ± 4.85 μg/L, respectively (Table 2). A significant correlation of Ba levels was observed in toenail and hair samples (r = 0.3370, p < 0.0001) or in urine and toenail samples (r = 0.1668, p = 0.0449), while no correlation was found in urine and hair samples (r = 0.0665, p = 0.4269). In addition, Ba levels (mean ± SD) in female subjects were 3.859 ± 3.226 μg/g in toenail samples and 11.155 ± 4.504 μg/g in hair samples, while those in male subjects were 2.489 ± 2.215 μg/g in toenail samples and 2.582 ± 1.907 μg/g in hair samples. There is no information about the correlation between Ba levels in biological samples and hearing loss in humans. Based on the determination of Ba levels significantly associated with hearing loss, we categorized the subjects into two groups at 12.62 μg/g in hair and 1.88 μg/g in toenails (Table 3). On the other hand, we used the median (2.55 μg/L) for urine samples (Table 3) since there was no correlation between Ba levels in urine samples and hearing levels. We then compared the auditory thresholds at 1, 4, 8 and 12 kHz between the two groups (Figure 2). The group with high Ba level in hair (n =26) showed significantly (8 kHz; $p = 0.0027$, 12 kHz; $p = 0.0045$) higher auditory thresholds at 8 kHz and 12 kHz than those in the group with low Ba level in hair (n = 119) (Figure 2A). The average auditory thresholds at 4 kHz, 8 kHz and 12 kHz in the group with high Ba level in toenails (n = 90) were significantly higher ($p < 0.0001$) than those in the group with low Ba level in toenails (n = 55) (Figure 2B). On the other hand, there were no significant differences in average auditory thresholds at all of the frequencies between the groups with the high and low Ba levels in urine samples (Figure 2C).

Binary logistic regression analysis

Binary logistic regression analysis with adjustment for age, sex, BMI and smoking showed that Ba levels in hair were significantly associated with hearing loss at 8 kHz [odds ratio (OR) = 4.75; 95% confidence interval (CI): 1.44, 17.68; $p = 0.0096$] and 12 kHz (OR = 15.48; 95% CI: 4.04, 79.45; $p < 0.0001$) (Table 4). Ba levels in toenails also showed significant associations with hearing loss at 4 kHz (OR = 2.76; 95% CI: 1.15, 6.85, $p = 0.0230$), 8 kHz (OR = 3.20; 95% CI: 1.35, 7.85; $p =0.0083$) and 12 kHz (OR = 3.63; 95% CI: 1.58, 8.55; $p = 0.0023$) in the adjusted model (Table 4). The model adjusted with continuous variable of arsenic as the additional confounder also showed a significant correlation between hearing loss and Ba levels in hair and toenails (Table 5). On the other hand, we did not find any relationship between Ba level in urine and hearing levels at all frequencies in the adjusted models (Tables 4 and 5). To verify the appropriateness of our model, we shifted the cut-off values of the dependent variable dichotomizing hearing levels from 10 to 15 dB at 4 kHz, from 25 to 30 dB at 8 kHz and from 40 to 45 dB at 12 kHz. In addition, we shifted the cut-off values of the independent valuable dichotomizing Ba levels from 10.62 to 13.62 μg/g in hair and from 1.88 to 2.38 μg/g in toenails. The results showed significant correlations between Ba levels in toenails and hair and hearing loss remained except for the correlation between Ba levels in toenails and hearing loss at 4 kHz. Thus, these results obtained in different classifications indicate that Ba levels in toenails and hair are associated with hearing loss at higher frequencies.

Discussion

In our previous study, exposure of mice to Ba by drinking water resulted in accumulation of Ba in inner ears that led to hearing loss[17]. However, there is no information about a correlation between ototoxicity and Ba in humans. This pilot study epidemiologically demonstrated a significant correlation between Ba levels in toenail and hair samples and hearing loss in humans. On the other hand, it has been shown that food is one of the major sources of Ba exposure in general[3], although the major source of Ba that accumulated in subjects in this study remains unclear. In our previous study, Ba levels in inner ears of mice not exposed to Ba were higher than those in other tissues including the liver, kidney and heart, in which Ba levels were undetectably low, while mice exposed to Ba by drinking water showed significantly increased Ba levels by about 10% in inner ears[17]. Our measurements of Ba levels in foods and drinking water for normal mouse breeding showed that Ba level in food was about 35 mg/kg and that in water was about 4 μg/L. Thus, it is possible that the accumulation of Ba in inner ears of mice bred under normal conditions is mainly derived from food and it could be accelerated in mice exposed to Ba by drinking water. Therefore, it is important to monitor Ba levels in food and drinking water worldwide in order to further analyze the correlation between ingestion of Ba and hearing loss in humans.

In this study, hearing levels at higher frequencies (8 and 12 kHz) were affected in groups with high Ba levels in hair and toenails, whereas our previous study with ICR mice showed that hearing levels at lower frequencies (4, 12 and 20 kHz) were affected in the Ba exposure group[17]. On the other hand, hearing levels at high frequency (32 kHz) in the non-exposed group and Ba-exposed group were comparable at the end of the exposure period, because ICR mice have been shown to progressively suffer from hearing loss[35], although a genetic reason has not been demonstrated. Therefore, it is possible that people who potentially have a genetic factor for hearing loss also suffer from hearing loss at lower frequency by exposure to Ba, although we could not find such a case in this study. On the other hand, there is no information about a correlation of Ba levels among inner ears, hair and toenails. It is crucial to analyze an association of Ba levels among those tissues in an experimental animals exposed to Ba in order to determine the threshold of Ba levels in hair and toenails associated with hearing loss.

This study showed that there was a significant correlation between hearing levels and Ba levels in hair and toenails, while there was no significant correlation between hearing levels and Ba level in urine. Major excretion routes of Ba ingested from daily diets (750 µg) have been shown to be feces (690 µg), hair (75 µg), urine (50 µg) and sweat (10 µg)[3]. Meanwhile, the growth cycle of a fingernail is generally known to be about 6 months[36] and that of a toenail has been shown to be about 10 months[37]. Also, the growth cycle of hair has shown to be several years[38,39], while detection of an element level in urine is generally regarded as being associated with daily exposure. Measurements of trace element levels in toenails and hair have been considered as reliable biomarkers that can reflect chronic exposure status[40-43]. Therefore, our results suggest that Ba levels in toenails and hair can reflect levels after chronic exposure to Ba. Thus, our results raise the possibility that chronic exposure to Ba causes hearing loss, although our experimental study showed sub-acute exposure to Ba by drinking water caused hearing loss in ICR mice[17]. Additional study is needed to analyze a correlation between duration of exposure to Ba and hearing loss with consideration for exposure conditions in humans and mice.

This study showed that hearing levels in female subjects were significantly worse than those in male subjects. In addition, Ba levels in female subjects were significantly higher than those in male subjects (hair; $p < 0.0001$, nail; $p = 0.0018$). Therefore, it is possible that higher Ba levels in female subjects cause higher auditory thresholds than those in male subjects, although the reason for the gender difference in Ba levels is not clear. On the other hand, hearing levels in males are generally known to be worse than those in females[32], while a previous study showed that females with depression tend to have hearing loss compared to males[44]. Hence, it is important for an understanding of the gender difference in hearing levels to compare Ba levels in biological samples from males and females and to consider other confounders including mental status. Meanwhile, since this study showed that the smoking group had greater hearing loss than did the non-smoking group as was previously reported[33], we compared Ba levels in the two groups. The results showed that there was no significant difference in Ba levels between the smoking group and the non-smoking group, although it has been shown that tobacco leaves contain barium hydroxide[45]. In this study, we found that there was a significant correlation between hearing loss and Ba levels in hair and toenails in binary logistic regression analysis adjusted with arsenic as the confounder, though univariate analyses have shown that arsenic levels in hair samples and in nail samples affect hearing in humans[46,47]. Thus, this study suggests that Ba could be an independent risk factor for hearing loss.

In conclusion, the present pilot study further provided epidemiological evidence that there is a correlation between Ba and hearing loss in humans in addition to our previous experimental study in mice[17]. Our study also suggested possible thresholds in hair and toenails that increase the risk for hearing loss in humans.

Conflict of interest statement

All authors declare to have no actual or potential conflicts of interest.

Acknowledgements

This work was supported in part by Grants-in-Aid for Scientific Research (A) (No. 15H01743 and 15H02588), (B) (No. 24390157 and 24406002) and (C) (No. 25460178, 25340052, 25461717), Grant-in-Aid for Challenging Exploratory Research (No. 23650241 and No. 26670525), Grant-in-Aid for Scientific Research on Innovative Areas (No. 24108001) and COE Project for Private Universities (Nutritional Health Science Research Center; No. S1201007) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT); the Mitsubishi Foundation; Toyoaki Scholarship Foundation; the Mitsui & Co., Ltd. Environment Fund; Aichi Health Promotion Foundation, Foundation from Center for Advanced Medical and Clinical Research Nagoya University Hospital and AEON Environmental Foundation.

References

1. Lalwani AK, Gu¨rtler N. Sensorineural hearing loss, the aging inner ear, and hereditary hearing impairment. In: Lalwani AK (ed). CURRENT diagnosis & treatment in otolaryngology—head & neck surgery. 2nd edn. McGraw-Hill: New York, 2008, pp 683-704.
2. WHO (World Health Organization). *Global estimates on prevalence of hearing loss.* Available: http://www.who.int/entity/pbd/deafness/WHO_GE_HL.pdf?ua=1 (Accessed: 7 January 2015). 2012.
3. ATSDR (Agency for Toxic Substances and Disease Registry). *Toxicological profile for barium.* Available: http://www.atsdr.cdc.gov/toxprofiles/tp24.pdf (Accessed: 12

December 2014). 2007.

4. Calabrese EJ. Excessive barium and radium-226 in Illinois drinking water. J Environ Health 1977; **39**,: 366-369.

5. EPA (Environmental Protection Agency). *Technical factsheet on: Barium.* Available: http://www.epa.gov/ogwdw/pdfs/factsheets/ioc/tech/barium.pdf (Accessed: 12 December 2014) 2005.

6. Robinson WO, Whetstone RR, Edgington G. The occurrence of barium in soils and plants. US Department of Agriculture technical bulletin 1950; 1013: 1-36.

7. Smith KA. The comparative uptake and translocation by plants of calcium, strontium, barium and radium. I. Bertholletia excelsa (Brazil nut tree). Plant and soil 1971; 34: 369-379.

8. Health Canada. *Canadian total diet study.* Available: http://www.hc-sc.gc.ca/food-aliment/cs-ipc/fr-ra/e_tds.html (Accessed: 12 December 2014). 2005.

9. González-Weller D, Rubio C, Gutiérrez AJ, González GL, Mesa JM, Gironés CR, et al. Dietary intake of barium, bismuth, chromium, lithium, and strontium in a Spanish population (Canary Islands, Spain). Food Chem Toxicol 2013; 62: 856-868.

10. Brenniman GR, Kojola WH, Levy PS, Carnow BW, Namekata T. Health effects of human exposure to barium in drinking water. Available: http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=2000Z0BX.txt (Accessed: 12 December 2014). 1979.

11. Brenniman GR, Namekata T, Kojola WH, Carnow BW, Levy PS. Cardiovascular disease death rates in communities with elevated levels of barium in drinking water. Environ. Res 1979; 20: 318-324.

12. Brenniman GR, Kojola WH, Levy PS, Carnow BW, Namekata T. High barium levels in public drinking water and its association with elevated blood pressure. Arch. Environ. Health 1981; 36: 28-32.

13. Brenniman GR, Levy PS. Epidemiological study of barium in Illinois drinking water supplies. In: Calabrese EJ, Tuthill RW, Condie L (eds). Inorganics in water and cardiovascular disease. Princeton Scientific Publishing Co: Princeton, 1985, pp 231-240.
14. Blaurock-Busch E, Busch YM, Friedle A, Buerner H, Parkash C, Kaur A. Comparing the metal concentration in the hair of cancer patients and healthy people living in the malwa region of punjab, India. Clin Med Insights Oncol 2014; 8:1-13.
15. Blaurock-Busch E, Friedle A, Godfrey M, Schulte-Uebbing CE. Metal exposure in the physically and mentally challenged children of Punjab, India. Maedica (Buchar) 2010; 5(2):102-110.
16. Blaurock-Busch E, Amin OR, Rabah T. Heavy metals and trace elements in hair and urine of a sample of arab children with autistic spectrum disorder. Maedica (Buchar) 2011; 6(4):247-257.
17. Ohgami N, Hori S, Ohgami K, Tamura H, Tsuzuki T, Ohnuma S, et al. Exposure to low-dose barium by drinking water causes hearing loss in mice. Neurotoxicology 2012; 33: 1276-1283.
18. Ohgami N, Kondo T, Kato M. Effects of light smoking on extra-high-frequency auditory thresholds in young adults. Toxicol Ind Health 2011; 27(2): 143-147.
19. Sumit AF, Das A, Sharmin Z, Ahsan N, Ohgami N, Kato M, et al. Cigarette smoking causes hearing impairment among Bangladeshi population. PLoS ONE 2015; **10**: e0118960.
20. Szudek J, Ostevik A, Dziegielewski P, Robinson-Anagor J, Gomaa N, Hodgetts B, et al. Can Uhear me now? Validation of an iPod-based hearing loss screening test. J Otolaryngol Head Neck Surg 2012; Suppl 1: S78-84.
21. Van Tasell DJ, Folkeard P. Reliability and Accuracy of a Method of Adjustment for Self-Measurement of Auditory Thresholds. Otol Neurotol 2013; 34(1): 9-15.
22. Seow WJ, Pan WC, Kile ML, Baccarelli AA, Quamruzzaman Q, Rahman M, et al.

Arsenic reduction in drinking water and improvement in skin lesions: a follow-up study in Bangladesh. Environ Health Perspect 2012; 120(12): 1733-1738.

23. Kato M, Kumasaka MY, Ohnuma S, Furuta A, Kato Y, Shekhar HU, et al. Comparison of Barium and Arsenic Concentrations in Well Drinking Water and in Human Body Samples and a Novel Remediation System for These Elements in Well Drinking Water. PLoS One 2013; 8(6): e66681.
24. Miller RC, Brindle E, Holman DJ, Shofer J, Klein NA, Soules MR, et al. Comparison of specific gravity and creatinine for normalizing urinary reproductive hormone concentrations. Clin Chem 2004; 50(5): 924-932.
25. Hauser R, Meeker JD, Park S, Silva MJ, Calafat AM. Temporal Variability of Urinary Phthalate Metabolite Levels in Men of Reproductive Age. Environ Health Perspect 2004; 112(17): 1734-1740.
26. Kato M, Iida M, Goto Y, Kondo T, Yajima I. Sunlight exposure-mediated DNA damage in young adults. Cancer Epidemiol Biomarkers Prev 2011; 20(8): 1622-1628.
27. Cantley LF, Galusha D, Cullen MR, Dixon-Ernst C, Rabinowitz PM, Neitzel RL. Association between ambient noise exposure, hearing acuity, and risk of acute occupational injury. Scand J Work Environ Health 2015; 41: 75-83.
28. Job A, Raynal M, Kossowski M, Studler M, Ghernaouti C, Baffioni-Venturi A, et al. Otoacoustic detection of risk of early hearing loss in ears with normal audiograms: A 3-year follow-up study. Hear Res 2009; 251: 1-2.
29. Bainbridge KE, Hoffman HJ, Cowie CC. Risk Factors for Hearing Impairment Among U.S. Adults With Diabetes. Diabetes Care 2011; 34(7): 1540-1545.
30. Fabry DA, Davila EP, Arheart KL, Serdar B, Dietz NA, Bandiera FC, et al. Secondhand Smoke Exposure and the Risk of Hearing Loss. Tob Control 2011; 20(1): 82-85.
31. CDC (Centers for Disease Control and Prevention). *Hearing levels of adults, by age and sex, United States, 1960-1962.* Available: http://stacks.cdc.gov/view/cdc/12597

(Accessed: 12 December 2014). 1965.

32. Jun HJ, Hwang SY, Lee SH, Lee JE, Song JJ, Chae S. The Prevalence of Hearing Loss in South Korea: Data From a Population-Based Study. Laryngoscope 2015; 125: 690-694.

33. Dawes P, Cruickshanks KJ, Moore DR, Edmondson-Jones M, McCormack A, Fortnum H, et al. Cigarette Smoking, Passive Smoking, Alcohol Consumption, and Hearing Loss. J Assoc Res Otolaryngol 2014; 15(4): 663-674.

34. Dąbrowski M, Mielnik-Niedzielska G, Nowakowski A. Impact of different modifiable factors on hearing function in type 1 and type 2 diabetic subjects. A preliminary study. Ann Agric Environ Med 2013; 20(4): 773-778.

35. Drayton M, Noben-Trauth K. Mapping quantitative trait loci for hearing loss in Black Swiss mice. Hear Res 2006; 212(1-2): 128-139.

36. Williams DD, Short R, Bowden DM. Fingernail growth rate as a biomarker of aging in the pigtailed macaque (Macaca nemestrina). Exp Gerontol 1990; 25(5): 423-432.

37. Yaemsiri S, Hou N, Slining MM, He K. Growth rate of human fingernails and toenails in healthy American young adults. J Eur Acad Dermatol Venereol 2010; 24(4): 420-423.

38. Lynfield YL. Effect of Pregnancy on the Human Hair Cycle. J Invest Dermatol 1960; 35: 323-327.

39. Lyle S, Christofidou-Solomidou M, Liu Y, Elder DE, Albelda S, Cotsarelis G. The C8/144B monoclonal antibody recognizes cytokeratin 15 and defines the location of human hair follicle stem cells. J Cell Sci 1998; 111(21): 3179-3188.

40. Airey D. Meacury in human hair due to environment and diet: a review. Environ Health Perspect 1983; 52: 303-316.

41. Longnecker MP, Stampfer MJ, Morris JS, Spate V, Baskett C, Mason M, et al. A 1-y trial of the effect of high-selenium bread on selenium concentrations in blood and toenails. Am J Clin Nutr 1993; 57(3): 408-413.

42. Garland M, Morris JS, Rosner BA, Stampfer MJ, Spate VL, Baskett CJ, et al. Toenail trace element levels as biomarkers: reproducibility over a 6-year period. Cancer Epidemiol Biomarkers Prev 1993; 2: 493-497.

43. He K. Trace elements in nails as bio-markers in clinical research. Eur J Clin Invest 2011; 41: 98-102.

44. Li CM , Zhang X , Hoffman HJ , Cotch MF , Themann CL , Wilson MR. Hearing impairment associated with depression in US adults, National Health and Nutrition Examination Survey 2005-2010. JAMA Otolaryngol Head Neck Surg 2014; 140(4): 293-302.

45. Van Duuren BL, Sivak A, Langseth L, Goldschmidt BM, Segal A. Initiators and promoters in tobacco carcinogenesis. World Conference on smoking and health: Toward a less harmful cigarette. National Cancer Institute Monograph 1968; 28: 173-180.

46. Bencko V, Symon K. Test of environmental exposure to arsenic and hearing changes in exposed children. Environ Health Perspect 1977; 19: 95-101.

47. Saunders JE, Jastrzembski BG, Buckey JC, Enriquez D, MacKenzie TA, Karagas MR. Hearing loss and heavy metal toxicity in a Nicaraguan mining community: audiological results and case reports. Audiol Neurootol 2013; 18(2): 101-113.

Figure legends

Figure 1. Correlations between hearing levels and confounding factors including age, BMI, sex and smoking. (*A*) Auditory thresholds (mean ± SD) at 1, 4, 8 and 12 kHz in the young group (< 30 years old; n = 77, diamonds) and the old group (≥ 30 years old; n = 68, squares) are displayed. (*B*) Auditory thresholds (mean ± SD) from frequencies of 1 kHz to 12 kHz in the underweight group [body mass index (BMI) < 18.5; n = 23, diamonds], normal range group (18.5 ≤ BMI < 25; n = 94, squares) and overweight group (25 ≤ BMI; n = 28, triangles) are displayed. (*C*) Auditory thresholds (mean ± SD) from frequencies of 1 kHz to 12 kHz in males (n = 69, diamonds) and females (n = 76, squares) are presented. (*D*) Auditory thresholds (mean ± SD) from frequencies of 1 kHz to 12 kHz in smokers (n = 31, diamonds) and non-smokers (n = 114, squares) are displayed. Smokers include people using tobacco powder. Significant differences (*p < 0.05, **p < 0.01) were analyzed by the Mann-Whitney *U* test (*A, C* and *D*) and Steel-Dwass test (*B*).

Figure 2. Correlations between barium levels in biological samples and hearing levels. (*A*) Auditory thresholds (mean ± SD) from frequencies of 1 kHz to 12 kHz in the high Ba level group (≥ 12.62 µg/g; n = 26, squares) and the low Ba level group (< 12.62 µg/g; n = 119, diamonds) in hair are displayed. (*B*) Auditory thresholds (mean ± SD) from frequencies of 1 kHz to 12 kHz in the high Ba level group (≥ 1.88 µg/g; n = 90, squares) and the low Ba level group (< 1.88 µg/g; n = 55, diamonds) in toenails are displayed. (*C*) Auditory thresholds (mean ± SD) from frequencies of 1 kHz to 12 kHz in the high Ba level group (≥ 2.55 µg/L; n = 72, squares) and the low Ba level group (< 2.55 µg/L; n = 73, diamonds) in urine are displayed. Ba level in urine was corrected by specific gravity. Significant differences (*p < 0.05, **p < 0.01) were analyzed by the Mann-Whitney *U* test.

Table 1. Variables for each factor

	Variables	Participants	Percentage (%)
Sex	Male	69	47.59
	Female	76	52.41
Age	< 30	77	53.1
	≥ 30	68	46.9
BMI	18.5 <	23	15.86
	18.5-25	94	64.83
	≥ 25	28	19.31
Smoking	Yes	31	21.38
	No	114	78.62

Table 2. Barium levels in biological samples

	Barium level (µg/g)		
	Mean ± SD	Minimum	Maximum
Hair	7.08 ± 5.54	0.33	23.96
Toenail	3.21 ± 2.87	0.2	16.56
Urine*	3.87 ± 4.85	0.13	36.72

*Barium concentration in urine was corrected by specific gravity (µg/L).

Table 3. Classification according to barium levels in biological samples

Barium level (μg/g)		Participants	Percentage (%)
Hair	Low (< 12.62)	119	82.07
	High (≥ 12.62)	26	17.93
Toenail	Low (< 1.88)	55	37.93
	High (≥ 1.88)	90	62.07
Urine*	Low (< 2.55)	73	50.34
	High (≥ 2.55)	72	49.66

*Barium concentration in urine was corrected by specific gravity (μg/L).

Table 4. Adjusted odds ratio (95% CI) for hearing levels and barium (Ba) levels in biological samples (n = 145)[a]

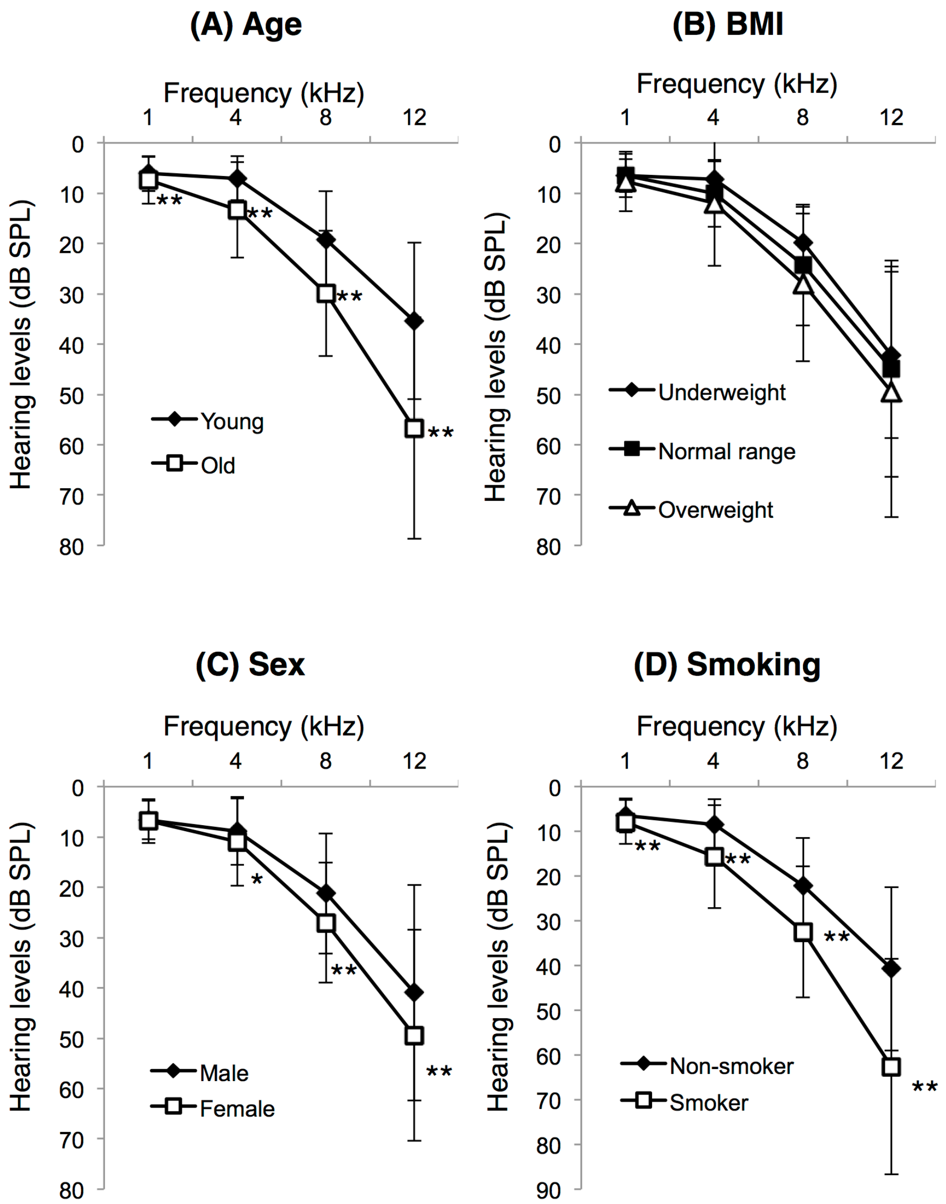
	1 kHz (≥ 10 dB)	4 kHz (≥ 10 dB)	8 kHz (≥ 25 dB)	12 kHz (≥ 40 dB)
Ba in hair	1.63	2.60	4.75**	15.48**
	(0.50, 5.19)	(0.85, 8.41)	(1.44, 17.68)	(4.04, 79.45)
Ba in toenail	0.82	2.76*	3.20**	3.63**
	(0.32, 2.10)	(1.15, 6.85)	(1.35, 7.85)	(1.58, 8.55)
Ba in urine	1.23	1.16	1.01	0.93
	(0.54, 2.85)	(0.52, 2.56)	(0.45, 2.23)	(0.43, 1.99)

[a]Models were adjusted for sex[26], age[43], BMI[44] and smoking[15], which were previously reported to affect hearing levels. Abbreviation: CI, confidence interval. *p < 0.05, **p < 0.01.

Table 5. Adjusted odds ratio (95% CI) for hearing levels and barium (Ba) levels in biological samples (n = 145)[a]

	1 kHz (≥ 10 dB)	4 kHz (≥ 10 dB)	8 kHz (≥ 25 dB)	12 kHz (≥ 40 dB)
Ba in hair	1.56	2.34	4.46*	21.72**
	(0.47, 4.98)	(0.75, 7.70)	(1.32, 17.24)	(4.95, 129.53)
Ba in toenail	0.84	3.04*	3.10*	2.72*
	(0.32, 2.21)	(1.23, 7.72)	(1.26, 7.79)	(1.10, 6.76)
Ba in urine	1.29	1.18	1.03	0.90
	(0.56, 3.01)	(0.53, 2.62)	(0.46, 2.28)	(0.41, 1.95)

[a]Models were adjusted for sex[26], age[43], BMI[44], smoking[15] and arsenic[29,30] which were previously reported to affect hearing levels. Abbreviation: CI, confidence interval. *$p < 0.05$, **$p < 0.01$.



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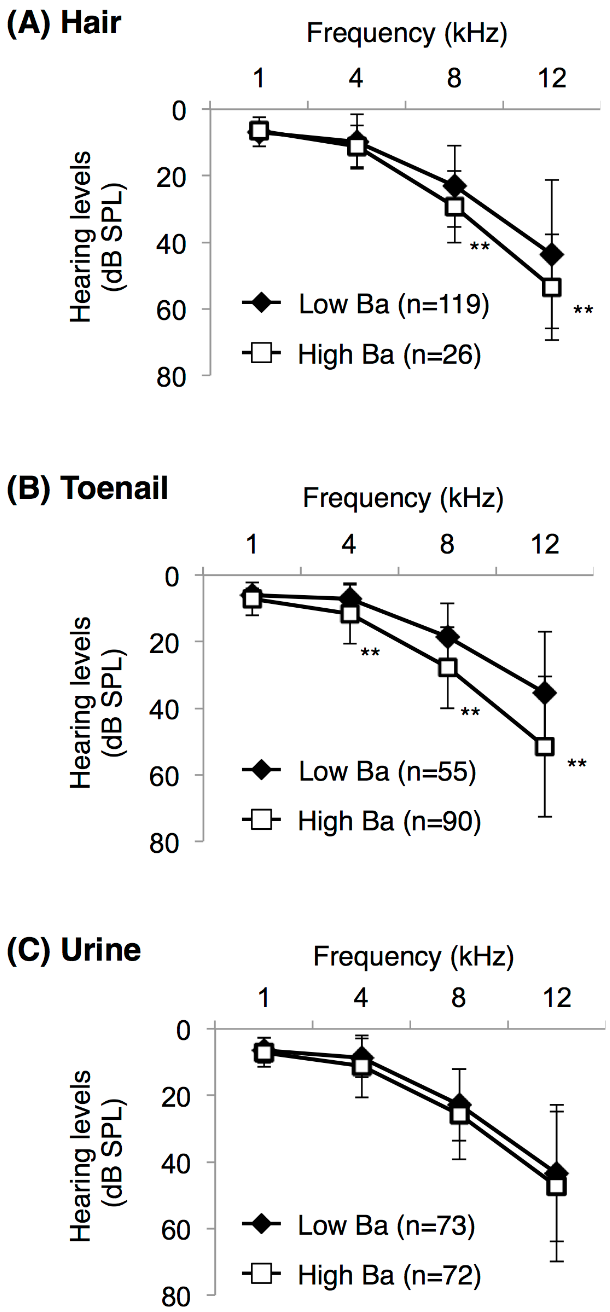

Ohgami et al., Figure 2