Sensorineural hearing loss and volatile organic compound metabolites in urine

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Abstract:

Purpose: Oxidative stress in the auditory system contributes to acquired sensorineural hearing loss. Systemic oxidative stress, which may predict auditory oxidative stress, can be assessed by measuring volatile organic compound metabolite concentrations in urine. The purpose of this retrospective study was to determine if hearing decreased in those with higher concentrations of urinary volatile organic compound metabolites. **Materials and methods:** Audiometric, demographic, and metabolite concentration data were downloaded from the 2011–2012 cycle of the U.S. National Health and Nutritional Examination Survey. Participants were first grouped by reported noise exposure. For each metabolite, an analysis of covariance was used to look for differences in age-adjusted hearing loss among urinary volatile organic compound metabolite concentration groups. Participants were grouped into quartiles based on concentration for each metabolite separately because many individuals were at the lower limit of concentration detection for several metabolites, leading to a non-normal distribution. **Results:** Age-adjusted high-frequency pure-tone thresholds were significantly (FDR < 0.05) increased by about 3 to 4 dB in high concentration quartile groups for five metabolites. All five metabolites were glutathione-dependent mercapturic acids. The parent compounds of these metabolites included acrylonitrile, 1,3 butadiene, styrene, acrylamide, and *N*,*N*-dimethylformamide. Significant associations were only found in those with no reported noise exposure. **Conclusions:** Urinary metabolites may help to explain susceptibility to oxidative stress-induced hearing loss.

Keywords: Sensorineural hearing loss | Volatile organic compounds | Mercapturic acids

Article:

1. Introduction

Volatile organic compound (VOC) metabolites are markers for oxidative stress, which is a molecular pathway linked to acquired sensorineural hearing loss (ASNHL) [1,2]. Inhibiting oxidative stress has been shown to reduce hearing loss in animals and may have therapeutic

effects in humans as well [3]. Oxidative stress damages the cochlea by producing reactive oxygen species that damage DNA, break down lipids, and induce apoptosis in the cochlea [2]. The mechanisms that regulate this damage are poorly understood. Furthering our understanding of these mechanisms may help to identify those at risk for ASHNL and lead to the development of pharmaceutical treatments.

Oxidative stress is caused by both environmental and endogenous toxins. Environmental toxins known to induce stress include organic solvents and cigarette smoke [4,5]. Endogenous factors associated with oxidative stress include polymorphisms in genes such as NOX, and concentrations of VOC metabolites such as 4-methyl-octane, 4-methyl-decane, hexane, and 5-methyl-pentadecane [1,5]. Exploring the association of VOC metabolism and hearing loss may help to explain how oxidative stress damages the auditory pathway because these metabolites are affected by both environmental and endogenous stress factors. For example, oxidative stress-inducing toxins such as cigarette smoke and organic solvents lead to increases in VOC metabolites [[6], [7], [8]]. Internally, genetic markers for glutathione S-transferase have been associated with metabolism rates of the VOCs benzene, acrolein, and crotonaldehyde [9]. Identifying specific metabolites associated with hearing loss may highlight the effects of specific environmental toxins and endogenous molecular pathways.

Urinary VOC metabolites that are produced by ototoxic organic solvents have been shown to be increased in those with hearing loss [[10], [11], [12], [13], [14], [15]]. However, to date, only this small subset of VOC metabolites has been measured in those with hearing loss. Specifically, increases in three urinary VOC metabolites, mandelic acid and phenylgloxylic acid, both of which are metabolites of styrene, and hippuric acid, a metabolite of toluene, have been associated with ASNHL [[10], [11], [12], [13], [14], [15]]. Mandelic acid is the only urinary VOC metabolite previously found to be increased in individuals with hearing loss among those without noise exposure [10]. All three metabolites have been associated with hearing loss in those with occupational noise exposure [[11], [12], [13], [14]]. Metabolites may not be associated with ASNHL in both noise-exposed and unexposed populations because noise and organic solvents have been shown to have a synergistic effect [16]. Hippuric acid levels have also been associated with auditory processing impairments in individuals with and without occupational noise exposure [14,15].

Mercapturic acids are a larger subset of VOCs that have not been measured in those with hearing loss. These VOC metabolites are produced by glutathione conjugation, which leads to oxidative stress, and therefore may be associated with ASNHL [2,17]. Mercapturic acids are also of interest because they have a short half-life, which makes many of them ideal markers for specific environmental exposures [17]. To date, urinary concentrations of mercapturic acids have not been compared to audiometric thresholds.

The purpose of this study was to examine the association among urinary VOC metabolites and ASNHL. Although previous studies have identified an association between ototoxic organic solvent metabolites and hearing loss, this is the first study to measure the association of hearing loss and a panel of VOC metabolites that include mercapturic acids [[11], [12], [13], [14], [15], [16]]. Identifying specific urinary markers may support the effect of specific environmental toxins and endogenous molecular pathways in the auditory system.

2. Methods

2.1. Data collection

The data for this retrospective analysis came from the 2011–2012 cycle of the National Health and Nutritional Examination Surveys (NHANES). This survey is an ongoing program designed to assess the health and nutritional status of the residential, civilian, non-institutionalized U.S. population by recruiting a nationally representative sample using a stratified, multistage, probability cluster design [18]. Participants who were between 20 and 69 years old filled out a questionnaire, underwent a series of examinations, and submitted biological samples for laboratory analyses. Data were extracted from the 2011–2012 cycle of this program because these were the only years when both audiometric data and urine samples were collected at the time of this study. From this dataset, inclusion criteria included a valid hearing test, a urine sample for VOC metabolite analyses, no middle ear issues, and a withdraw from exposure to loud noises for 12 h before testing. Individuals with recent noise exposure were excluded because it is difficult to differentiate those with ASNHL and those with temporary threshold shifts. Questionnaires were used to determine age, gender, and recent and long-term noise exposure history of all participants.

2.1.1. Auditory assessment

Auditory data downloaded for this study included tympanometry and pure-tone threshold measurements. Tympanometry was conducted with the Earscan Tympanometer (Micro Audiometrics, Murphy, NC). Individuals with tympanograms that were flat or indicated negative middle ear pressure were excluded from this study to reduce the number of people with hearing loss caused by other pathologies.

Hearing tests were performed with the AD226 audiometer (Interacoustics, Middlefart, Denmark). Calibration checks and noise measurements were performed daily with the bioacoustic simulator and 1800 sound level meter (Quest Technologies, Miami, FL) [19]. Hearing was assessed by calculating the mean bilateral high-frequency thresholds at 4000, 6000, and 8000 Hz ($PTA_{4,6,8}$). Individuals with reported thresholds outside of the limits of the audiometer at any threshold were excluded from the study.

2.1.2. Urinary volatile organic compound metabolites

Urinary concentrations of 27 VOC metabolites were measured with ultra-performance liquid chromatography coupled with electrospray tandem mass spectrometry [20]. This concentration data was downloaded for all 27 metabolites, 21 of which were mercapturic acids [17].

2.2. Statistical analysis

Participants were first separated by a present or absent history of reported noise exposure. Then, within these groups, participants were placed into groups based on the quartiles of concentrations for each VOC metabolite. Participants were placed into groups because the concentrations of

VOC metabolites strongly deviated from normality, typically because of a floor effect where many participants were at the lowest level of detection. A Levene's test was run to measure homogeneity of variance in age-adjusted hearing loss across groups. Analyses of covariances (ANCOVA) were run for all VOC metabolites to measure the effect of VOC metabolite concentration groups on age-adjusted hearing loss. The family-wise error rate was controlled across all tests within each noise exposure group by assessing the false discovery rate [21,22]. All analyses were run in SPSS (IBM Corp., Chicago) except for the false discovery rate, which was calculated using a publicly available excel spreadsheet [23].

3. Results

Of the 9756 individuals in the 2011/2012 NHANES data set, only about 10% of the participants in the original dataset had both a hearing test and urine analysis. With this, only 557 participants met the inclusion criteria for the group without reported noise exposure, and 292 met the inclusion criteria for the group with reported noise exposure. In the group without reported noise exposure, the mean age was 42.9 years old, 61% percent were female, and the mean bilateral $PTA_{4,6,8}$ was 12.8 dB HL. In the group with reported noise exposure, the mean age was 45.81 years old, 29% percent were female, and the mean bilateral $PTA_{4,6,8}$ was 17.7 dB HL.

Table 1. Common and full names of 21 VOC metabolites and associated parent compounds analyzed in this study.

Parent	Metabolite	Metabolite full name[a]
Mercapturic acids		
1,3-Butadiene	DHBMA	(3,4-Dihydroxybutyl)
1,3-Butadiene	MHBMA2	(2-Hydroxy-3-butenyl)
1-Bromopropane	BPMA	(*n*-Propyl)
Acrolein	CEMA	(2-Carboxyethyl)
Acrolein	3HPMA	(3-Hydroxypropyl)
Acrylamide	AAMA	(2-Carbamoylethyl)
Acrylamide	GAMA	(2-Carbamoyl-2-hydroxyethyl)
Acrylonitrile	CYMA	(2-Cyanoethyl)
(Multiple)	HEMA	(2-Hydroxyethyl)
Benzene	PMA	(Phenyl)
Crotonaldehyde	HPMMA	(3-Hydroxypropyl-1-methyl)
N,*N*-Dimethylformamide	AMCC	(*N*-methylcarbamoyl)
Propylene oxide	2HPMA	(2-Hydroxypropyl)
Styrene	PHEMA	([1-2]-Phenyl-2-hydroxyethyl)
Toluene	BMA	(Benzyl)
Other metabolites		
Carbon-disulfide	TTCA	2-Thioxothiazolidine-4-carboxylic acid
Cyanide	ATCA	2-Aminothiazoline-4-carboxylic acid
Ethylbenzene, styrene	PGA	Phenylglyoxylic acid
Styrene	MA	Mandelic acid
Xylene	2MHA	2-Methylhippuric acid
Xylene	3,4MHA	[3-4]-Methylhippuric acid

[a] Only the R-group is listed for the full names of mercapturic acids.

Six of the 27 VOC metabolites in the NHANE's database were excluded because the measured concentrations did not vary among the participants included in the study. Of the 21 VOC

metabolites remaining, 15 were mercapturic acids. Two of the non-mercapturic acids, mandelic acid and phenylglyoxylic acid, were previously associated with hearing loss, Table 1 [10,11,13].

Table 2. F-tests and *p*-values from analysis of covariance for 21 volatile organic compound metabolites in individuals reporting a history with and without noise exposure. Bilateral high-frequency pure-tone thresholds were different among quartile groups created from 5 VOC metabolite concentrations in those without reported noise exposure.

	No noise group		**Noise exposed group**	
VOC metabolites	**F-test**	***p*-Value**	**F-test**	***p*-Value**
Mercapturic acids				
CYMA	5.40	**0.001***	1.27	0.285
PHEMA	5.97	**0.003***	0.062	0.94
DHBMA	4.69	**0.003***	0.688	0.56
MHBMA2	4.22	0.04	1.11	0.292
GAMA	4.37	**0.005***	0.435	0.728
AMCC	3.73	**0.011***	0.489	0.69
HPMMA	2.48	0.061	1.02	0.383
BMA	1.88	0.133	1.24	0.295
AAMA	2.04	0.108	0.415	0.742
HEMA	1.63	0.182	0.790	0.500
CEMA	2.16	0.092	0.212	0.888
2HPMA	0.809	0.489	1.45	0.23
3HPMA	1.43	0.233	0.355	0.785
PMA	1.33	0.265	0.38	0.767
BPMA	1.21	0.307	0.461	0.71
Other metabolites				
(3MHA + 4MHA)	1.96	0.118	3.76	0.011
2MHA	2.27	0.080	1.06	0.364
PGA	2.54	0.056	0.748	0.524
MA	2.18	0.089	0.566	0.638
ATCA	1.1	0.349	0.642	0.589
TTCA	0.393	0.758	0.231	0.875

* Statistically significant (FDR < 0.05).

Table 3. Median volatile organic compound metabolite concentrations of markers significantly associated with hearing loss and mean bilateral pure tone thresholds (standard error) for each quartile group. Thresholds are age-adjusted; a value of 0 indicates the expected hearing loss of some given their age. The thresholds in the highest quartile group are approximately 3–4 dB higher compared to those in the lower groups. For PHEMA, quartiles 1 and 2 were combined because over half of the participants were at the lower limit of detection, 0.495 ng/mL.

	Q1		**Q2**		**Q3**		**Q4**	
Metabolite	**Med (ng/mL)**	**HL mean (SE)**	**Med (ng/mL)**	**HL mean (SE)**	**Med (ng/mL)**	**HL mean (SE)**	**Med (ng/mL)**	**HL mean (SE)**
AMCC	31.5	−0.76 (0.72)	85.1	−1.41 (0.74)	161	−0.34 (0.95)	382	2.26 (0.96)
CYMA	0.527	−0.695 (0.75)	1.24	−1.12 (0.78)	2.76	−1.06 (0.79)	94.6	2.88 (0.99)
DHBMA	80.3	−1.73 (0.76)	185	−1.36 (0.73)	310	1.2 (0.90)	533	1.88 (0.92)
GAMA	6.65	−1.31 (0.78)	11	−1.52 (0.81)	16.7	0.408 (0.86)	32.2	2.3 (0.90)
PHEMA	n/a	n/a	0.495	−0.88 (0.78)	0.857	−0.44 (0.83)	1.69	2.49 (0.97)

Five of the 21 VOC metabolites were significantly associated (FDR < 0.05) with mean bilateral $PTA_{4,6,8}$ after adjusting for age, Table 2. Thresholds were approximately 3–4 dB higher in

increased metabolite concentration groups compared to the lowest concentration group, Table 3. According to the Levene's test, the variances were not statistically different across concentration groups for these five metabolites. All five of these metabolites were mercapturic acids. The parent compounds for these metabolites included acrylonitrile, styrene, 1,3-butadiene, acrylamide, and *N*,*N*-dimethylformamide.

4. Discussion

This is the first study to show a decrease in hearing thresholds in those with increased concentrations of mercapturic acid in urine. Increases in these metabolites have been linked to diseases and biological aging, possibly because they indicate systemic oxidative stress [17,24,25]. Oxidative stress damages nucleic acids and lipids and can induce cell death through apoptosis or necrosis. Mercapturic acids are associated with oxidative stress because they are created in a detoxification route for endogenous and exogenous oxidative stress-inducing toxins [17,24,26]. Mercapturic acids can also create oxidative stress by depleting glutathione [17]. Given that oxidative stress has been linked to ASNHL, it is possible that systemic markers of oxidative stress are increased in those with hearing loss because the mechanisms that create oxidative stress in the blood and urine are similar to the mechanisms that create this stress in the auditory system. Also, oxidative stress intermediates created in the blood may enter the auditory system and generate stress in an otherwise healthy ear.

Identifying increased levels of specific mercapturic acids in those with hearing loss may support the effects of environmental toxins on auditory function. Mercapturic acids have a short half-life, making them reliable markers for environmentally exposed toxins [26]. All five mercapturic acids associated with hearing loss in this study are downstream markers of VOCs found in cigarette smoke, which is known to cause hearing loss [7,27,28]. Furthermore, the parent compounds of all five metabolites are also linked to specific toxins, including synthetics and polymers. Acrylonitrile, *N*,*N*-dimethylformamide, and 1,3 butadiene are found in synthetic fibers, leathers, and rubbers, respectively [[29], [30], [31]]. Styrene and acrylamide are found in plastics and polymer gels, respectively [32,33]. Urinary metabolites may serve as more accurate markers for environmental toxin exposure than reports from individuals.

Urinary metabolites may also identify individuals susceptible to toxin exposure by discerning those that are more likely to metabolize these toxins into hazardous, rather than inert, intermediates. For example *N*,*N*-dimethylformamide may be metabolized into either AMCC or other less toxic pathways [34]. Our findings demonstrated that those with higher levels of AMCC are more likely to have hearing loss. It is possible that those who are exposed to *N*,*N*-dimethylformamide and metabolize it into AMCC may be at a higher risk of hearing loss than those who metabolize it into less toxic intermediates. This advantage may also be true for styrene. In this study, we evaluated the association of three metabolites with hearing loss. Of these three, only the mercapturic acid demonstrated a significant association. It is possible that individuals who are both exposed to styrene and metabolize it through the mercapturic acid pathway are susceptible to ASHNL compared to those who metabolize styrene through less toxic intermediates.

The findings of this study may also explain endogenous pathways key to regulating oxidative stress induced hearing loss. For instance, acrylamide is a downstream product of lipid peroxidation [35]. The association between hearing loss and this metabolite of may specifically support the damaging effect of lipid peroxidation over other harmful effects of oxidative stress, such as apoptosis and necrosis. Investigating the relationship of these metabolites may explain how individuals who favor specific metabolic pathways may be more susceptible to hearing loss.

Associations between hearing loss and VOC metabolites were only found in individuals without reported noise exposure. This is surprising considering that organic solvents and noise synergistically affect hearing loss [16]. There has also been more research to support an association of hearing loss and VOC metabolites in noise exposed participants compared to those without reported noise-exposure [[10], [11], [12], [13], [14], [15]]. We may have not been able to identify a significant association between hearing loss and noise exposure in this group because there were less individuals in this group. It is also possible that the increased variance in pure-tone thresholds caused by varying degrees of noise exposure among the participants made it difficult to detect a significant association with VOC metabolite concentrations.

Mandelic acid, phenylgloxylic acid, and hippuric acid, have been associated with ASNHL in previous research [[10], [11], [12], [13], [14], [15]]. In this current retrospective study, we evaluated the association of hearing loss with mandelic acid and phenylgloxylic acid, but data on hippuric acid concentrations were not available. Although neither mandelic acid or phenylgloxylic acid were associated with hearing loss in either noise exposure group, a trend in increased mean $PTA_{(4,6,8)}$ was found in quartile groups based on higher concentrations for both mandelic acid ($p = 0.089$) and phenylgloxylic acid ($p = 0.056$) in individuals without reported noise exposure. Significant findings may have been obtained with a larger sample size.

One limitation of this study is that differences between metabolic concentration quartile groups may have been explained by lurking variables such as noise exposure, cardiovascular health, or exposure to other toxins. These variables were excluded in this study because we tested 22 VOC metabolites and did not have the sample size to include more variables. However, even with this limitation, it is still interesting that associations were specific to these five VOC metabolites. Of these lurking variables, noise likely has the greatest influence on hearing thresholds. Although all significant associations were found within individuals without reported noise exposure, it is possible that unreported noise exposure was higher in those in higher metabolite concentration groups. However, if noise was a significant lurking variable, then it likely would have had a larger difference in metabolite concentration groups among those with reported noise exposure. Despite this, a follow up study is needed to focus on the interaction of the five VOC metabolites associated with hearing loss in this study and lurking variables such as noise and environmental exposure and cardiovascular health.

5. Conclusion

This was the first study to detect an increase of ASNHL in those with higher levels of mercapturic acids in urine. Thresholds were about 3–4 dB higher in high metabolite concentration groups compared to low concentration groups for five mercapturic acids. The parent compounds of these mercapturic acids included acrylonitrile, styrene, 1,3-

butadiene, acrylamide, and *N*,*N*-dimethylformamide. These findings support further investigation into the effect of environmental toxins and metabolic regulation on urinary concentrations of VOC metabolites in those with ASNHL.

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