Chemosphere 306 (2022) 135571

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Decreased hearing levels at frequencies for understanding speech in tannery workers exposed to a high level of trivalent chromium in Bangladesh

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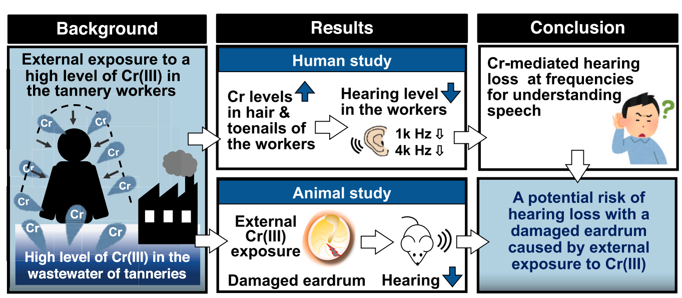
HIGHLIGHTS

- Examined the effect of Cr(III) on hearing, a crucial sense, in tannery workers (TWs).
- Decrease of hearing levels (DHLs) at 1 k and 4 k Hz for understanding speech in the TWs.
- DHL with damaged eardrum in mice treated with eardrops containing Cr(III).
- General classification of DHL into sensorineural DHL and conductive DHL (CDHL).
- CDHL caused by Cr(III) with corrosiveness in TWs exposed to a high level of Cr (III).

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling Editor: Jian-Ying Hu

Keywords:
Chromium
Decrease of hearing level
Eardrum
External exposure
Tannery

ABSTRACT

Hexavalent chromium [Cr(VI)], which has a strong corrosive effect, has been reported to cause perforation of the eardrum. Trivalent chromium [Cr(III)] also has a weak corrosive effect. However, there has been no study on the effects of exposure to Cr, either Cr(VI) or Cr(III), on hearing levels in animals or humans. In this study, the effect of Cr(III) exposure on hearing levels was determined in a human study. Then the reproducibility of the results obtained in the human study and the etiology were investigated in an animal study. The mean levels of total chromium (t-Cr) in hair and toenails from 100 Bangladeshi tannery workers were >20-fold and >360-fold higher, respectively, than those in hair and toenails from 49 Bangladeshi non-tannery workers (office workers). Multivariate analysis revealed decreases of hearing levels (DHLs) at 1 k and 4 k Hz, frequencies that are crucial

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https://doi.org/10.1016/j.chemosphere.2022.135571
Received 28 March 2022; Received in revised form 21 June 2022; Accepted 28 June 2022
Available online 4 July 2022
0045-6535/

for understanding language, but not at 8 k and 12 k Hz, in the tannery workers. Since >99.99% of t-Cr in the wastewater that the workers were in direct contact with in the tanneries was Cr(III), the epidemiological results suggest Cr(III)-mediated DHLs in the tannery workers. The results of animal experiments in this study further showed that treatment with eardrops but not intraperitoneal injection with the same amount of Cr(III) that tannery workers might be exposed to resulted in DHL with a damaged eardrum in mice. Previous studies suggested that Cr(III) can directly reach the eardrums of tannery workers via droplets in the air. Cr(III) could also reach the eardrum via picking an ear canal with a finger contaminated with tannery wastewater including Cr(III). Taken together, the results of both human and animal studies suggest the risk of DHLs caused by damage of the eardrum through external exposure to Cr(III) via the ear canal.

1. Introduction

It is estimated that more than 360 million people worldwide suffer from a decrease of hearing level (DHL) (Olusanya et al., 2014). DHL is one of the most important public health issues that directly affect social life worldwide (Emmett and West, 2015). Hearing levels at lower frequencies of 0.5 k-4 k Hz centered on 1 k Hz are especially important for understanding speech, while those at higher frequencies such as 8 k and 12 k Hz have less effects on daily life (Yueh et al., 2003; CDC, 2005). DHL is classified into sensorineural disorder and conductive disorder. Sensorineural DHL, represented by an age-related or noise-induced disorder, is caused by neural impairments including inner ear and auditory nerve impairments. This type of DHL generally occurs at higher frequencies (NIH, 1990; Yueh et al., 2003; Li et al., 2017). Conductive DHL is caused by disorders of the outer and middle ears such as traumatic damage of the eardrum and auditory ossicle that conduct sound. Conductive DHL can develop from lower frequencies for understanding speech (Ahmad and Ramani, 1979). Because the diagnostic characteristics including initial-affected frequencies of DHL are different between sensorineural and conductive disorders, pure-tone audiometry (PTA) could be a useful tool for distinguishing them. Since preventive methods as well as therapies for sensorineural DHL and conductive DHL are quite different, their differential diagnosis is indispensable for health risk assessment of environmental factors. However, there have been very few studies on the discrimination of sensorineural and conductive DHLs in the field of environmental health sciences.

There have been few *in vitro* and *in vivo* studies on the toxicities of trivalent chromium [Cr(III)] compared to the number of studies for toxicities of hexavalent chromium [Cr(VI)] (Gibb et al., 2000; WHO, 2013; Tsuchiyama et al., 2020). There have been even fewer studies focusing on disorders in humans caused by exposure to Cr(III). Therefore, it has been believed that Cr(III) is a safe chemical and it is used worldwide in various industries including leather, coating material and commodity industries as a fungible chemical of Cr(VI) (WHO, 2009).

Since the global trade value of leather products is estimated to be 100 billion USD per year (UNIDO, 2010), leather industry plays an important role in the economy of the world. However, a previous study showed that only 10% of tannery workers in Bangladesh could survive after 50 years of age (Maurice, 2001), though the causes of mortality have remained unknown. Our previous study revealed that the levels of total chromium (t-Cr) in hair and toenails from tannery workers were >20-fold and >360-fold higher, respectively, than those in non-tannery workers in Bangladesh (Al Hossain et al., 2019). The t-Cr levels in hair and toenails of non-tannery workers were comparable to those in general people in other countries (Randall and Gibson, 1989; Soininen et al., 2003; Rajpathak et al., 2004; Son et al., 2018; Al Hossain et al., 2019). These results indicate that tannery workers were exposed to higher levels of Cr than the levels to which general people were exposed (Al Hossain et al., 2019; Tsuchiyama et al., 2020). The operation of leather manufacture is classified into 3 steps consisting of pre-tanning, tanning and post-tanning (Al Hossain et al., 2019). A large amount of chemicals including Cr(III) represented by basic chromium sulfate (BCS) is consumed in the tanning step (Thanikaivelan et al., 2005; Tsuchiyama et al., 2020). Our previous study revealed that >99.99% of t-Cr in the wastewater to which workers were directly exposed inside the tanneries was Cr(III) (Tsuchiyama et al., 2020). Exposure of tannery workers to a large amount of Cr(III) has also been shown in other studies (Aitio et al., 1984; Randall and Gibson, 1989). On the other hand, the health risk of Cr(VI) exposure in the tannery workers who participated in this study was limited because the mean concentration of Cr(VI) in wastewater to which the workers were exposed was lower than the standard level (50 μg/L) of Cr(VI) in drinking water in other countries (NHMRC, 2011; Kato et al., 2016; Tsuchiyama et al., 2020). Taken together, the results of our previous environmental and epidemiological studies (Al Hossain et al., 2019; Tsuchiyama et al., 2020; Yuan et al., 2021) indicated that the tannery workers who participated in this study were exposed to a high level of Cr(III).

The Cr(III)-mediated health disorders in the tannery workers remain unclear, although cutaneous and renal disorders were shown to be caused by external exposure to Cr(III) via skin and internal exposure to Cr(III) via digestion, respectively, in Bangladeshi tannery workers in our previous studies (Al Hossain et al., 2019; Tsuchiyama et al., 2020). Tannery-originating Cr(III) pollution could be a health risk for the residents around the tannery built-up area (Yoshinaga et al., 2018; Yuan et al., 2021). There is the possibility of not only external Cr(III) exposure to the skin, eyes and eardrums through cosmetics, soaps and leather products but also internal Cr(III) exposure through ingestion of supplements and diabetes medicines for people worldwide (WHO, 2009). Therefore, the results of health risk assessment of Cr(III) in tannery workers exposed to a high level of Cr(III) are useful in various situations.

Recently, internal exposure to various elements including arsenic (As) (Li et al., 2017), barium (Ba) (Ohgami et al., 2016), manganese (Mn) (Ohgami et al., 2018) and iron (Fe) (He et al., 2019) has been reported to cause sensorineural DHL in humans. However, there has been no study on the effects of sole exposure to chromium, either Cr(VI) or Cr(III), on DHL in animals or humans. The effect of exposure to Cr(III) on hearing levels was first investigated in our epidemiological study. The reproducibility of Cr(III)-mediated DHL in humans and the etiology were further examined in our animal study. This study sheds light on a rare type of DHL caused by exposure to Cr(III).

2. Materials and methods

2.1. Basic information and ethical permission for the study

A total of 149 male subjects including 100 tannery workers in a tannery built-up area in Dhaka City and 49 non-tannery workers (office workers) in Gazipur City in Bangladesh participated in this cross-sectional study. Basic information including the investigation area, subjects, collection of biological samples and environmental conditions inside tanneries was the same as our previous studies (Al Hossain et al., 2019; Tsuchiyama et al., 2020). As shown in our previous study (Al Hossain et al., 2019), t-Cr levels in humans were determined by using hair and toenail samples because elements stably remained in the samples (Salcedo-Bellido et al., 2021). Since most of the participants were Muslim, for whom alcohol consumption is forbidden, no information on alcohol consumption was obtained as in other studies conducted in Bangladesh (Yajima et al., 2018; Kato et al., 2020; Tsuchiyama et al., 2020). Noise levels in the pre-tanning, tanning and post-tanning steps in the tanneries and noise levels in offices were measured by using a digital sound level meter (GS-04, Be-s, Japan). Mean noise levels (A-weighted) in the pre-tanning, tanning and post-tanning steps in tanneries were 70.85 dB sound pressure level (SPL), 84.25 dB SPL and 88.55 dB SPL, respectively. Based on the results, the workers who were engaged in the pre-tanning step and in the tanning and post-tanning steps were classified into a no occupational noise-exposed group and an occupational noise-exposed group, respectively, as described previously (Li et al., 2021), because there was no information on individual noise exposure levels in this study. The workers did not use any personal protective equipment for noise (e.g., earplugs) in the tanneries.

Since the mean noise level in the offices was 58.81 dB SPL, the non-tannery workers were classified into the no occupational noise-exposed group. The subjects participated in this study after providing signed informed consent forms. This study was performed after approval by Nagoya University Bioethics Committee following the regulations of the Japanese government (2013-0070 and 2016-0036) and by the Faculty of Biological Science, University of Dhaka (5509/Bio.Sc).

2.2. Hearing levels in humans

Hearing levels of subjects were presented as auditory thresholds at frequencies of 1 k, 4 k, 8 k and 12 k Hz as shown in previous studies (Ohgami et al., 2016; Li et al., 2017; He et al., 2019). Auditory thresholds were determined by PTA with air conduction by the method described previously (Ohgami et al., 2016).

2.3. Levels of t-Cr in biological samples from humans

Levels of t-Cr in hair and toenails were measured by inductively coupled plasma mass spectrometry (ICP-MS, 7500cx, Agilent Technologies) following the method previously described (Kato et al., 2013; Kurniasari et al., 2022). Briefly, all of the hair and toenail samples were carefully washed with detergent using ultrasonication followed by acetone treatment to remove possible substances that had adhered to the surfaces of samples. In the next step, samples were incubated in 61% HNO_3 (Grade: EL, Kanto Chemical Co., Inc) at 80 °C for 3 h followed by further incubation in 30% H_2O_2 (Grade: Atomic Absorption Spectrometry, Kanto Chemical Co., Inc) at 80 °C for 3 h. Finally, levels of Cr, As, Ba, Fe and Mn in the samples were measured by ICP-MS. Custom Assurance Standard (SPEX: XSTC-622, NJ, USA) was used to develop standard curves. A certified reference material (Trace Elements in River Water, CRM 7202-C, National Metrology Institute of Japan, Tsukuba, Japan) was used to verify the standard curve of elements.

2.4. Mice

Male ICR mice of 3–4 weeks of age were purchased from Japan SLC Inc (Hamamatsu, Japan). The animal experiments were approved by the Institutional Animal Care and Use Committee of Nagoya University (approval number: M210309-002) and were performed in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals.

2.5. Exposure of eardrums to BCS via eardrops in mice

BCS (Chromitan® B, Stahl, Netherlands), which is actually used in tanneries in Bangladesh, was prepared for external treatment of eardrums with Cr(III). One eardrum of each of the mice was used in order to prevent leaking of the solution after the external treatment with Cr(III). A unilateral eardrum was treated once with 10 μL of BCS-dissolved distilled water that contained the mean concentration (1.9 μg/μL) of Cr(III) in wastewater samples [19 μg of Cr(III) as the total amount per ear] previously measured in Bangladeshi tanneries (Tsuchiyama et al., 2020). A unilateral eardrum of each of the control mice was also treated once with 10 μL of distilled water. Hearing levels were measured by auditory brainstem response (ABR) following the method previously described (Ohgami et al., 2010; Negishi-Oshino et al., 2019) before and 3 days after the treatment with ear drops. In addition, eardrums treated and those not treated with Cr(III) were morphologically analyzed by a scanning electron microscope (SEM) and SEM-energy dispersive X-ray (SEM-EDX) (JSM-7610F; JEOL Ltd, Japan) by methods previously described (Inoué and Osatake, 1988; Kakoi and Anniko, 1996; Zhang et al., 2012). The EDX analyses were performed with an acceleration voltage of 20.00 kV and 1000× magnification.

2.6. Statistical analyses

Categorical data were presented as numbers and percentages and analyzed by the chi-squared test, while quantitative data were presented as medians and interquartile ranges (IQRs) and analyzed by the Mann-Whitney *U* test or the Kruskal-Wallis *H* test (Xu et al., 2021). Linear regression models were used in multivariate analyses and in trend tests to evaluate the correlation between t-Cr levels in biological samples and hearing levels. Confounders included age (Davis et al., 2016), BMI (Hu et al., 2020), smoking (Sumit et al., 2015) and noise exposure (Basner et al., 2014; Mirza et al., 2018), which have been reported to be associated with hearing level. Nagelkerke's R^2 value and Pseudo R^2 value were calculated to evaluate the goodness of fit of regression models and the relative contributions of the variables to DHL, respectively (Al Hossain et al., 2019; Tsuchiyama et al., 2020). Cut-off values of hearing levels at 1 k, 4 k, 8 k and 12 k Hz were defined as ≥15 dB, ≥25 dB, ≥30 dB and ≥50 dB, respectively, following previous studies (Parving et al., 2000; Ak et al., 2004; Hunt et al., 2017; Lee et al., 2018). All statistical analyses were performed using SPSS 24.0 (SPSS Inc., Chicago, IL, USA). A two-sided P value < 0.05 was judged as being statistically significant.

3. Results

3.1. Basic characteristics of the subjects in this study

The characteristics of the subjects in this study are shown in Table 1. In order to eliminate a gender bias, only male workers participated in this study. To examine a dose-dependent effect of Cr exposure, Bangladeshi tannery workers were divided into a group of workers who were exposed to a low level of t-Cr (tannery workers with low Cr, n = 50) and a group of workers who were exposed to a high level of t-Cr (tannery workers with high Cr, n = 50) by the median levels of t-Cr in their hair and toenails. Levels of t-Cr in hair and toenails from Bangladeshi officer workers (non-tannery workers, n = 49) were comparable to those in hair and toenails from general people (Randall and Gibson, 1989; Soininen et al., 2003; Rajpathak et al., 2004; Son et al., 2018). Levels of t-Cr in hair and toenails of the tannery workers with low Cr were 6.1-fold and 22.1-fold higher, respectively, than those in non-tannery workers (Bangladeshi officer workers, n = 49). Levels of t-Cr in hair and toenails of the tannery workers with high Cr were 21.7-fold and 582.7-fold higher, respectively, than those in non-tannery workers. Although there was no significant difference in age or smoking among the three groups, body mass index (BMI) in tannery workers with a low level of t-Cr was significantly higher than those in tannery workers with a high level of t-Cr and non-tannery workers. The auditory thresholds in the older group (≥30 years old, n = 102) were higher than those in the younger group (<30 years old, n = 47) at 1 k Hz ($P = 0.038$), 8 k Hz ($P = 0.002$) and 12 k Hz ($P = 0.001$) but not at 4 k Hz ($P = 0.056$) (Supplemental Figure S1A). The auditory thresholds in the occupational noise-exposed group (n = 76) were also higher than those in the no occupational noise-exposed group (n = 73) at 1 k Hz ($P < 0.001$) and 4 k Hz ($P < 0.001$) but not at 8 k Hz ($P = 0.277$) and 12 k Hz ($P = 0.607$) (Supplemental Figure S1B).

Table 1
Basic information for tannery and non-tannery workers in Bangladesh.

Variables		Non-tannery workers	Tannery workers with low Cr [c]	Tannery workers with high Cr [c]	*P* value
		(n = 49)	(n = 50)	(n = 50)	
Age (years)[a]		32.00 (12.00)	37.50 (22.00)	33.50 (20.00)	0.300
Age group (%)[b]	<30 years old	14 (28.6)	14 (28.0)	19 (38.0)	0.483
	≥30 years old	35 (71.4)	36 (72.0)	31 (62.0)	
BMI (kg/m^2)[a]		22.72 (3.72)	24.56 (3.81)	22.24 (5.42)	0.005
BMI group (%)[b]	Underweight (<18.5)	5 (10.2)	2 (4.0)	4 (8.0)	0.029
	Normal weight (18.5–25)	39 (79.6)	29 (58.0)	33 (66.0)	
	Overweight (≥25)	5 (10.2)	19 (38.0)	13 (26.0)	
Smoking (%)[b]	Smoker	24 (49.0)	16 (32.0)	26 (52.0)	0.095
	Non-smoker	25 (51.0)	34 (68.0)	24 (48.0)	
Occupational noise exposure[b]	Yes	0	30 (60.0)	46 (92.0)	<0.001
	No	49 (100.0)	20 (40.0)	4 (80.0)	
Cr levels (μg/g)[a]	in hair	0.169 (0.167)	1.032 (0.838)	3.672 (3.433)	<0.001
	in toenail [d]	0.277 (0.226)	6.111 (9.353)	161.415 (229.086)	<0.001

[a] Continuous data are presented as medians (IQR) and *P* values were calculated by using the Kruskal-Wallis *H* test.
[b] Categorical data are presented as n (%) and *P* values were calculated by using the chi-squared test.
[c] Tannery workers are divided by the median of t-Cr levels in hair.
[d] Tannery workers are divided by the median of t-Cr levels in toenails.

3.2. *Univariate analysis of hearing levels in tannery workers and non-tannery workers*

Hearing levels were compared among non-tannery workers (controls), tannery workers with low levels of t-Cr (n = 50) and tannery workers with high levels of t-Cr (n = 50) in hair (Fig. 1A) and toenails (Fig. 1B) by univariate analysis. The hearing levels at 1 k and 4 k Hz, but not those at 8 k and 12 k Hz, in tannery workers with low and high levels of t-Cr in hair and toenails were significantly decreased compared with those in non-tannery workers. These results suggest that excess exposure to Cr decreases hearing levels at 1 k and 4 k Hz in humans.

3.3. *Multivariate analysis of hearing levels in tannery workers and non-tannery workers*

Hearing levels were again compared among non-tannery workers (controls), tannery workers with low levels of t-Cr (n = 50) and tannery workers with high levels of t-Cr (n = 50) in hair (Fig. 2A) and toenails (Fig. 2B) by multivariate analysis. The hearing levels at 1 k and 4 k Hz, but not those at 8 k and 12 k Hz, in tannery workers with low and high levels of t-Cr in hair and toenails were significantly decreased compared with those in non-tannery workers after adjustments for age, BMI and smoking (Model 1 in Fig. 2) by linear regression models. The DHLs in tannery workers with low and high levels of t-Cr were maintained after additional adjustment with noise exposure (Model 2 in Fig. 2) and after further additional adjustment with As, Ba, Fe and Mn levels in hair and toenails (Model 3 in Fig. 2), which have been reported to be associated with hearing levels in Bangladeshi (Li et al., 2017; Ohgami et al., 2016, 2018; He et al., 2019). These results indicated that excess exposure to Cr decreases hearing levels at 1 k and 4 k Hz in humans. Comparable DHLs in tannery workers with exposure to a low level of Cr and tannery workers with exposure to a high level of Cr indicate that a dose-dependent effect of Cr exposure on hearing level is limited. The characteristics of hearing levels seem to be different from those of sensorineural DHL caused by internal exposure via ingestion to various elements in previous studies (Ohgami et al., 2012, 2016; Li et al., 2017; Kato et al., 2020).

3.4. *Relative contributions of t-Cr levels to hearing levels in tannery workers and non-tannery workers*

The relative effects of t-Cr levels in hair and toenails and other confounders on hearing levels were further investigated by the Nagelkerke R^2 and Psuedo R^2 values (Table 2). The results indicated that Cr levels in hair and toenails were the strongest contributor to hearing levels at 1 k and 4 k Hz. The contributions of the Cr levels in hair (12.5%) and toenails (12.5%) to hearing levels at 1 k Hz were especially high. While age was the strongest contributor to hearing levels at 8 k and 12 k Hz as previously shown in Bangladeshi (Li et al., 2018; Kato et al., 2020), noise exposure showed limited effects on the hearing levels at all frequencies after adjusting for other confounders.

3.5. *DHLs with damaged eardrums caused by treatment with eardrops containing BCS in mice*

One ear in each of the intervention mice was treated with 10 μL of eardrops consisting of BCS including 19 μg Cr(III), which corresponds to the mean concentration of Cr(III) in wastewater from Bangladeshi tanneries (Tsuchiyama et al., 2020). One ear in each of the control mice was treated with 10 μL of eardrops consisting of the solvent of BCS (distilled

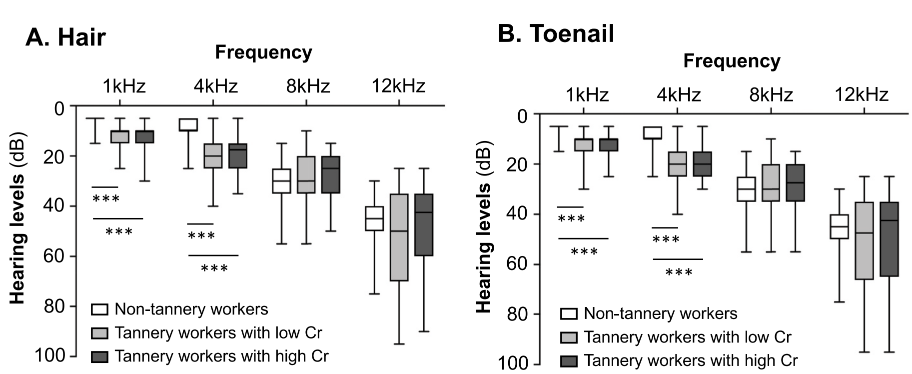

Fig. 1. Comparison of hearing levels by univariate analysis. (A, B) Hearing levels at 1 k, 4 k, 8 k and 12 k Hz in non-tannery workers (n = 49) and tannery workers with a low level of Cr exposure (n = 50) and a high level of Cr exposure (n = 50) are presented. Levels of Cr exposure were investigated by using hair (A) and toenail (B) samples. The differences were examined by pairwise comparison after the Kruskal-Wallis *H* test. ***$P < 0.001$.

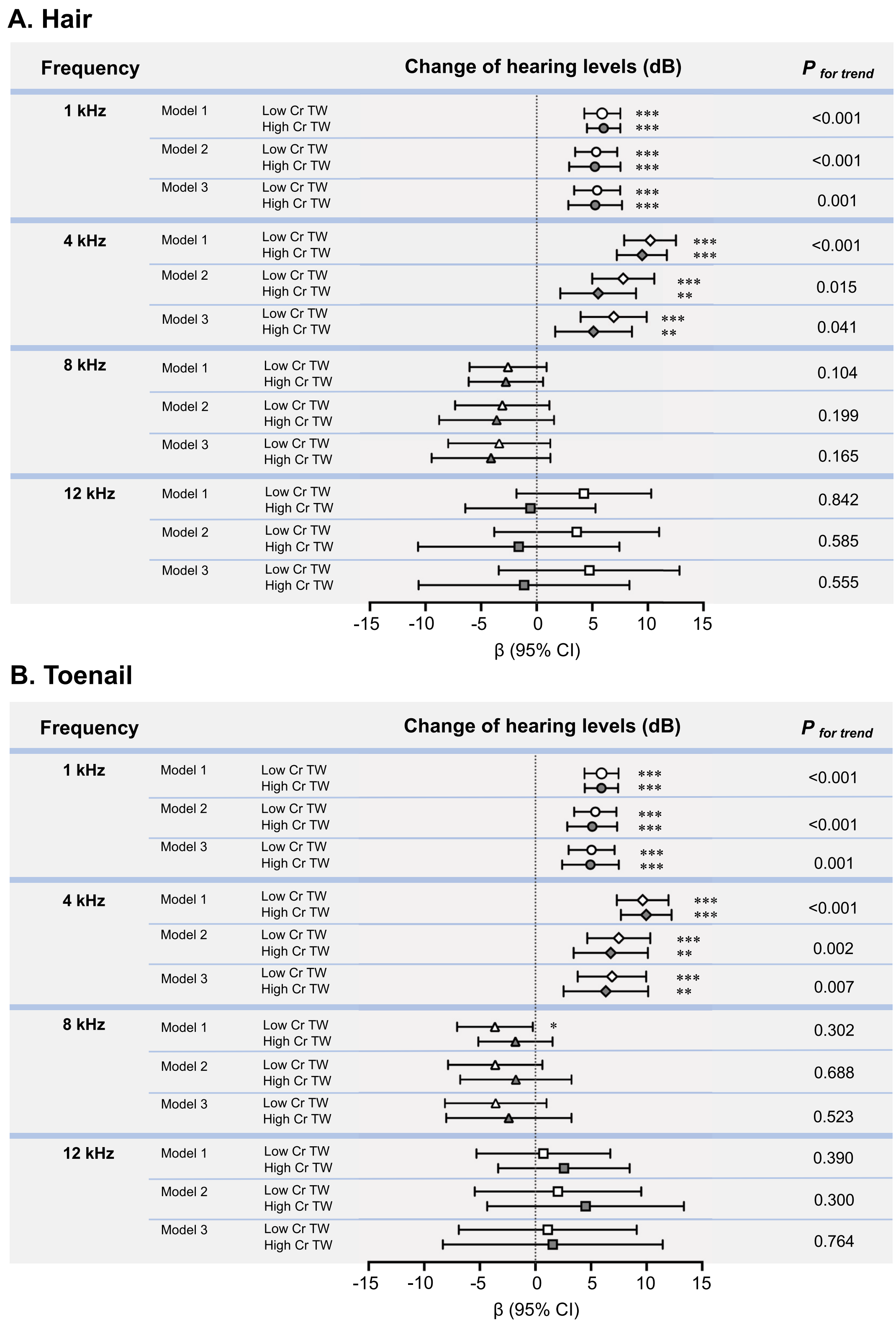

Fig. 2. Comparison of hearing levels by multivariate analysis. Changes [β (95% confidential interval)] in hearing levels (dB) at 1 k, 4 k, 8 k and 12 k Hz in tannery workers with a low level of Cr exposure (Low Cr TW, n = 50) and a high level of Cr exposure (High Cr TW, n = 50) were compared with those in non-tannery workers who were included as a reference group in the multiple linear regression model. Cr exposure levels were investigated by using hair (A) and toenail (B) samples. Model 1: adjusted with age, BMI and smoking. Model 2: adjusted with variables in Model 1 and occupational noise exposure. Model 3: adjusted with variables in Model 2 and levels of As, Ba, Fe, and Mn in hair (A) and toenails (B). Age, BMI and levels of As, Ba, Fe and Mn in human samples were included in the models as continuous data. Smoking and occupational noise exposure were included in the models as categorical data. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 2
Relative contributions of variables determined by Pseudo R^2 (%).

		1 kHz (≥15 dB)	4 kHz (≥25 dB)	8 kHz (≥30 dB)	12 kHz (≥50 dB)
Model 1[a]					
Pseudo R^2 (%)	Hair chromium	12.5	5.5	3.7	1.7
	Age	6.2	2.6	6.6	15.9
	BMI	1.4	0.1	0.1	1.9
	Smoking	0.1	0.2	0.5	0
	Occupational noise exposure	0.1	2.7	1.3	0
	Redundancy	79.7	88.9	87.8	80.5
Nagelkerke R^2 (%) of model		32.1	22.4	11.8	20.9
Model 2[a]					
Pseudo R^2 (%)	Toenail chromium	12.5	4.5	2.3	0.2
	Age	6.1	2.7	7.0	16.2
	BMI	1.6	0.0	0.1	2.3
	Smoking	0.1	0.1	0.3	0.0
	Occupational noise exposure	0.0	2.0	0.5	0.1
	Redundancy	79.7	90.7	89.8	81.2
Nagelkerke R^2 (%) of model		32.1	21.4	10.4	19.4

[a] Results from logistic regression models showing relative contributions of variables to hearing levels at 1 k, 4 k, 8 k and 12 k Hz in hair (Model 1) and toenails (Model 2) from non-tannery workers (n = 49) and tannery workers with a low level of Cr exposure (n = 50) and a high level of Cr exposure (n = 50) are presented. The goodness of fit of models is shown as Nagelkerke's R^2 (%) and relative contributions of variables were determined by Pseudo R^2 (%). Cut-off values of hearing levels at different frequencies are based on those used in previous studies (Parving et al., 2000; Ak et al., 2004; Hunt et al., 2017; Lee et al., 2018). All covariates were included in the models as categorical data.

water). Physiological (Fig. 3) and morphological (Fig. 4) analyses were performed for the intervention mice and the control mice. Considering the difference of audible frequencies in mice (1 k-40 k Hz) and humans (20-20 k Hz) as shown previously (Heffner and Heffner, 2007), hearing levels at 4 k Hz as a representative frequency of lower frequencies (Fig. 3A) and at 32 k Hz as a representative frequency of higher frequencies (Fig. 3B) were measured. Hearing level at the lower frequency (Fig. 3A), but not that at the higher frequency (Fig. 3B), in the intervention mice was significantly lower than that in the control mice. Increased damage of the eardrum (Fig. 4A and B) externally treated with 19 μg of Cr(III) (Fig. 4C and D) was observed by analyses of SEM (Fig. 4A and B) and SEM-EDX (Fig. 4C and D) and was confirmed by semi-quantitative analysis (Fig. 4E) based on the method shown in Supplemental Figure S2. These results suggest the DHL with a damaged eardrum at a lower frequency is caused by external exposure to Cr(III).

4. Discussion

Internal exposure to Cr(III) in tannery workers via ingestion of large droplets including Cr(III) in the air inside tanneries has been reported (Aitio et al., 1984). External exposure to Cr(III) in tannery workers also occurs via wastewater on the floor and large droplets in the air because tannery workers suffer from disorders of external organs including otorhinolaryngologic, dermatological, ophthalmologic and oral disorders (Aitio et al., 1984; Cuberos et al., 2009; Al Hossain et al., 2019). An otorhinolaryngologic disorder has been reported to be the most frequent disorder in tannery workers despite limited information on the disorder (Cuberos et al., 2009). Therefore, the present study was carried out to clarify the effects of Cr(III) exposure on ear function in tannery workers.

This cross-sectional study first demonstrated that there were significantly decreased hearing levels at lower frequencies (1 k and 4 k Hz) but not at higher frequencies (8 k and 12 k Hz) in the tannery workers compared to those in the non-tannery workers. A previous study for participants with the same basic information as that shown in Table 1 indicated positive correlations of duration of tannery work (years) with Cr(III) levels in hair ($r = 0.62$, $P < 0.001$) and toenails ($r = 0.61$, $P < 0.001$) in tannery workers (Al Hossain et al., 2019). The present study further indicated positive correlations of duration of tannery work (years) with hearing levels at 1 k Hz ($r = 0.667$, $P < 0.001$) and 4 k Hz ($r = 0.678$, $P < 0.001$) but not at 8 k Hz ($r = 0.020$, $P = 0.811$) and 12 k Hz ($r = 0.130$, $P = 0.114$) (Supplemental figure S3). These results suggest that tannery workers with longer working years have higher risks of DHLs at lower frequencies (1 k and 4 k Hz) due to higher exposure levels of Cr(III). The peak frequency of Japanese speech has been reported to be 1 k Hz (Shiraishi et al., 2022), while the peak frequency of Bengali, a native language in Bangladesh, has been reported to be about 4 k Hz (Narne et al., 2021). These results also suggest that exposure to Cr(III) results in DHL at frequencies that are important for understanding various languages. Clinical characteristics of the DHL caused by exposure to Cr(III) in the tannery workers were then considered. Age-related DHL and also excess internal exposure to As (Li et al., 2017; Kato et al., 2020), Ba (Ohgami et al., 2012), Mn (Ohgami et al., 2018) and Fe (He et al., 2019) via drinking water cause sensorineural DHL that starts at higher frequencies. Noise-induced DHL, a representative sensorineural disorder, typically starts from a decreased hearing level at 4 k Hz and then progresses to a decreased hearing level at 8 k Hz in humans (NIH, 1990; Mirza et al., 2018; Nieman and Oh, 2020). However, the characteristics of decreased hearing levels in the tannery workers are quite different from the previously reported characteristics of sensorineural DHL caused by aging, noise and internal exposure to elements such as As, Ba, Mn and Fe. Correspondingly, the correlation between t-Cr levels in hair and toenails and DHLs was maintained after considering confounding factors such as age, noise and elements that affect hearing

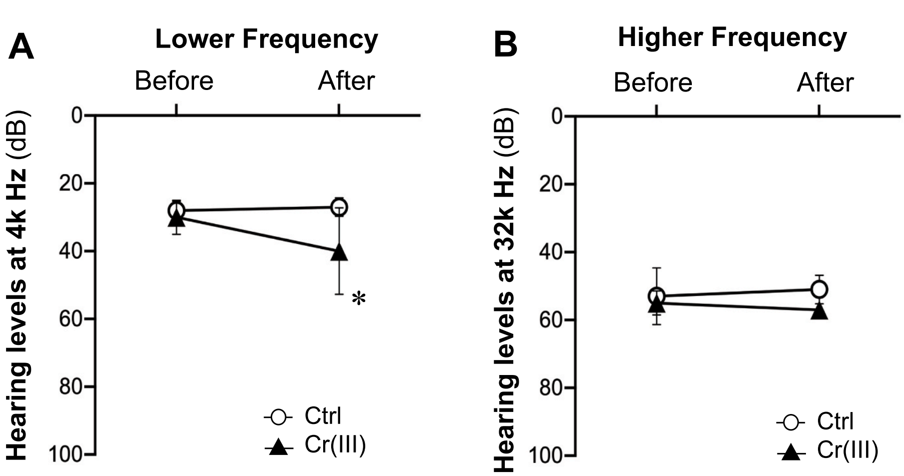

Fig. 3. Effect of Cr(III) treatment by eardrops on hearing levels in mice. (A, B) Hearing levels in 3-4-week-old ICR mice were measured before and 3 days after one treatment with eardrops containing 10 μL distilled water (Ctrl, n = 5) or 10 μL distilled water including BCS [Cr(III), n = 5] that contained the mean concentration (1.9 μg/μL) of Cr(III) in wastewater samples in the tanning step inside Bangladeshi tanneries (Tsuchiyama et al., 2020). Hearing levels at 4 k Hz (A) and at 32 k Hz (B) were measured by auditory brainstem response (ABR). The differences were examined by the Mann-Whitney U test. $^*P < 0.05$.

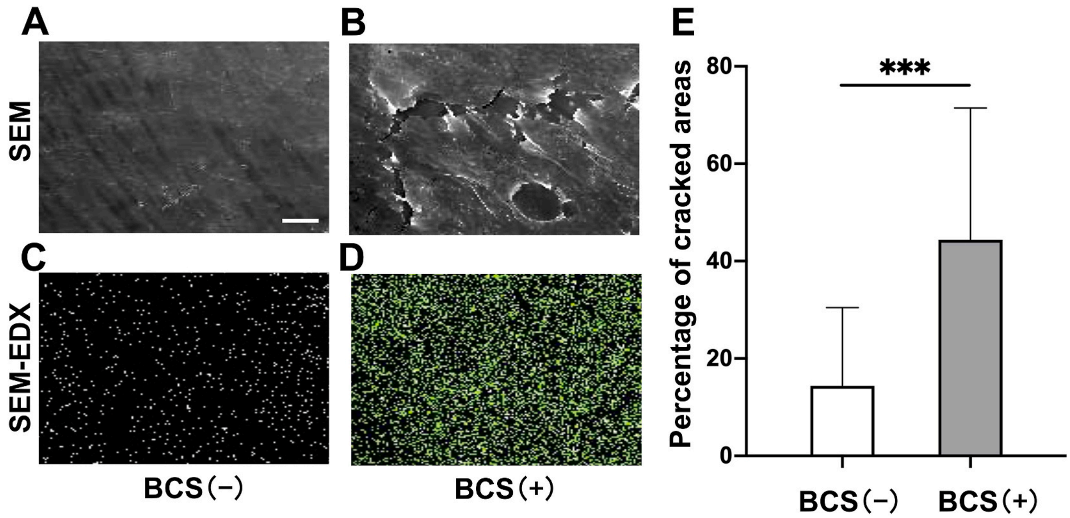

Fig. 4. Damage of eardrums treated with Cr(III) in mice. (A–E) Effects of basic chromium sulfate (BCS) treatment by eardrops on eardrums were morphologically investigated. Identical images of an eardrum treated with BCS (B, D) and an untreated control eardrum (A, C) that were analyzed by a scanning electron microscope (SEM) (A, B) and SEM-energy dispersive X-ray (SEM-EDX) (C, D) are presented. Intensity of the green color in photographs (C, D) shows the distribution of t-Cr on the eardrum treated with BCS (D) and the untreated control eardrum (C). A graph (E) of the percentages of cracked areas in eardrums treated and not treated with BCS determined by semi-quantitative analysis for which the method is shown in Supplemental Figure S2 is presented. Fifteen images from 3 mice were investigated in each group. The difference was analyzed by the Mann-Whitney U test. ***$P < 0.001$. Scale bar: 10 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

level. Taken together, the clinical characteristics of Cr(III)-mediated DHL in the tannery workers were different from the characteristics of sensorineural DHL.

Cr(VI), which has a strong corrosive effect, has been reported to cause perforation of the eardrum, though there is no information on the effect of Cr(VI) on hearing level (Gibb et al., 2000). Cr(III) also has a corrosive effect, but its corrosive effect is weaker than that of Cr(VI) (WHO, 2009). Cr(III) can directly reach the eardrums of tannery workers via droplets in the air (Aitio et al., 1984; Cuberos et al., 2009). Cr(III) could also indirectly reach the eardrum via picking an ear canal with a finger contaminated with wastewater including Cr(III). Therefore, the results in the human study led to a hypothesis that conductive DHL via damage of the eardrum caused by external exposure to Cr(III) could occur in tannery workers. The animal study showed that external exposure to BCS containing the mean level of Cr(III) in wastewater inside tanneries (Tsuchiyama et al., 2020) through eardrops decreased the hearing level at a lower frequency but not that at a higher frequency with damage of eardrums in mice. On the other hand, there was a limited effect of internal exposure to BCS containing the same amount of Cr(III) through intraperitoneal injection on hearing levels at lower and higher frequencies in mice (Supplemental Figure S4). These results suggest that DHL can occur more easily by external exposure to Cr(III) than by internal exposure to Cr(III). Consideration should be given to the use of ear protectors for preventing Cr(III)-mediated conductive DHL in workplaces in which there might be high levels of external exposure to Cr(III).

This study has a few limitations. A cross-sectional study may be inadequate to determine the causal relationship, although corresponding results were obtained in the animal study. The number of participants in this study was also insufficient. A longitudinal study with a larger number of participants is needed to assess the causal relationship.

5. Conclusion

Hearing is one of the indispensable senses for maintenance of good quality of life in humans. Hearing capability at 1 k and 4 k Hz is essential for communication (Yueh et al., 2003; Narne et al., 2021; Shiraishi et al., 2022). In this study, our epidemiological investigation showed for the first time diagnostic DHLs caused by Cr(III) exposure in tannery workers. After confirming the development of Cr(III)-mediated DHL, the mechanism of DHL via eardrum damage caused by external exposure to Cr(III) was proposed. Considering the increasing use of Cr(III) instead of Cr(VI) in various industries in addition to the general use of Cr(III) for commodities worldwide, attention should be given to the health risk of sensory organs by external exposure to Cr(III).

Author Contributions Statement

Yishuo Gu: Data curation, Formal analysis, Investigation, Validation, Writing – original draft. **Nobutaka Ohgami**: Conceptualization, Data curation, Formal analysis, Methodology, Validation, Writing – original draft. **M.M. Aeorangajeb Al Hossain**: Investigation, Methodology, Project administration. **Akira Tazaki**: Investigation, Project administration, Data curation. **Tomoyuki Tsuchiyama**: Data curation, Formal analysis, Methodology. **Tingchao He**: Data curation, Formal analysis. **Masayo Aoki**: Methodology. **Nazmul Ahsan**: Resources, Investigation. **Anwarul Azim Akhand**: Resources, Investigation. **Masashi Kato**: Conceptualization, Funding acquisition, Data curation, Project administration, Supervision, Writing – review & editing.

Ethics statement

This study was performed after approval by Nagoya University Bioethics Committee following the regulations of the Japanese government (2013-0070 and 2016-0036) and by the Faculty of Biological Science, University of Dhaka (5509/Bio.Sc). The subjects participated in this study after providing signed informed consent forms. Animal experiments were approved by the Institutional Animal Care and Use Committee of Nagoya University (approval number: M210309-002) and were performed in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by the Grants-in-Aids for Scientific Research (A) (19H01147) and (B) (17KT0033, 20H03929) and Challenging Exploratory Research (20K21708) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Kobayashi International Scholarship Foundation, Chukyo Longevity Medical and Promotion Foundation, Heiwa Nakajima Foundation and CSC (Chinese Scholarship Council) Scholarship (201806010422). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2022.135571.

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