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Serum Bilirubin Level as a Potential Marker for the Hearing Outcome in Severe-Profound Bilateral Sudden Deafness

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Objective: To investigate the association of serum bilirubin level with hearing outcomes in bilateral sudden sensorineural hearing loss (BSSHL) patients.
Participants: One hundred thirteen in-patient BSSHL patients were consecutively enrolled between July 2008 and December 2015 in a tertiary center.
Main Outcome Measures: Multivariable linear regression, generalized estimating equations (GEE), and stratified analyses were applied to examine the association between serum bilirubin level and hearing outcome measures such as final hearing threshold and absolute and relative hearing gains in BSSHL.
Results: After full adjustment for potential confounders, total bilirubin levels (TBIL) were observed to be positively and independently associated with hearing outcomes as measured by final hearing (β [95% confidence interval {CI}]: −1.5 [−2.7, −0.2] dB HL per 1 μmol/L increase in TBIL) and absolute and relative hearing gains (β [95% CI]: 1.4 [0.2, 2.7] dB and 1.6 [0.2, 3.1] dB, respectively) in the severe to profound hearing loss subpopulation.
Conclusions: Higher TBIL levels, within the normal or mildly elevated ranges, were independently and significantly associated with better hearing outcome in BSSHL patients with severe to profound hearing loss. Given bilirubin elevation treatments exist, our finding suggests a novel pharmacological strategy for this specific subpopulation.
Key Words: Bilateral sudden sensorineural hearing loss—Bilirubin—Hearing outcome.

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Bilateral sudden sensorineural hearing loss (BSSHL) has been reported to account for 1.4 to 4.9% of all cases of sudden sensorineural hearing loss (SSHL) patients (1–4). In contrast with a majority of idiopathic etiologies found in unilateral SSHL, BSSHL cases are typically linked with systemic disorders including cardio-cerebrovascular insufficiency, intracranial infection, toxicity, and malignancies (5–8). Despite of significant disparity between unilateral and bilateral SSHL, the current pathomechanisms proposed for unilateral SSHL have also been used to explain for BSSHL. Among the multiple pathogenic hypotheses, vascular dysfunction and viral infection are the most popular and commonly accepted ones (1,2,4,9,10). As a result, anti-inflammatory compounds such as steroids and drugs ameliorating microcirculation have been widely prescribed as therapeutic approaches for both unilateral and bilateral SSHL patients.

Traditionally, bilirubin was considered as a toxic waste product of heme metabolism. In the field of otology, hyperbilirubinemia has long been documented as one of the risk factors in the development of auditory impairment. Two decades ago, however, a lack of sensorineural hearing loss was reported in a cohort of Crigler–Najjar syndrome type 1 patients, who had lifelong unconjugated hyperbilirubinemia leading to significant risk of bilirubin encephalopathy and death. This provided evidence that bilirubin may not be as ototoxic as is generally thought (11).

Recent studies indicated that bilirubin is a potent antioxidant with in vivo and in vitro anti-inflammatory properties (12–14). Several studies have reported that serum bilirubin concentrations were negatively associated with cardio-cerebrovascular risk (15–20). Moreover, mildly elevated serum bilirubin may provide protective effects against diabetes, metabolic syndrome

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(21–23), and all-cause mortality in adults (24). As has been shown by many authors, there was overlap between risk factors for cardiovascular disease and SSHL (25–28); in particular, cochlear dysfunction resulting from systemic cardiovascular disorders was even more obvious in bilateral than in unilateral SSHL (4). Therefore, the present study hypothesized that bilirubin concentrations may serve as a potential marker for hearing outcomes in BSSHL patients. In addition, we further determined which bilirubin subtype accounted for the observed association.

MATERIALS AND METHODS

Study Population

This retrospective study included exclusively BSSHL patients treated in otolaryngology ward between July 2008 and December 2015 in a tertiary teaching medical center. The inclusion criteria consisted of following parameters: 1) subjective perception of hearing loss affecting both ears either simultaneously (i.e., the second ear is affected within 3 days of the first ear) or sequentially (the second ear is affected $\geq$3 days after the first ear); 2) abrupt onset of sensorineural hearing loss developed within 72 hours; 3) hearing levels were calculated as average audiometric threshold across affected frequencies ($\geq$3 consecutive frequencies) in the affected ear(s) and must be 30 dB HL or higher. It should be noted that the standard pure tone average (PTA) across 500 to 4000 Hz was not used. All patients must undergo detailed evaluation, including medical and otologic history and extensive systems review, otolaryngologic and neurologic physical examination, audiometry, and imaging. A total of 196 patients were reviewed for eligibility (Fig. 1). Those who met one of the following criteria were excluded: 1) recurrent vertigo; 2) recurrent sudden deafness in same ear; 3) self-recovery in one or two ears at presentation; 4) genetic hearing loss or family history of hearing loss; 5) previous history of autoimmune disorder, hematologic malignancies, head and neck cancer, mumps, syphilis, parotitis, radiation therapy, and ear surgery; 6) exposure to high intensity noise and/or ototoxic substances; 7) general anesthesia within 1 month of deafness onset; 8) head trauma immediately preceding sudden deafness; 9) structural or retrocochlear pathology revealed by computed tomographic scanning or magnetic resonance (MR) imaging, such as craniofacial or temporal bone malformations, cerebellopontine angle tumor, stroke, or demyelination. Since BSSHL has long been deemed as an ominous sign for a more sinister underlying disorder (29), all the patients were followed through telephone call for a median time of 77 months (interquartile range: 59–98 mos). The authors then excluded three patients who experienced life-threatening disease (heart attack and stroke) after discharge during follow-up period. There were only four participants with total bilirubin levels (TBIL) beyond normal range (reference: $\leq$21 μmol/L in the present study), three of whom had a slight elevation (22.7–24.2 μmol/L). While one presented a moderate increase (50.5 μmol/L) and was excluded from the analysis as an outlier.

The investigation was conducted in accordance with the standards set by the ''Declaration of Helsinki'' and approved by the Committee of Medical Ethics of Chinese PLA General Hospital (No. S2017–024–01). The Committee granted a waiver of written informed consent for this retrospective study because the patient records were deidentified. The case series have been reported in previous publication for comparison between unilateral and bilateral SSHL (30), however, the relationships between bilirubin and hearing outcomes have never been published.

Data Availability

Due to the restriction of data management in military hospital, the original data of this study is currently not available in public data deposition. All interested researchers can get access to the data by submitting their requests to the corresponding author and the Committee of Medical Ethics of Chinese PLA General Hospital.

Evaluation of Clinical Characteristics

The basic clinical characteristics of participants were age, sex, body mass index (BMI), accompany symptoms, and relevant cardiovascular risk factors, including comorbid hypertension, diabetes, and dyslipidemia. Smoking and alcohol use were evaluated as current or past by self-report.

Blood samples were collected at the first early morning after admission. Serum total bilirubin levels (TBIL) and direct bilirubin levels (DBIL) were measured in the standard laboratory of our

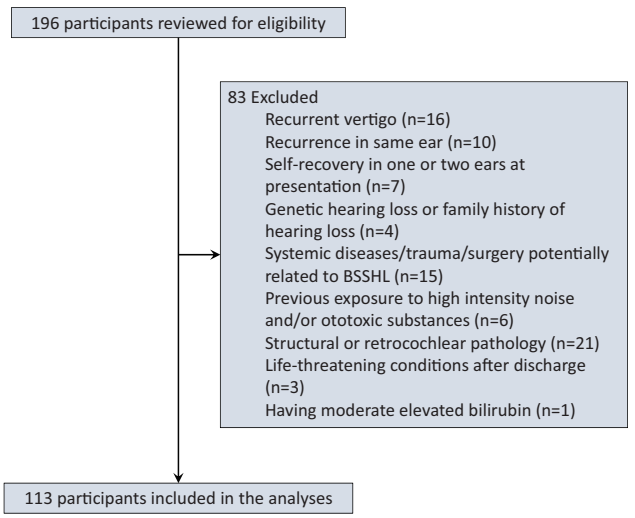

FIG. 1. Study flowchart.

hospital using automated enzymatic methods (Cobas, Roche, Germany). Indirect bilirubin levels (IBIL) were not directly measured in our hospital thus not being included in the present study. Other serology indicators included blood routine, coagulation indexes, lipid panel, fast plasma glucose, liver and renal function. The convalescent bilirubin levels were not obtained.

Treatment

The information of administered medications was collected from electronic medical record system, including the use of systemic steroids, local steroids, batroxobin, and Ginkgo Bilola Extract (EGb761). Basically, five units of batroxobin were given intravenously every other day except for the first treatment day when double dosage was used. The maximum dose of batroxobin was 50 units. Intravenous EGb761 was administered 70 to 105 mg/d. In addition, patients also received dexamethasone 10 mg/d for 3 days followed by a dose of 5 mg/d for another 3 to 5 days by intravenous injection. Local steroids were applied every other day, maximumly five times in total, through postotic subperiosteal injection with 40 mg of methylprednisolone sodium succinate when hearing recovery was not satisfying within 15 days from symptom onset.

Audiometric Assessment and Outcome Measures

In this study, the initial and final hearing thresholds were defined as the average audiometric thresholds across all the affected pure-tone frequencies measured, rather than the traditional pure tone average (PTA, 500–4000 Hz), at study entry and 2 to 4 weeks after the final treatment in our hospital, respectively. Initial hearing level was analyzed both as continuous variable and dichotomized variables (mild to moderate hearing loss, $\leq$70 dB HL; severe to profound hearing loss, $>$70 dB HL).

Hearing outcome measures included final hearing threshold, absolute hearing gain (the difference between final and initial hearing threshold), and relative hearing gain (hearing gain normalized by initial hearing threshold).

The patterns of hearing loss were divided into five categories as previously described (31), and were further dichotomized into having higher recovery rate (low-frequency and middle-frequency hearing loss) and not (descending, flat, and cophosis type) so as to achieve sufficient statistical power.

Statistical Analysis

A total of 113 participants but 180 ears constituted our final dataset. In the 46 sequential BSSHL patients only the recently affected ears were analyzed since it is not practical to obtain initial hearing loss and corresponding serum bilirubin for the former sudden deafness attack. While in the 67 simultaneous BSSHL patients both ears were included for analysis. Similar to ophthalmology, there are at least two levels of nesting, i.e., subject-specific and ear-specific levels, existed in otologic research. The ear is the unit of measurement for outcomes, and also for covariates. Under such circumstance, it is recommended to introduce generalized estimating equations to regression models for appropriate consideration of the correlation between paired outcomes. This approach generally makes maximum use of available data and efficient use of information from subjects that contribute only one ear to analyses, enhancing interpretability of covariate-outcome associations (32).

We performed analyses based on bilirubin tertile categories as well as using bilirubin as continuous variables. The baseline characteristics with normal distribution were presented as mean (SD) and compared using analyses of variance (ANOVA), while the non-normal distributed variables were presented as medians (interquartile range) and compared by Kruskal–Wallis tests. The categorical variables were presented as numbers (percentages) and compared through χ^2 tests or Fisher's exact test. In addition, post-hoc pair-wise comparisons with Bonferroni adjustment of α-level were performed ($\alpha = 0.05$).

We firstly performed simple linear regression analyses for crude models which included only one independent variable and one outcome variable. Secondly, multiple regression analyses were used to isolate the effect of specific variable by adjusting for potentially confounding variables. Regression coefficients (β) represent the mean change in final hearing thresholds and hearing gains for per μmol/L change in bilirubin levels while holding covariates in the model constant. The criteria for selected covariates were based on their association with the outcomes of interest or a more than 10% change in effect estimate after removal from the full model or introduction into the basic model. In this study, a fully adjusted model included covariates as following: age, sex, time duration, audiogram, alcohol use, diabetes, antecedent respiratory infections, initial hearing level, magnesium, hematocrit, alkaline phosphatase, low-density lipoprotein cholesterol, neutrophil, and platelet. Due to correlation of ears in the same individual, generalized estimating equations (GEE) regression were combined with linear regression models for examining the associations of serum bilirubin levels with hearing outcomes in BSSHL patients. An interaction term was added to the models to evaluate whether the effect of bilirubin on hearing outcome varied by the severity of initial hearing level. All the analyses were conducted using Empower (R) (www.empowerstats.com, X&Y solutions, Inc. Boston, MA) and R (http://www.R-project.org). A two-sided p value of <0.05 was considered to represent statistical significance.

RESULTS

Characteristics of BSSHL Patients According to Tertiles of Serum TBIL Levels

As shown in Table 1, participants with higher level of on-admission TBIL tended to be man, having better hearing before and after treatment in our hospital, more irregular audiogram pattern but less cophosis type, higher level of red blood cell counts, hemoglobin, hematocrit, magnesium, and gamma-glutamyl transferase. There were no significant differences in age, medication, assumption of tobacco and alcohol, incidences of hypertension, diabetes, and dyslipidemia among the tertile groups.

No Statistical Significance Was Found in Association Between Hearing Outcomes and TBIL in the Overall Sample After Full Adjustment

The crude full sample analyses showed that 1 μmol/L increase in serum TBIL was significantly associated negatively with final hearing threshold (β [95% CI]: −1.8 [−2.9, −0.6] dB). The highest TBIL tertile group had better hearing outcome which was denoted by a significant lower final hearing threshold (β [95% CI]: −17.3 [−29.6, −4.9] dB) as compared with the lowest tertile group. In contrast, DBIL was not significant associated with any outcome of interest even in the crude analysis. However, all the estimates were clearly decreased and no association remained significant after

TABLE 1. *Clinical characteristics of bilateral sudden sensorineural hearing loss participants according to serum total bilirubin tertiles*

Variables	Overall	TBIL (μmol/L)			p
		T1 (<10.6)	T2 (10.6–13.4)	T3 (>13.4)[#]	
Number of participants	113	40	38	35	
TBIL (μmol/L)*	11.9 (4.2)	7.8 (1.9)	11.9 (0.9)	16.6 (3.1)	<0.001[a]
DBIL (μmol/L)*	3.2 (1.2)	2.3 (0.9)	3.2 (0.8)	4.3 (1.1)	<0.001[a]
Age (yrs)*	51.0 (38.0–58.0)	47.5 (33.8–59.2)	51.0 (39.8–56.8)	52.0 (42.2–62.0)	0.490
Gender*					0.003[b]
Female	45 (39.8%)	24 (60.0%)	13 (34.2%)	8 (22.9%)	
Male	68 (60.2%)	16 (40.0%)	25 (65.8%)	27 (77.1%)	
Neutrophil (10^3/μl)*	4.0 (3.0–5.7)	4.1 (3.0–6.9)	3.8 (2.9–4.8)	4.1 (3.0–5.6)	0.650
Lymphocyte (10^3/μl)*	2.1 (1.6–2.4)	2.2 (1.5–2.7)	2.1 (1.8–2.4)	1.9 (1.4–2.3)	0.308
RBC (10^6/μl)*	4.6 (0.5)	4.5 (0.5)	4.6 (0.5)	4.7 (0.4)	0.027[c]
Hemoglobin (g/L)*	139.7 (16.6)	133.1 (17.8)	142.5 (14.0)	144.1 (15.8)	0.006[c]
Platelet (10^3/μl)*	225.5 (53.6)	241.4 (61.8)	211.4 (52.3)	222.8 (39.6)	0.042[d]
Hemocrit*	0.41 (0.05)	0.39 (0.05)	0.42 (0.04)	0.42 (0.04)	0.005[c]
GGT (U/L)*	23.1 (15.7–35.4)	18.4 (12.4–25.2)	23.6 (17.5–33.0)	27.0 (19.0–45.0)	0.011[e]
ALP (U/L)*	64.9 (53.4–74.6)	66.0 (55.1–92.4)	56.9 (50.9–73.2)	65.6 (59.5–68.5)	0.061
Mg (mmol/L)*	0.89 (0.07)	0.87 (0.07)	0.88 (0.07)	0.92 (0.06)	0.020[e]
TG (mmol/L)*	1.4 (0.9–1.9)	1.3 (0.7–1.6)	1.6 (0.7)	1.6 (0.7)	0.055
LDL (mmol/L)*	3.0 (0.8)	2.8 (0.8)	3.0 (0.8)	3.2 (0.9)	0.191
HDL (mmol/L)*	1.3 (1.1–1.5)	1.4 (1.1–1.7)	1.2 (1.0–1.5)	1.3 (1.2–1.5)	0.790
Treatment (d)*					
Local steroids	1.0 (0.0–3.0)	2.0 (0.0–3.0)	1.0 (0.0–3.0)	1.0 (0.0–3.0)	0.488
Systemic steroids	0.0 (0.0–5.0)	0.0 (0.0–4.0)	0.0 (0.0–6.0)	0.0 (0.0–5.5)	0.648
Batroxobin	4.0 (2.0–5.0)	5.0 (2.0–5.2)	4.0 (3.0–5.0)	3.0 (1.0–5.0)	0.101
EGb761	10.0 (6.0–13.0)	11.0 (8.5–13.2)	10.0 (6.2–12.0)	9.0 (4.5–11.5)	0.089
Number of ears	180	62	60	58	
Time duration (d)[Δ]	10.0 (4.0–16.2)	14.0 (8.0–17.0)	7.5 (4.0–14.5)	10.0 (3.0–15.0)	0.043
Initial hearing (dB HL)	65.3 (46.7–88.4)	78.2 (58.4–98.8)	63.2 (45.0–84.1)	57.6 (44.5–75.3)	0.002[c]
Final hearing (dB HL)	58.3 (29.2)	67.4 (29.7)	56.6 (29.1)	50.1 (26.3)	0.004[c]
Relative hearing gain (dB)	10.4 (1.2–30.9)	7.1 (0.0–23.7)	12.3 (1.2–31.4)	15.7 (4.2–32.6)	0.092
Absolute hearing gain (dB)	7.3 (0.8–17.9)	4.6 (0.0–12.8)	10.0 (1.1–18.2)	8.7 (2.5–17.7)	0.283
Audiogram					<0.001
Flat	45 (25.0%)	15 (24.2%)	19 (31.7%)	11 (19.0%)	
Descending	60 (33.3%)	18 (29.0%)	19 (31.7%)	23 (39.7%)	
Cophosis	57 (31.7%)	27 (43.5%)	18 (30.0%)	12 (20.7%)	[b]
Irregular	9 (5.0%)	0 (0.0%)	0 (0.0%)	9 (15.5%)	[e]
Ascending	9 (5.0%)	2 (3.2%)	4 (6.7%)	3 (5.2%)	

Continuous variables were presented as mean (standard deviation) for normal distribution, or medians (interquartile range) for non-normal distribution. Categorical variables were presented as n (%).

*These were subject-specific covariates, while the rest were ear-specific covariates.

[Δ]The time elapse between symptom onset and the study entry.

[#]Only three out of the 113 patients had levels of TBIL above the normal range.

[a]Significant differences were found in all pairs of groups after post-hoc comparison.

[b]The only significant difference was between T1 and T3 groups after post-hoc comparison.

[c]Significant differences were found between T1 and T2, T3 groups after post-hoc comparison.

[d]The only significant difference was between T1 and T2 groups after post-hoc comparison.

[e]Significant differences were found between T1, T2, and T3 groups after post-hoc comparison.

ALP indicates alkaline phosphatase; BSSHL, bilateral sudden sensorineural hearing loss; DBIL, direct bilirubin; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; IBIL, indirect bilirubin; LDL, low-density lipoprotein; Mg, magnesium; RBC, red blood cell counts; TBIL, total bilirubin; TC, total cholesterol; TG, triglyceride.

full adjustment with potential confounding variables, including age, sex, time duration, audiogram, alcohol use, diabetes, antecedent respiratory infections, initial hearing level, magnesium, hematocrit, alkaline phosphatase, low-density lipoprotein cholesterol, neutrophil, and platelet (Table 2).

Significant Associations Between Bilirubin Levels and Hearing Outcomes Were Only Observed in the Subgroup With Severe to Profound Initial Hearing Loss

Since initial hearing loss exhibited great impact on the bilirubin-final hearing relationship (Table S1, http://

TABLE 2. *The crude and adjusted regression coefficient (β) and 95% confidence interval (CI) for final hearing, absolute and relative hearing gain in relation to TBIL and DBIL in full sample*

	Crude		Adjusted*	
	β (95% CI)	p	β (95% CI)	p
Final hearing				
TBIL (per 1 μmol/L)	−1.8 (−2.9, −0.6)	0.003	−0.3 (−0.9, 0.3)	0.289
TBIL tertiles				
T1	0 [Reference]		0 [Reference]	
T2	−10.8 (−23.4, 1.9)	0.094	2.6 (−4.0, 9.2)	0.437
T3	−17.3 (−29.6, −4.9)	0.006	−2.6 (−8.5, 3.4)	0.394
DBIL (per 1 μmol/L)	−1.6 (−6.0, 2.8)	0.469	0.1 (−2.1, 2.3)	0.896
DBIL tertiles				
T1	0 [Reference]		0 [Reference]	
T2	−3.9 (−15.8, 8.1)	0.525	−1.4 (−8.4, 5.6)	0.697
T3	−3.4 (−17.2, 10.4)	0.624	−0.7 (−9.2, 7.7)	0.868
Absolute hearing gain				
TBIL (per 1 μmol/L)	0.2 (−0.5, 0.9)	0.551	0.3 (−0.3, 0.9)	0.299
TBIL tertiles				
T1	0 [Reference]		0 [Reference]	
T2	−1.1 (−7.6, 5.4)	0.744	−3.1 (−9.5, 3.2)	0.337
T3	2.3 (−4.8, 9.5)	0.522	2.3 (−3.6, 8.2)	0.435
DBIL (per 1 μmol/L)	−0.1 (−2.2, 2.0)	0.925	−0.2 (−2.4, 2.0)	0.845
DBIL tertiles				
T1	0 [Reference]		0 [Reference]	
T2	0.0 (−6.5, 6.4)	0.989	0.8 (−5.9, 7.4)	0.825
T3	0.9 (−6.3, 8.0)	0.808	0.3 (−8.0, 8.5)	0.950
Relative hearing gain				
TBIL (per 1 μmol/L)	0.6 (−0.4, 1.5)	0.261	0.3 (−0.5, 1.2)	0.417
TBIL tertiles				
T1	0 [Reference]		0 [Reference]	
T2	2.9 (−6.9, 12.7)	0.568	−1.3 (−10.3, 7.7)	0.774
T3	6.6 (−3.0, 16.3)	0.178	4.0 (−4.3, 12.4)	0.347
DBIL (per 1 μmol/L)	0.2 (−2.8, 3.1)	0.913	−0.1 (−2.9, 2.8)	0.962
DBIL tertiles				
T1	0 [Reference]		0 [Reference]	
T2	1.3 (−8.1, 10.6)	0.788	3.5 (−5.8, 12.8)	0.456
T3	1.9 (−8.4, 12.2)	0.722	2.4 (−8.3, 13.1)	0.657

*Adjusted for age, sex, time duration, audiogram, alcohol use, diabetes, preceding infection, baseline hearing level, magnesium, hematocrit, alkaline phosphatase, low-density lipoprotein cholesterol, neutrophil, and platelet.

DBIL indicates direct bilirubin levels; TBIL, total bilirubin levels.

links.lww.com/MAO/A787), the effect of bilirubin on hearing outcomes may be offset by the effect of initial hearing. We then conducted stratified analysis by this covariate (Table 3). In the subgroup with severe to profound initial hearing loss, a higher level of TBIL was observed to be significantly associated with better final hearing threshold (−1.9 [−3.6, −0.2] dB per 1 μmol/L increase in TBIL) without adjustment. The effect of TBIL on final hearing was smaller but remained statistically significant after full adjustment (−1.5 [−2.7, −0.2] dB per 1 μmol/L increase in TBIL). In contrast, the adjusted estimates of TBIL on relative and absolute hearing gain (absolute hearing gain: 1.4 [0.2, 2.7]; relative hearing gain: 1.6 [0.2, 3.1]) were even increased as compared with those in crude analysis (absolute hearing gain: 1.2 [−0.3, 2.7]; relative hearing gain: 1.3 [−0.3, 2.9]). The R-squared is 0.4348, 0.2423, and 0.2775 for outcome variables of final hearing threshold, absolute and relative hearing gain, respectively.

In addition, there were significant interactions between TBIL and initial hearing in relation to all the hearing outcomes of interests after full adjustment (P interaction = 0.0141 for final hearing; P interaction = 0.0144 0.0144 for absolute hearing gain; P interaction = 0.0149 for relative hearing gain).

In contrast, DBIL were not significantly associated with any hearing outcomes of interest in either subgroup.

DISCUSSION

In the present study, higher TBIL level was observed to be associated with better hearing outcomes as measured by final hearing threshold, absolute and relative hearing gain with bilirubin level in the normal or mildly elevated range. This beneficial effect was only significant and robust for BSSHL patients with severe to profound initial hearing loss (>70 dB HL).

TABLE 3. *The crude and adjusted regression coefficient (β) and 95% confidence interval (CI) for final hearing threshold, absolute and relative hearing gain in relation to TBIL and DBIL stratified by initial hearing threshold*

	≤70 dB HL (104 Ears)		>70 dB HL (76 Ears)		
Outcomes	β (95% CI)	*p*	β (95%CI)	*p*	*p* Interaction
Final hearing					
TBIL (per 1 μmol/L)					
Crude	−0.2 (−1.1, 0.6)	0.5517	−1.9 (−3.6, −0.2)	0.026	0.0601
Adjusted*	0.2 (−0.3, 0.7)	0.4265	−1.5 (−2.7, −0.2)	0.0258	0.0141
DBIL (per 1 μmol/L)					
Crude	−0.4 (−3.4, 2.5)	0.7735	−2.0 (−7.9, 3.9)	0.5118	0.6593
Adjusted*	1.4 (−0.7, 3.5)	0.1945	−2.1 (−6.1, 2.0)	0.3171	0.1000
Absolute hearing gain					
TBIL (per 1 μmol/L)					
Crude	−0.2 (−0.7, 0.3)	0.4172	1.2 (−0.3, 2.7)	0.1267	0.0561
Adjusted*	−0.2 (−0.7, 0.3)	0.4226	1.4 (0.2, 2.7)	0.0263	0.0144
DBIL (per 1 μmol/L)					
Crude	−1.3 (−3.1, 0.5)	0.1576	1.8 (−2.4, 5.9)	0.4022	0.1632
Adjusted*	−1.4 (−3.5, 0.6)	0.1732	2.0 (−2.0, 6.0)	0.3364	0.1027
Relative hearing gain					
TBIL (per 1 μmol/L)					
Crude	−0.1 (−1.2, 0.9)	0.8041	1.3 (−0.3, 2.9)	0.1079	0.0783
Adjusted*	−0.2 (−1.1, 0.6)	0.6015	1.6 (0.2, 3.1)	0.024	0.0149
DBIL (per 1 μmol/L)					
Crude	−1.4 (−5.0, 2.3)	0.4564	2.2 (−2.5, 6.8)	0.3597	0.2313
Adjusted*	−1.7 (−5.0, 1.6)	0.317	2.8 (−1.5, 7.1)	0.2021	0.0782

*Adjusted for age, sex, time duration, audiogram, alcohol use, diabetes, preceding infection, magnesium, hematocrit, alkaline phosphatase, low-density lipoprotein cholesterol, neutrophil, and platelet.

DBIL indicates direct bilirubin levels; TBIL, total bilirubin levels.

Within the otology domain, bilirubin has long been considered as an ototoxic substance and hyperbilirubinemia has been well documented to be linked with hearing disorders such as auditory neuropathy. To the best of our knowledge, few previous literatures have proposed an otoprotective effect of bilirubin. Thus, many questions remain regarding how a high normal or mildly elevated TBIL improves hearing outcome in BSSHL patients. It is now clear that bilirubin is an important vasoprotective molecule with properties of anti-oxidant, anti-inflammatory, vasodilatory, anti-mutagenic, immune-modulatory, anti-proliferative, and anti-apoptotic (33,34). It should be noted that oxidative stress may be also involved in the pathogenesis of sudden deafness (35). Given that vascular compromise and oxidative stress may play important role in the development of sudden deafness and bilirubin has a protective function in general, it is possible that inner ear injury resulted from BSSHL can benefit from high normal or mildly elevated bilirubin level.

Unconjugated bilirubin (also known as indirect bilirubin, IBIL) is formed based on biliverdin reduction by biliverdin reductase (BVR) during heme catabolism where heme oxygenase (HO) catalyzes the initial and rate-limiting step (36,37). IBIL is then transported to the liver and conjugated with glucuronic acid by uridine diphosphate glucuronosyltransferase Family 1 Member A1 (UGT1A1) to a water-soluble form (DBIL) for elimination (38). The serum IBIL usually accounts for the majority of TBIL in human. IBIL can enter into the central nervous system through the blood–brain barrier due to its high lipid solubility (39,40) thus exerting an impact on the peripheral auditory system including spiral ganglion neurons and inner hair cells. Gilbert's syndrome is the most common cause of mild elevations of IBIL due to decreased UGT1A1 activity, exhibiting lower risk of cardiovascular and metabolic diseases such as diabetes, than that of normobilirubinemic participants (41,42). Coincidently, although we did not measure and analyzed the IBIL level in this study, out result shows TBIL but not DBIL is correlated with the hearing outcome in BSSHL subgroup with severe to profound initial hearing impairment. In this context, we speculate the protective effect of bilirubin observed in BSSHL mainly derives from IBIL.

Although no previous study has provided direct evidence supporting such a positive association between bilirubin and the improvement of hearing loss, the favorable role of higher bilirubin levels observed in this study may be mediated through heme oxygenase (HO) or by other substrates involved in the bilirubin signaling pathway, such as, biliverdin and carbon monoxide (CO). It has been shown by some authors that HO-1 acts as an additional mediator against noise or ototoxic drug induced cochlear damage in vivo and in vitro (43–45). Furthermore, not only can HO-mediated CO generation protect against vascular dysfunction and immune-mediated diseases (46,47) but it also plays an essential role in modulation of tympanogenic cochlear inflammation (48).

Based on the present finding, TBIL may serve as a potential outcome predictor for BSSHL. Moreover, it suggests a novel therapeutic target for BSSHL patients. Pharmacological approaches that cause mild-to-moderate elevations in circulating levels of bilirubin, including heme oxygenase-1 inducers and UGT1A1 inhibitor in addition to direct administration of bilirubin or its precursor biliverdin (49), may be plausible tools for BSSHL treatment in the future. Probably patients with initial hearing threshold worse than 70 dB HL would derive greater benefit from bilirubin elevation treatment compared with those with lower thresholds. Nonetheless, among three included patients with TBIL above normal range, two experienced mild-moderate hearing loss had a recovery of near normal hearing. Although the patient with the highest level of bilirubin (50.5 μmol/L) was discarded from the present study due to being an ''outlier,'' he experienced a marked improvement in one ear (Table S2, http://links.lww.com/MAO/A787). It seems that BSSHL patients with TBIL above normal have better recovery rate than those within normal limits. Under such circumstance, we might even expect that BSSHL patients with severe-profound hearing loss are not the only subgroup that could benefit from higher level of bilirubin. This needs to be elucidated after incorporating more samples. Given that markedly elevated bilirubin levels may exert toxic effects in the neural and auditory system, caution is definitely required.

To the best of our knowledge, this is the first study to clarify the potential favorable role of TBIL on hearing outcome in BSSHL patients with severe to profound initial hearing threshold. Interestingly, a 100% of patients with irregular audiogram pattern (though the number of patients is small) had TBIL level in the highest tertile. In addition, all four patients with abnormal TBIL were men and had simultaneous bilateral sudden hearing loss. Underlying mechanism remains unknown how sex effect and hearing loss pattern interact in the association between bilirubin and hearing outcome in BSSHL patients. It suggests the need to re-examine the role of bilirubin, in particular bilirubin within the normal and near-normal range, on the auditory system. Given bilirubin elevation treatments are already existing, novel strategies for improving BSSHL prognosis is implied.

However, there are several drawbacks. First, the research was conducted at a single Chinese medical center in patients treated in otolaryngology ward. It was reported that causes of approximately 70% BSSHL cases were identified via MR imaging (29). However, the abnormal rate in MR imaging is only 10.5% in the present study. This is probably because in our hospital most BSSHL patients with severe systemic disease have been firstly diagnosed and admitted in relevant departments, such as neurology, cardiology, hematology, and so on. It is not practical to collect complete medical information from patients who were not treated in otolaryngology ward, thus the results may not be extrapolated to the general BSSHL patients. Second, the possible association between BSSHL and other relevant markers within the bilirubin signaling such as, biliverdin reductase and HO-1, which have been reported to be otoprotective in noise, ototoxic drug induced cochlear damage, were not assessed. Therefore, it is not possible to conclude whether bilirubin per se exerts the protective effect on BSSHL or it is actually a mediator or a marker. Third, bilirubin was only measured at admission, which prevented us from examining its changes over time. Fourth, our findings were inherently limited for elucidating causal relationships between serum bilirubin levels and BSSHL hearing outcomes due to its cross-sectional study design. Fifth, since it is impractical to obtain the baseline pre-loss hearing for BSSHL patients, ''relative hearing gain'' that defined as hearing gain normalized by initial hearing threshold in this study is much likely underestimated. Sixth, the strength of associations between TBIL and hearing outcomes is not satisfying enough. The percentage of variability in outcomes accounted for by TBIL, denoted by the R-squared measure, implies that some other factors are at play. Further research is warranted to clarify the exact interaction between serum bilirubin level and the development of BSSHL.

CONCLUSION

In the present study, we investigated the hearing outcome in BSSHL patients in relation to serum bilirubin levels. TBIL is independently and positively associated with hearing outcomes as measured by final hearing threshold, absolute and relative hearing gain in BSSHL patients with severe to profound initial hearing loss (>70 dB HL). The potential otoprotective effect of normal and near-normal bilirubin level may be attributed to its anti-inflammation and anti-oxidant capacity. This finding extends our understanding of the diverse roles of bilirubin in auditory system. Since bilirubin levels are standardized and cost-effective serum indicators and treatments for elevating bilirubin levels are already available, our results suggest a novel and plausible strategy for improve hearing outcome in specific subpopulation of BSSHL.

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