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Effects of Intralabyrinthine Hemorrhage on the Cochlear Elements

A Human Temporal Bone Study

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Hypothesis: To compare histopathologic findings in the cochlea of human temporal bones with versus without intralabyrinthine hemorrhage.
Background: Hemorrhagic labyrinthitis can cause sensorineural damage, sudden hearing loss, and vertigo. Yet, to our knowledge, no studies have quantitatively described histopathologic effects of intralabyrinthine hemorrhage on the elements of the cochlea.
Methods: We analyzed 46 human temporal bone samples from 23 patients with unilateral intralabyrinthine hemorrhage (23 samples from ears with intralabyrinthine hemorrhage and 23 samples from contralateral ears without). We noted the location of hemorrhage in the inner ear, the degree of endolymphatic hydrops, the number of spiral ganglion cells and hair cells, mean loss of fibrocytes in spiral ligament, and areas of the stria vascularis and spiral ligament.
Results: Intralabyrinthine hemorrhage caused significant loss of outer hair cells in the lower basal ($p = 0.001$), upper basal ($p = 0.005$), and lower middle ($p = 0.012$) cochlear turns. The degree of endolymphatic hydrops was significantly different between the hemorrhagic and contralateral sides ($p = 0.011$). But we found no significant difference between the 2 sides in the number of inner hair cells, spiral ganglion cells, and fibrocytes, or in the areas of the stria vascularis and spiral ligament between the two groups ($p > 0.05$).
Conclusion: These findings suggest that such patients could be good candidates for hearing aid or cochlear implant if they have profound sensorineural hearing loss.
Key Words: Cochlea—Endolymphatic hydrops—Hair cells—Hemorrhage—Histopathology—Human temporal bones—Inner ear—Labyrinthitis—Spiral ganglion cells—Spiral ligament—Stria vascularis.

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Intralabyrinthine hemorrhage (IH), though a rare problem, can result in sudden sensorineural hearing loss and vertigo. The most common underlying cause is hemorrhagic diathesis, but other important causes may include trauma, leukemia, metastatic malignancy, Wegener's granulomatosis, intracranial hemorrhage, and cocain consumption (1,2). Intracranial hemorrhages can include intracerebral, subarachnoid, and subdural locations (2).

The purpose of this study was to evaluate the otopathologic effects of IH on the structures of the cochlea. Specifically, we compared histopathologic findings in inner ears of human temporal bones with versus without IH. A more thorough understanding of the pathology and mechanism of cochlear damage after IH will help clinicians plan medical or surgical treatment for such patients.

MATERIALS AND METHODS

We obtained samples for our study from the human temporal bone collection at the University of Minnesota. All temporal bones had previously been removed at autopsy and fixed in formalin solution. Each bone was decalcified, embedded in celloidin, and then serially sectioned in the horizontal plane at a thickness of 20 μm. Every 10th section was stained with hematoxylin-eosin (H&E) and mounted on a glass slide for light microscopic observation.

For our study, we selected temporal bones with unilateral IH. We excluded bones from patients with a history of, or histopathologic findings of, ear surgery, otitis media, suppurative labyrinthitis, otosclerosis, or Ménière's disease. Our final selection included 46 temporal bone samples from 23 patients with

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The authors disclose no conflicts of interest.

TABLE 1. *Causes of IH in 23 patients*

Cause	Number of Patients
Intracerebral hemorrhage	5
Subarachnoid hemorrhage	4
Leukemia	4
Head trauma	3
Subdural hemorrhage	2
Metastatic malignancy	1
Multiple myeloma	1
Wegener's granulomatosis	1
Scuba diving	1
Acute renal failure	1

IH indicates intralabyrinthine hemorrhage.

unilateral IH (23 samples from ears with IH and 23 samples from contralateral ears without). The patients included 17 men (74%) and 6 women (26%), ranging in age from 1 day to 71 years (mean, 29.9 ± 25.5 yr).

The underlying causes of IH are listed in Table 1. Of the 23 patients, 11 (48%) had intracranial hemorrhage: 5 intracerebral, 4 subarachnoid, and 2 subdural. Another 4 patients (17%) had leukemia: 2 acute myelogenous, 1 acute lymphocytic, and 1 erythromyelogenous. In three patients, IH was secondary to head trauma. The remaining five causes of IH (in one patient each) were metastatic malignancy, multiple myeloma, Wegener's granulomatosis, scuba diving, and acute renal failure.

We documented the location of hemorrhage in the cochlea, and compared findings of endolymphatic hydrops, the number of spiral ganglion cells, hair cells, and fibrocytes, and areas of spiral ligament and stria vascularis with the contralateral ears.

Spiral Ganglion Cells

We divided Rosenthal's canal into 4 segments as described previously: segment I (from base to 6 mm); II (6 to 15 mm); III (15 to 22 mm); and IV (22 mm to apex) (3). All nuclei were counted in each section. The number of ganglion cells was determined for each segment and for the cochlea as a whole by multiplying their summed counts by 10 to account for the unmounted sections and by a factor of 0.9 to account for cells that would be counted because of their location at the interface between sections.

Hair Cells

In each section, we counted the number of present and missing cochlear hair cells. In all turns of the cochlea, we calculated the percentage of hair cell loss by dividing the number of missing hair cells by the total number of hair cells possible in that turn.

Stria Vascularis

In all of the cochlear turns at the midmodiolar level, as well as on the adjacent two sections, we obtained morphometric measurements of the stria vascularis. We acquired each image with a digital camera at a magnification of $\times 200$. Using a computer, we quantified the areas of the cut surfaces of the stria vascularis. For the measurements, we used image analyses software (SPOT Advanced, SPOT Imaging Solutions, Sterling Heights, MI, U.S.A.). Excluded from the area of the stria vascularis were any secondary changes, such as cystic-like structural areas or concretions.

Spiral Ligament

We divided the spiral ligament into four segments according to the appearance of different types of fibrocytes, per previous studies by Spicer and Schulte (4). Type I fibrocytes lie circumferentially aligned, between the stria vascularis and bone. Type II fibrocytes occupy the superficial inferior spiral ligament between the basilar crest and the stria. Type III fibrocytes are longitudinally located in the deepest part of the inferior spiral ligament. Type IV fibrocytes lie radially oriented, inferior to the basilar crest. Morphometric measurements of spiral ligament's area were made in all turns of the cochlea at the midmodiolar level and the adjacent two sections. The image was obtained with a charge-coupled device camera that was connected to a personal computer. The calibrated image was obtained at an original magnification of $\times 40$. The areas of spiral ligament were quantified by determining the areas of their cut surfaces, with the aid of the computer. Measurements were made using commercially available image analysis software (SPOT Advanced, SPOT Imaging Solutions).

Fibrocytes

To estimate and evaluate the mean loss of fibrocytes in each segment, we used a rating scale: 0, within normal limits (missing less than 1/3 of the fibrocytes); 1, missing 1/3 of the fibrocytes; 2, missing 2/3 of the fibrocytes; and 3, severe or complete loss of the fibrocytes on sections at the midmodiolar level, per the methods of Hequembourg and Liberman (5). The calibrated image was obtained at an original magnification of $\times 40$.

Endolymphatic Hydrops

We subdivided our temporal bone samples according to degree of hydrops, per classification of Cureoglu et al. (6): 1) *slight* hydrops, i.e., bulging of Reissner's membrane without contact with the bony wall of the scala vestibuli; 2) *moderate* hydrops, i.e., displacement of Reissner's membrane with contact with the wall of the scala vestibuli, but with an angle with the osseous spiral lamina of less than 90 degrees; and 3) *profound* hydrops, i.e., displacement of Reissner's membrane with bony contact, with an angle with the osseous spiral lamina of more than 90 degrees.

Statistical Analysis

To analyze any differences between the hemorrhagic and contralateral sides, we used the paired *t* test. Significance was defined as $p < 0.05$.

RESULTS

We observed IH in the scala tympani of the basal turn in 20 (87%) of our temporal bone samples; of the middle turn, in 8 (35%). But we observed no IH in the scala media of the middle and apical turns. Nor did we observe IH in the scala vestibuli of the apical turn. The locations of IH in the inner ears are shown in Table 2.

We found no significant differences in the total number of spiral ganglion cells between the hemorrhagic (mean $\pm$ SD, $26{,}458 \pm 5{,}764$), and contralateral sides (mean $\pm$ SD, $27{,}522 \pm 5{,}391$) ($p > 0.05$). We found no significant differences in any segment between the two sides ($p > 0.05$) (Fig. 1).

The hemorrhagic, as compared with the contralateral, sides had significantly fewer outer hair cells in the lower

TABLE 2. *Locations of IH in 23 temporal bone samples*

Location	Number of Temporal Bone Samples
Superior SC	1
Lateral SC	1
Posterior SC	3
Utricle	3
Saccule	4
Lower basal turn	
SV	2
SM	1
ST	18
Upper basal turn	
SV	1
SM	1
ST	11
Lower middle turn	
SV	2
SM	0
ST	7
Upper middle turn	
SV	1
SM	0
ST	2
Apical turn	
SV	0
SM	0
ST	2

IH indicates intralabyrinthine hemorrhage; SC, semicircular canal; SM, scala media; ST, scala tympani; SV, scala vestibuli.

basal ($p = 0.001$), upper basal ($p = 0.005$), and lower middle turns ($p = 0.012$) ($p < 0.05$) (Fig. 2). The location of lost outer hair cells was adjacent to the location of IH (Table 2 and Fig. 2).

We found no significant differences between the hemorrhagic and contralateral sides in the number of outer hair cells in the upper middle and apical turns ($p > 0.05$). We found no significant differences between the two sides in the number of inner hair cells in any turn ($p > 0.05$). And we found no significant differences in the areas of the stria vascularis in any turn ($p > 0.05$) (Fig. 3).

We found no significant differences between the hemorrhagic and contralateral sides in the numbers of fibrocytes (for any of the types) and the area of spiral ligament in any cochlear turn ($p > 0.05$).

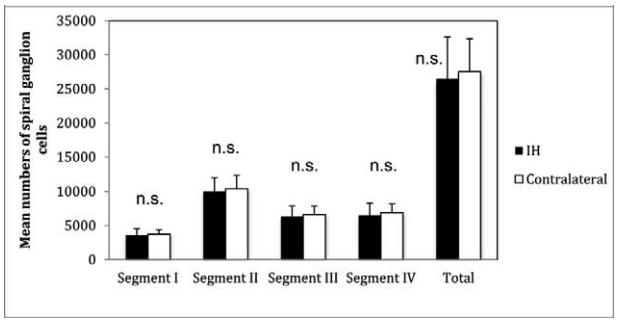

FIG. 1. Mean number of the spiral ganglion cells by segment (error bars = standard deviation). IH indicates intralabyrinthine hemorrhage; n.s., not significant.

A slight endolymphatic hydrops was observed in six (26%) ears with IH, but not in any of the contralateral ears ($p = 0.011$) (Fig. 4).

DISCUSSION

IH is the presence, for any reason, of red blood corpuscles in the cochlea, vestibule, and/or semicircular canals. In the literature, reported cases of IH in the inner ear have been because of head trauma (7), subarachnoid hemorrhage (8), leukemia (9,10), anticoagulant therapy (11,12), sickle cell disease (13), systemic lupus erythematosus (14), scuba diving (15), multiple myeloma (16), and metastatic malignancy (17). To our knowledge, no studies, until ours, have quantitatively investigated the effects of IH on the elements of the cochlea.

We observed hemorrhage in the scala tympani of the basal turn in 87% of our temporal bone samples with IH. Possible reasons implicate the opening of the cochlear aqueduct to the scala tympani, the close proximity of the modiolus to the scala tympani, the vessels located in the tympanic covering layer of the cochlea, or the effect of gravity. Since we observed pathologic changes in the cochlea adjacent to areas of hemorrhage, we speculate that local effects of hemoglobin breakdown products (such as thrombin) and/or the lack of blood supply to the cochlear elements could be the underlying mechanisms of cochlear damage. Hemolysis of erythrocytes gives rise to the release of hemoglobin, whose breakdown products include carbon monoxide, iron, and biliverdin (18). Carbon monoxide, which is a free radical, can cause tissue damage (19). Iron is able to activate the formation of free radicals, leading to neuronal damage (19). In addition, hemoglobin inhibits sodium/potassium (Na/K) adenosinetriphosphatase (ATPase) activity; in tissues, it activates lipid peroxidations (20,21). Moreover, the physiologic response of tissues against hemorrhage is the activation of hemostatic system, including thrombin, which in turn has an adverse effect on tissues (22–24). In an animal model, Radeloff et al. showed the harmful effects of IH on the cochlea (25). After inserting blood into the scala tympani, they observed permanently elevated hearing thresholds. In our study, we observed a significant loss of outer hair cells in the lower basal, upper basal, and lower middle turns of the cochlea.

Several clinical reports have shown that IH, for a variety of reasons, leads to sudden deafness and vertigo (13,14,26). Naganawa et al. (27) described a patient, on anticoagulant therapy, who had IH in the lateral and posterior semicircular canals and then suffered from sudden hearing loss and vertigo. Paparella et al. (10) described a patient with leukemia who had IH in the saccule, posterior semicircular canal, and scala tympani. In our analysis of 23 temporal bone samples with IH, we have observed hemorrhages in the vestibular area in multiple sites (Table 2).

We found the degree of endolymphatic hydrops to be significantly higher in temporal bone samples with IH. The reason might be electrolyte imbalance as a result of

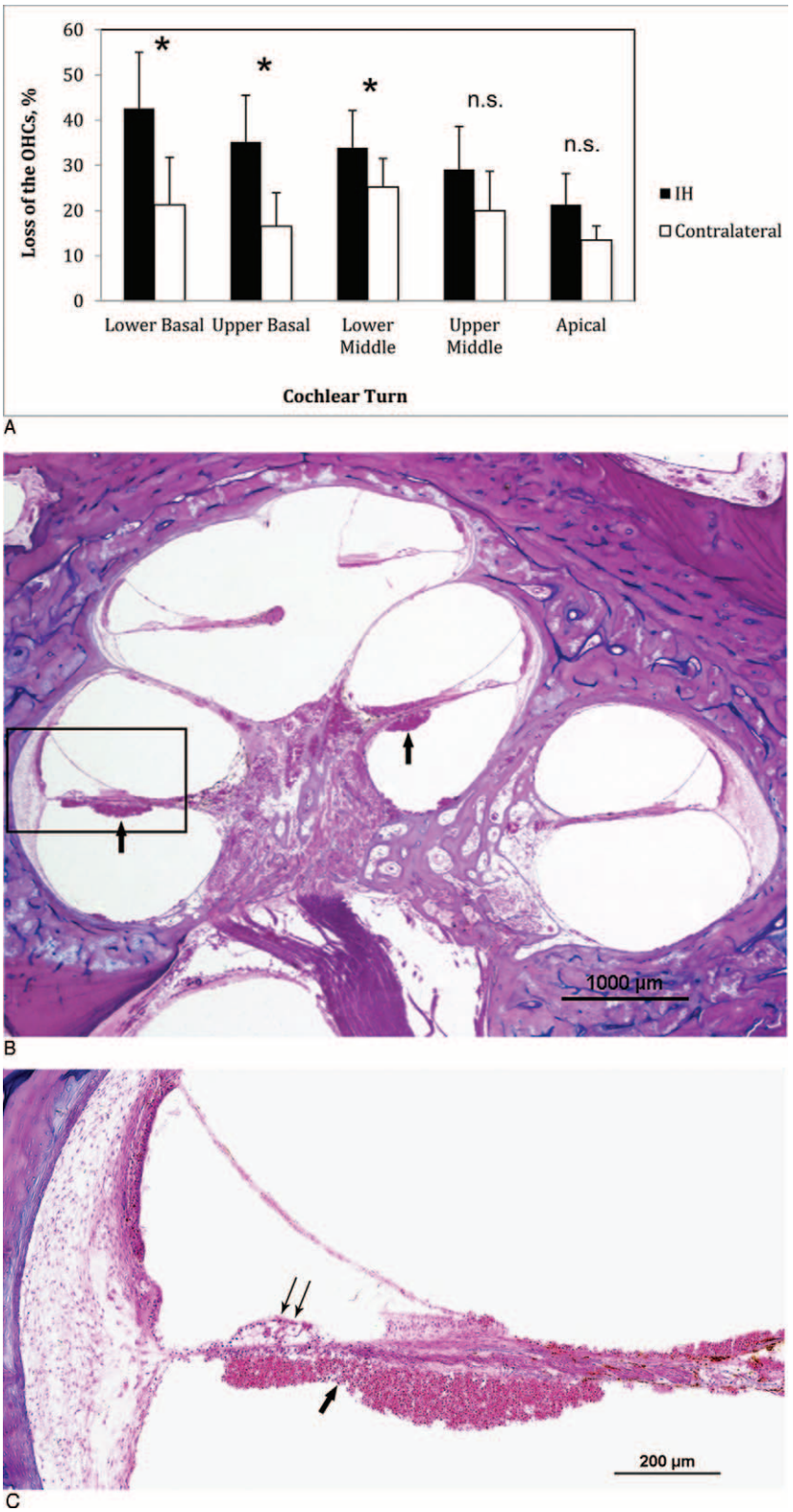

FIG. 2. Outer hair cells (OHCs) loss in a 25-year-old man with intralabyrinthine hemorrhage (IH). (*A*) Graph shows mean OHCs loss (error bars = standard deviation). (*B*) Lower magnification image. (*C*) Magnified view of boxed area. Thick arrow: intralabyrinthine hemorrhage in the upper basal, and lower middle cochlear turn; thin arrow: OHCs loss in upper basal turn; $*p < 0.05$; n.s. indicates not significant. Staining with H&E.

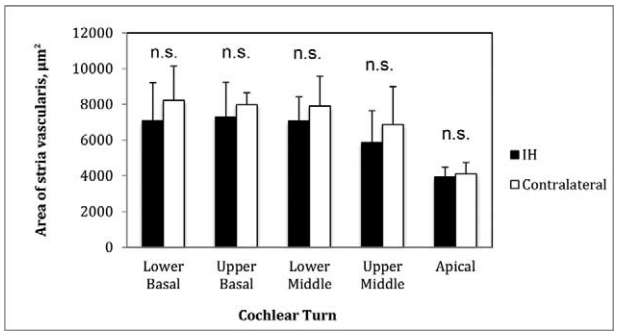

FIG. 3. Mean area of the stria vascularis (μm^2) (error bars = standard deviation). IH indicates intralabyrinthine hemorrhage; n.s., not significant.

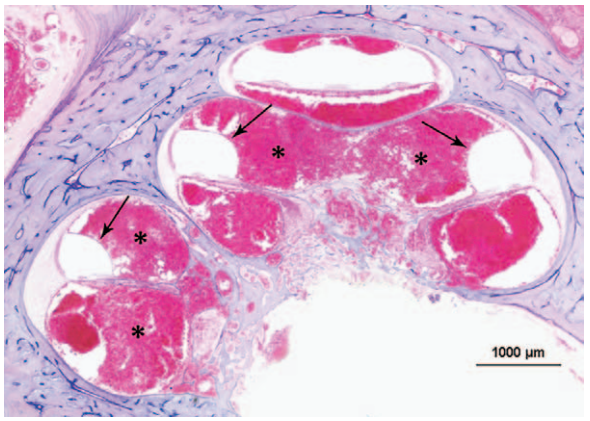

FIG. 4. Slight cochlear endolymphatic hydrops in a 16-year-old boy with acute lymphocytic leukemia. Arrows = endolymphatic hydrops. (*) hemorrhage. Staining with H&E.

an increase in reactive oxygen species and/or enzymatic dysregulation. We did not observe significant differences between the hemorrhagic and contralateral sides in the number of inner hair cells, in the number of spiral ganglion cells, in the number of fibrocytes of spiral ligament, or in the areas of the stria vascularis and spiral ligament. But that finding might because of the fact that most of our temporal bone samples were from patients with acute problems (intracerebral hemorrhage, subarachnoid hemorrhage, subdural hemorrhage, head trauma, and scuba diving); damage to these structures can take longer to develop.

In conclusion, we found that IH can lead to a significant loss of outer hair cells in the cochlea and endolymphatic hydrops, which could account for hearing loss and vertigo. Lack of damage to the inner hair cells, spiral ganglion cells, stria vascularis, or spiral ligament suggests that patients with IH could be candidates for amplification with hearing aid or cochlear implant.

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