Original Article

Cochlear lateral wall and vestibular aqueduct in temporal bones with endolymphatic hydrops from patients with and without vestibular symptoms

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ABSTRACT

Objectives: This study aims to compare the stria vascularis and spiral ligament, and evaluate the bony arch around the vestibular aqueduct, in temporal bones with endolymphatic hydrops (EH) from patients with (EH+), and without (EH-) vestibular symptoms, compared to control temporal bones, to better understand the mechanism of vestibular symptoms in Meniere disease.

Patients and Methods: A total of 30 temporal bones (twelve EH+, six EH-, and twelve controls from 27 subjects [mean age 72 years; range 61-89 years]) were retrospectively evaluated in this study. Exclusions were ototoxicity, systemic or neurologic diseases. Stria vascularis areas were measured in all cochlear turns. The spiral ligament was divided into four sections according to fibrocytes. The bony area around the vestibular aqueduct was evaluated according to cellular appearance.

Results: Stria vascularis area between EH+ and EH- in any turn except upper middle was statistically insignificant. Loss of fibrocytes was not statistically different between any groups. Denuded osteoblasts, and diminished edges around bony layer of vestibular aquaduct were statistically different in EH+ and EH- (p=.0003).

Conclusion: Lack of histopathologic differences in cochlear lateral walls between any group suggest that these changes might not be responsible for generation of vestibular symptoms. Significant degenerative changes in the vestibular aqueduct in EH+ compared to EH- bones may suggest a relationship of the vestibular aqueduct and vestibular symptoms.

Keywords: Endolymphatic hydrops; Meniere disease; temporal bone.

Endolymphatic hydrops (EH) has been considered to be the principal histopathologic finding in Meniere disease. It has also been described in a variety of conditions, including genetic anomalies, otitis media, otosclerosis, trauma, viral infection, and autoimmune disease.[1] A histopathologic report described EH in patients who did not exhibit the classic symptoms of Meniere disease.[2] Because hydrops is the only consistent pathologic abnormality observed at the level of light microscopy, a central hypothesis has been articulated regarding the pathophysiology of hydrops and vestibular symptoms. If this

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hypothesis were true, every case of hydrops should show the clinical symptoms, unless the chain of neural events is interrupted. A recent study concluded that EH was only a histologic marker for Meniere syndrome and was not directly responsible for its symptoms, including sensorineural hearing loss, tinnitus, and vertigo.[3]

In a recent histological study, Michaels et al.[4] described the inner layer of the bony vestibular aqueduct. The cylindrical lining extends into the posteromedial portion of the vestibule in an arch-like form which was named the 'vestibular arch.' It closely surrounds the endolymphatic duct, being associated with it throughout most of its length. The vestibular arch is a thin, osseous structure containing numerous small skeletal cells. They observed a striking loss of these cells in 20 cases of Meniere disease.[4] The vestibular arch has the following features: heavy infiltration with osteoblasts throughout the whole length of vestibular arch; evidence of a minor degree of apoptosis by the presence of degenerative changes in some of the osteoblast nuclei; the presence of numerous Volkmann canals of large and small size, many containing blood vessels within their lumina; and the presence of numerous 'microcanals,' particularly around Volkmann canals, in close proximity to the endolymphatic duct.[5]

The purpose of this study is to evaluate the areas of the stria vascularis and spiral ligament and the bony arch around the vestibular aqueduct in temporal bones from patients with EH with (EH+), and without (EH-) vestibular symptoms in an effort to understand the mechanism of vestibular symptoms in Meniere disease.

PATIENTS AND METHODS

The study included 30 temporal bones, 18 with EH (12 with vestibular symptoms EH+, six without vestibular symptoms EH-), and 12 controls without hydrops, from 27 subjects (mean age 72 years; range, 61 to 89 years). The temporal bones were obtained from the collection at the University of Minnesota, Minneapolis, Minnesota. All of the temporal bones had been removed at autopsy, fixed in formalin solution, decalcified, and embedded

in celloidin. Temporal bones were serially sectioned in the horizontal plane at a thickness of 20 mm. Every 10th section was stained with hemotoxylin and eosin and mounted on glass slides for light microscopic observation. Morphometric measurements were made in all turns of the cochlea at the mid-modiolar level and two adjacent sections. Temporal bones were excluded if patients had a history of ototoxicity, systemic and/or neurologic diseases. The study was approved by the Institutional Review Board of the University of Minnesota with 35 temporal bones, but five were excluded from the study because of a history of ototoxicity.

Stria vascularis

Images were acquired with a Nikon Eclipse E400 imaging system equipped with measurement software (NIS-Elements Basic Research, version 4.30.01, Nikon, Instruments Inc., Tokyo, Japan) connected to a personal computer (Dell XPS Inter core 2014, Dell, USA). The calibrated image was obtained at a magnification of 20× (AxioCam, Carl Zeiss Microscopy, Pleasanton, CA, USA) for the stria vascularis. Areas of stria vascularis were quantified by determining the areas of their cut surfaces, with the aid of the computer. The measurements were made using Nikon Eclipse E400 imaging system equipped with measurement software (NIS-Elements Basic Research, version 4.30.01, Nikon, Instruments Inc., Tokyo, Japan). Areas of stria vascularis in each segment were compared between cases of EH+ and EH-, and between the control group using two-tailed t-test.

Spiral ligament

The spiral ligament was divided into four segments according to the appearance of different types of fibrocytes based on the results of previous studies by Spicer and Schulte.[6] Type 1 fibrocytes lay circumferentially aligned between the stria vascularis and bone. Type 2 fibrocytes occupied the superficial inferior spiral ligament between the basilar crest and the stria. Type 3 fibrocytes were longitudinally located in the deepest part of the inferior spiral ligament. Type 4 fibrocytes lay radially oriented inferior to the basilar crest. Drs. Kocdor and Cureoglu performed the evaluation and scoring according to the rating score used in the study.
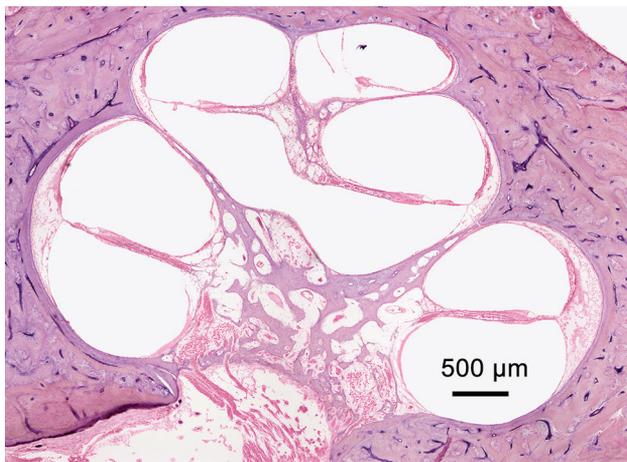
Figure 1. *A temporal bone mid-modiolar section with endolymphatic hydrops (H-E×40).*

The average loss of fibrocytes in each segment was estimated. The score was classified as: 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 at the mid-modiolar level, according to methods of Hequembourg and Liberman.[7] The average loss of fibrocytes in each segment was estimated and evaluated using the above scale. The loss of fibrocytes in each segment was compared between EH+ and EH-, in addition to that we also compared the loss of fibrocytes in each segment between the hydropic group and the control group by using two-tailed t-test.

Bony layer of the vestibular aqueduct

Temporal bone sections that included the vestibular arch from 18 temporal bones with EH and 12 temporal bones without hydrops

were evaluated seperately and blinded at a magnification of 20× by two authors on the basis of structural changes.

Statistical analysis

Data for each location were summarized by group using means and standard deviations (SDs), and compared statistically via two-sample t-tests using Excel 2013 (Microsoft Corp., Redmond, WA, USA). P-values less than .05 were considered statistically significant.

RESULTS

Of the 30 temporal bones from 27 subjects; there were 12 control temporal bones from 12 subjects (age range, 51 to 95 years, mean, 69 years); 12 temporal bones from 10 patients (age range, 61 to 89 years, mean, 72 years) were classified as EH+; and six temporal bones from five patients (age range 66 to 79 years, mean 72 years) were classified as EH-.

Every patient with the diagnosis of EH+ and EH- had EH on histopathologic examination in the affected ear (Figure 1). All hydrops cases were idiopathic, except one in the EH group that had a history of cochlear implantation. They all showed dilatation of the cochlear duct and the saccule. In the EH- group none of the five individuals had a history of episodic vertigo, despite hydrops of the cochlea and/or vestibular apparatus. In the EH- group one case had mild high frequency hearing loss; the remaining four cases had profound sensorineural hearing loss, which was progressive in nature.

The mean areas of the stria vascularis in the EH+ cases were 8,802.59 μm^2 in the lower basal, 8,044.37 μm^2 in the upper basal, 7,150.05 μm^2 in the lower middle, 5,453.93 μm^2 in the upper

Table 1. Mean area of stria vascularis in MD, EH and Control groups

	Lower basal turn	Upper basal turn	Lower middle turn	Upper middle turn	Apical turn
MD (mean area of stria vascularis, μm^2)	8802.59	8044.37	7150.05	5453.93	3999.14
EH (mean area of stria vascularis, μm^2)	6835.18	7748.86	5974.09	2279.34	4343.66
Control (mean area of stria vascularis, μm^2)	7324.30	7352.40	5368.30	4679.90	4043.40
P1	0.28	0.88	0.38	0.038	0.82
P2	0.89	0.85	0.53	0.57	0.66

EH: Endolymphatic hydrops; MD: Meniere disease; P1: P-value for comparison between MD and EH; P2: P-value for comparison between control and MD+EH.
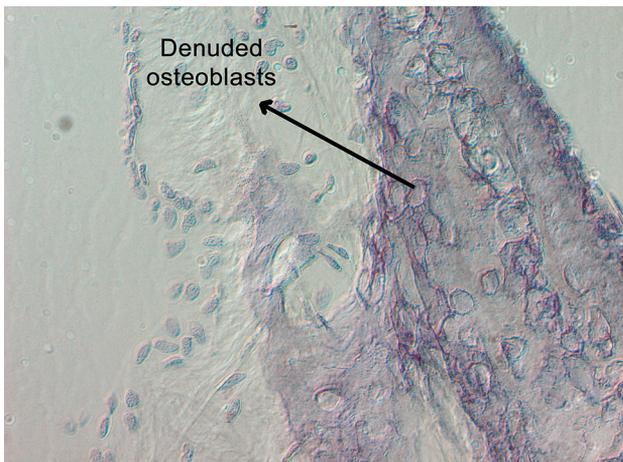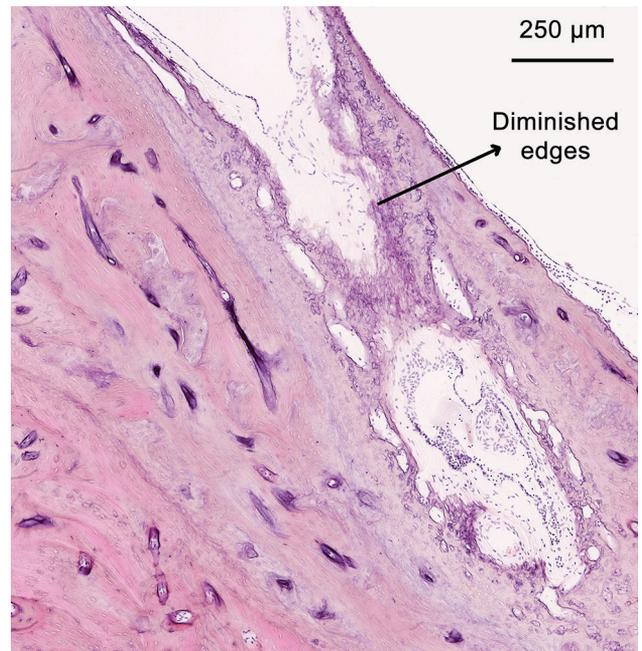
Figure 2. *Diminished, denuded osteoblasts around the endolymphatic duct in an EH+ case.*

Figure 3. *Diminished edges around the endolymphatic duct in an EH+ case.*

middle, and 3,999.14 μm² in the apical turn. In the EH- cases, the mean areas were 6,835.18 μm² in the lower basal, 7,748.86 μm² in the upper basal, 5,974.09 μm² in the lower middle, 2,279.34 μm² in the upper basal, and 4,343.66 μm² in the apical turn. There was no significant correlation between the areas of stria vascularis in any turn of the cochlea except the upper middle turn (p=.28 in the lower basal, .88 in the upper basal, .38 lower middle, .82 in the apical, and .038 in the upper middle turn). There was a significant decrease in the areas of stria vascularis in the upper middle turn in EH- compared to EH+ temporal bones (p=.038).

The mean areas of the stria vascularis were also measured in the group without MD and EH. These areas were compared with the hydrops group stria vascularis mean areas and again there was no statistical difference (Table 1).

There were no significant correlations between the loss of type 1, type 2, type 3 or type 4 fibrocytes in any turns of cochlea between all groups including controls.

One temporal bone each from the EH+ and EH- group did not show degenerative changes in the canals of the vestibular arch. Otherwise, there were significant degenerative changes in the canals of the vestibular arch in hydrops cases (both EH+ and EH-) compared to controls and a significant difference between EH+ compared to EH- temporal bones (p=.0003). The degenerative

changes in osteoblasts and irregular areas around the endolymphatic duct were greatly diminished or absent in the EH+ group (Figure 2, 3).

DISCUSSION

The central hypothesis for Meniere syndrome is that there are many possible etiologic factors that can lead to EH, which in turn generates the clinical symptoms. If this hypothesis were true, every case of hydrops should show the clinical symptoms, unless the chain of neural events is interrupted. Merchant et al.[3] indicated that EH of the cochlea is invariably associated with sensorineural hearing loss and hydrops of the cochlea and/or vestibular system and is not necessarily associated with a history of episodic vertigo. Our similar histopathologic findings between the groups with hydrops with, and without vestibular symptoms support their findings. Much research has focused on the pathology and pathophysiology of hydrops in the guinea pig model in an attempt to better understand Meniere disease. In a study in guinea pigs, Albers et al.[8] found that accumulation of endolymph due to obliteration of the endolymphatic sac and duct could lead to an

increase in reabsorptive activity of the stria vascularis to restore the disturbed fluid balance. Recent studies suggest that the stria vascularis and spiral ligament may have an important function in the regulation of ionic fluid balance in the inner ear.[2] Nadol et al.[9] observed that in patients with Meniere disease, there was a decrease in the immunoreactivity of the stria vascularis and spiral ligament for a variety of antibodies, including carbonic anhydrase, Ca^2 adenosine triphosphatase, aldehyde dehydrogenase, vimentin, Na^2K^2 adenosine triphosphatase, calcium-binding proteins, and connexin-26. It can be hypothesized that in EH, the enzyme system located in the Reissner membrane, stria vascularis, and spiral ligament immediately reverses the ionic imbalance between perilymph and endolymph due to leakage, but in Meniere disease there is a failure of this enzyme system resulting in ionic imbalance leading to symptoms.

Shinomori et al.[10] blocked the endolymphatic duct to induce hydrops in 22 guinea pigs and studied the cytochemistry of the inner ear at various postoperative survival times ranging from one day to three months. A striking finding was that there were changes in the cytochemistry of Type 1 and Type 2 fibrocytes and of nonsensory epithelial cells one day after surgery, before the development of hydrops. The connective tissue surrounding the endolymphatic duct is in communication with the perilymphatic fluid spaces of the vestibular and cochlear end-organs.[11] In our study, there was no significant difference in the areas of stria vascularis other than in the upper middle turn or loss of fibrocytes between EH+ and EH-.

In a recent histological study Michaels et al.[4] described the inner layer of the bony vestibular aqueduct. The cylindrical lining extends into the posteromedial portion of the vestibule in an arch-like form which they named the vestibular arch. It closely surrounds the endolymphatic duct, being associated with it throughout most of its length.[4] In their study all of the eight cases of Meniere disease showed degenerative changes in the canals of the vestibular arch. They made a list of terms for findings in the vestibular arch, including: *(i)* osteoblasts, for numerous small cells with well-defined edges

filing the "normal" vestibular arch; *(ii)* Volkmann canals, for numerous large or moderate-sized channels; and *(iii)* microcanals denuded of osteoblasts, for small empty spaces with thin, crazy pavement boundaries.[5] Changes such as denuded osteoblasts and diminished edges around the bony layer of the vestibular aqueduct were statistically different in both hydropic groups compared to the EH+ and EH- groups, in agreement with the results of Michaels and Soucek.[5]

In conclusion, the lack of histopathologic differences in the cochlear lateral wall between EH+ and EH- temporal bones with EH suggest that these changes might not be responsible for generation of vestibular symptoms, but significant degenerative changes in the vestibular aqueduct in EH+ compared to EH- bones may suggest a relationship of vestibular aqueduct and vestibular symptoms.

Declaration of conflicting interests

Funding

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