Effects of Type 2 Diabetes Mellitus on Cochlear Structure in Humans

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Objective: To evaluate the effects of type 2 diabetes mellitus on cochlear elements in humans.

Design: Comparative study of the histopathologic characteristics of human temporal bones.

Setting: Otopathology laboratory in a tertiary academic medical center.

Patients: Temporal bones from 18 patients with type 2 diabetes mellitus were divided into 2 groups according to the method of management of diabetes: insulin in 11 patients (mean age, 51.9 years; age range, 44-65 years) and oral hypoglycemic agents in 7 patients (mean age, 54.4 years; age range, 45-64 years). The diabetic groups and 26 age-matched controls (mean age, 52.9 years) were examined using light microscopy, and the cochlear changes were compared between groups.

Main Outcome Measures: Morphometric measurements of vessel wall thickness in the basilar membrane and stria vascularis were made in all turns of the cochlea at the midmodiolar level. Area measurements of the stria vascularis were made in all turns of the cochlea at the midmodiolar level. Cochlear reconstructions and standard cytococheleograms were prepared using an oil immersion objective. The number of spiral ganglion cells was determined for each segment of the cochlea. Comparisons were made in each segment between diabetic and control groups.

Results: In the insulin group, walls of the vessels of the basilar membrane and stria vascularis in all turns were significantly thicker than those of controls. Walls of the vessels of the stria vascularis in the basal turn were also significantly thicker in the oral hypoglycemic group than in controls. Atrophy of the stria vascularis in most turns of the insulin group and the lower middle turn of the oral hypoglycemic group was significantly greater than in the controls. Loss of cochlear outer hair cells was significantly greater in the lower and upper basal turns in both diabetic groups. No significant difference was found in the number of spiral ganglion cells or inner hair cells between groups.

Conclusion: This study demonstrates that cochlear microangiopathy and degeneration of the stria vascularis and cochlear outer hair cells are found in patients with type 2 diabetes mellitus.

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The relationship between diabetes mellitus and sensorineural hearing loss has been studied for more than a century. Many authors agree that diabetes mellitus can lead to sensorineural hearing loss.1 Histopathologic temporal bone studies in diabetic animal models have shown thickening of the basement membranes of capillaries,2 loss of outer hair cells (OHCs),3,4 loss of inner hair cells,4 atrophy of spiral ganglion cells,4,5 and edematous changes of the intermediate cells and atrophy of marginal cells in the stria vascularis.6,7 Although a few histopathologic case reports of human temporal bones from patients with diabetes mellitus exist, only 2 statistically comparable histopathologic studies have been reported. One study8 compared the luminal narrowing of the internal auditory arteries of nondiabetic patients with that of 4 diabetic patients. Another study9 compared the percentage of histologically normal hair cells and normal stria vascularis of nondiabetic patients with those of 8 diabetic patients. The authors noted no significant differences in the mean estimated percentage of hair cells and stria vascularis cells between groups; however, they did not differentiate diabetes mellitus into types 1 and 2, cochlear hair cells were not distinguished as outer or inner, and analysis was not performed by cochlear turn. To date, no other comparative study has reported histopathologic changes in cochlear elements in a large series of patients with type 2 diabetes mellitus.
In this article, we describe cochlear changes in patients with type 2 diabetes mellitus divided into 2 groups: an insulin management group and an oral hypoglycemic agent management group. Because type 2 diabetes mellitus in humans often occurs in elderly patients, it can be difficult to differentiate the effect of diabetes from that of presbycusis on hearing acuity. We therefore conducted a study in temporal bones from patients with type 2 diabetes mellitus, excluding patients older than 65 years. To our knowledge, this study is the first to quantitatively document the changes in the cochlea due to type 2 diabetes mellitus in humans.

**METHODS**

**PATIENTS**

Eighteen patients (10 men and 8 women) with type 2 diabetes mellitus were studied. Specimens of patients with diabetes mellitus were divided into 2 groups. The method of management of diabetes was insulin in 11 patients (mean age, 51.9 years; age range, 44-65 years) and oral hypoglycemic agents in 7 patients (mean age, 54.4 years; age range, 45-64 years). Duration of diabetes in all patients was more than 7 years. For the control group, 26 healthy cases (12 men and 14 women) were studied. Their ages ranged from 40 to 65 years, with a mean age of 52.9 years. Because in some cases only 1 temporal bone was removed, we randomly selected only 1 side from those cases that included both left and right ears. We excluded patients who had a history of acoustic trauma, systemic autoimmune disorders, ototoxic drug use, or otologic surgery and those with any other otologic diseases, such as otosclerosis and otitis media, since all of these conditions may contribute to changes of the inner ear. Temporal bones from patients older than 65 years were also excluded. All temporal bones in this study had previously 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 μm. Every 10th section was stained with hematoxylin-eosin and mounted on a glass slide for light-microscopic observation.

**VESSELS OF THE BASILAR MEMBRANE AND STRIA VASCULARIS**

Morphometric measurements of the wall thickness of the vessels of the basilar membrane (VBM) were made in all turns of the cochlea at the midmodiolar level and on the adjacent 2 sections. The wall thickness of the vessels of the stria vascularis (VSV) was measured using the maximum outer diameter of the VSV in each turn of the cochlea at the midmodiolar level. The image was acquired with a CCD camera (Dage-MTI Inc, Michigan City, Ind) connected to a personal computer. The calibrated image was obtained at a magnification of ×1000 for the VBM and ×600 for the VSV. Thickness of the vessel walls was measured using image analysis software (Image-Pro Plus, version 3.0; Media Cybernetics, Silver Springs, Md) and calculated using a modification of the method described by Robison et al.10

Vessel wall area (VWA) and vessel wall length (VWL) per vessel cross section were determined with the following formulas:

\[
\text{VWA} = T - (E + Lu)
\]

\[
\text{VWL} = \text{Length of Lines Delimiting VWA}/2,
\]

where T indicates the total cross-sectional area of each vessel; E, the endothelial cell nuclear area; and Lu, the luminal area. From these calculations, the relative thickness could be expressed in terms of vessel wall area per unit length (VWA/VWL).

**STRIA VASCULARIS**

Morphometric measurements of area counts of the stria vascularis were made in all turns of the cochlea at the midmodiolar level and on the adjacent 2 sections. The image was acquired with a CCD camera at a magnification of ×200 for the stria vascularis. The areas of stria vascularis were quantified by determining the areas of their cut surfaces with the aid of a computer. Measurements were made using image analysis software (Image-Pro Plus).

**HAIR CELLS**

Cochlear reconstructions and standard cytocochleograms were prepared using the oil immersion objective according to the method of Schuknecht and Gacek.11 Hair cells were identified as present or absent. Comparisons of the percentage of loss of hair cells in each turn were made between diabetic and control groups.

**SPIRAL GANGLION CELLS**

The Rosenthal canal was divided into 4 segments, described previously as follows: I, from base to 6 mm; II, 6 to 15 mm; III, 15 to 22 mm; and IV, 22 mm to apex.11 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. Comparisons of the numbers of spiral ganglion cells in each segment were made between diabetic and control groups.

**STATISTICAL ANALYSIS**

Results were presented as mean±SD. Statistical evaluation was performed with the nonparametric Mann-Whitney test. The level of significance was set at P<.05. Correlations were calculated with the Spearman rank correlation coefficient.

**VESSELS OF THE BASILAR MEMBRANE**

The insulin group had significant wall thickening of the VBM compared with the control group in the basal, middle, and apical turns of the cochlea (P=.02, P=.01, and P=.04, respectively) (Figure 1).

**VESSELS OF THE STRIA VASCULARIS**

The insulin group had significant wall thickening of the VSV compared with the control group in the basal, middle, and apical turns of the cochlea (P=.002, P=.02, and P=.03, respectively) (Figure 2). The oral hypoglycemic agent group had significant wall thickening of the VSV in the basal turn of the cochlea compared with the control group (P=.02) (Figure 2).

**STRIA VASCULARIS**

Atrophy of the stria vascularis in the lower basal (P=.002), lower middle (P=.002), upper middle (P=.03), and api-
cal turns \( (P = .002) \) in the insulin group was significantly greater than in the control group \( (P < .05) \) (Figure 3A). In addition, the insulin group had significant atrophy of the stria vascularis compared with the oral hypoglycemic agent group in the apical turn of the cochlea \( (P = .03) \) (Figure 3A).

HAIR CELLS

A significantly greater loss of OHCs occurred in the insulin group compared with the control group in the lower basal \( (P < .001) \) and upper basal turns \( (P = .003) \) \( (P < .05) \) (Figure 4). The oral hypoglycemic agent group had a significantly greater loss of OHCs compared with the control group in the lower and upper basal turns of the cochlea \( (P = .006 \text{ and } P = .04, \text{ respectively}) \) (Figure 2). No significant difference was found between the diabetic and control groups in the percentage of loss of inner hair cells in any turn.

SPIRAL GANGLION CELLS

No significant difference was found in the total number of spiral ganglion cells between the insulin \( (\text{mean} \pm \text{SD}, 27,832 \pm 4,689) \), oral hypoglycemic agent \( (\text{mean} \pm \text{SD}, 29,453 \pm 4,063) \), and control groups \( (\text{mean} \pm \text{SD}, 28,966 \pm 6,836) \). In addition, no significant difference was found in the number of spiral ganglion cells in the Rosenthal canal in any turn between groups. No relationship occurred between the decreasing spiral ganglion cells and loss of either OHCs or inner hair cells.

Figure 1. Mean wall thickness of the vessels of the basilar membrane (VBM) in each cochlear turn. Error bars indicate standard deviation. There is a significantly increased thickening of the VBM in the insulin group compared with the control group in all turns. \( *P < .05 \).

Figure 2. Mean wall thickness of the vessels of the stria vascularis (VSV) in each turn. Error bars indicate standard deviation. There is a significantly increased thickening of the VSV in the insulin and oral groups compared with the control group. \( *P < .05 \).

Figure 3. A, Mean area of the stria vascularis. Error bars indicate standard deviation. Atrophy of the stria vascularis in the insulin group is significantly higher than in the control and oral groups. The oral group also had significant atrophy of the stria vascularis compared with the control group. \( *P < .05 \). \( \dagger P < .01 \). B, This temporal bone from a 56-year-old woman with a 16-year history of type 2 diabetes mellitus has increased thickness of the vessel walls (arrowhead) and atrophy of the stria vascularis (arrow) in the lower middle turn (hematoxylin-eosin, original magnification \( \times 200 \)).
Only 3 patients with type 2 diabetes mellitus, 2 patients from the insulin group and 1 patient from the oral hypoglycemic group, had pure-tone audiometry. The audiogram of the 45-year-old patient with oral hypoglycemic therapy, performed just 2 months before death, showed a high-frequency sensorineural hearing loss. The audiometry from the 49-year-old patient with insulin therapy was performed just before death, and the results were within normal limits. However, the audiogram of the 51-year-old patient who had insulin therapy showed high-frequency sensorineural hearing loss.

Thickening of the vessels of the inner ear in a diabetic animal model and in patients with diabetes has previously been reported. In the present study, we showed a significantly increased wall thickness of the VBM and VSV in the diabetic groups. Thickness of vessel walls of the insulin group showed a tendency to increase when compared with those of the oral hypoglycemic agent group.

Some studies have reported damage of the cells of the stria vascularis in animal models with diabetes. Ishikawa et al reported edematous changes in the intermediate cells. Nakae and Tachibana observed that the marginal cells were atrophic and tended to be replaced by swollen intermediate cells. However, they did not observe any changes of the vessel walls in the cochlea.5,6 In addition, they believed that the pathologic changes observed in the diabetic mice were unlikely to be due to thickening of the vessels, indicating other possible precipitating factors. In this study, we observed not only a significantly increased thickness of the vessel walls but also significant atrophy of the stria vascularis in both of the diabetic groups compared with the control group, especially in the insulin group. However, no significant correlation occurred between wall thickness of the VSV and atrophy of the stria vascularis in any turn. Although angiopathic change occurs as a result of activation of the polyol pathway in the hyperglycemic state and the stria vascularis is known to be vulnerable to hyoxia, atrophy of the stria vascularis may be due not only to impairment of blood flow caused by microangiopathy but also to other factors, such as oxidative stress as a result of activation of the polyol pathway in the hyperglycemic state. Shi and Nuttall reported oxidative damage to the marginal cells of the stria vascularis attributable to noise stress. Recently, some authors reported a relationship between diabetes mellitus and apoptosis in the kidney. Kang et al observed that p38 mitogen-activated protein kinase activity was increased in early diabetic glomeruli. In the stria vascularis, apoptotic cell death induced by cisplatin and noise stress has been reported. We speculate that diabetes-induced apoptotic cell loss may occur not only in renal glomeruli but also in the diabetic stria vascularis.

Some animal studies have reported a significant loss of OHCs in the diabetic model. Most of them observed damage of OHCs mainly in the lower turns. A study by Wackym and Linthicum was the only one in which statistical analysis of human cochlear hair cells was performed. These authors noted no significant differences in mean estimated percentage of hair cells and stria vascularis cells between diabetic and nondiabetic patients. They noted, however, that diabetic patients with basilar membrane microangiopathy had significantly lower percentages of histologically normal hair cells and stria vascularis cells and significantly greater hearing loss than diabetic patients without such pathologic changes. They did not distinguish between outer and inner hair cells of the cochlea, and analysis was not performed by turn. Our study shows a significant decrease in the number of OHCs in the lower and upper basal turns of the cochlea in the type 2 diabetic groups when compared with the control group. In addition, loss of OHCs in the lower and upper basal turns of the insulin group were greater when compared with the control group. However, no significant correlation was found between wall thickness of the VBM and loss of OHCs in the lower and upper turns. Raynor et al reported that loss of hair cells in noise-exposed diabetic animals was significantly greater than in diabetic
animals without noise exposure. Another study reported a highly significant OHC loss in diabetic rats that were also hypertensive. In both studies, most of the damage was localized to the basal and middle turns of the cochlea. Although microangiopathy is an important factor in cochlear disease in diabetic patients, other precipitating factors such as oxidative stress and apoptosis due to the hyperglycemic state, noise, and hypertension can work synergistically to cause the observed pathologic changes in the stria vascularis and OHCs.

Loss of spiral ganglion cells has been reported in some animal models of diabetes. No study has reported statistically comparable histologic findings in human diabetic spiral ganglion cells. Accumulation of polyols leads to a decrease in myo-inositol and Na+/K+ adenosine triphosphatase in nerves, contributing to diabetic neuropathy. However, we did not observe a significant decrease in the number of spiral ganglion cells in temporal bones of diabetic patients compared with controls. As reported in our study, no correlation was found between the loss of hair cells and the number of spiral ganglion cells. This finding may be because there are 2 types of ganglion cells; the larger type 1 cells appear to constitute approximately 90% to 95% of all spiral ganglion cells and exclusively innervate inner hair cells, whereas the smaller type 2 cells innervate exclusively OHCs. Therefore, because no significant decrease in the number of inner hair cells occurred in the diabetic group, we would probably not observe a significant loss of spiral ganglion cells in any turn, even though a significant decrease occurred in the number of OHCs in the lower and upper basal turns of the cochlea in the diabetic groups compared with the control group.

This article demonstrates that type 2 diabetes mellitus results in changes of the cochlea, such as significant atrophy of the stria vascularis and OHC loss in the basal turn, that are likely to result in hearing loss. Significant wall thickening of the VBM and VSV was observed in diabetic patients. In addition, these pathologic changes of the insulin group showed a tendency to increase when compared with the oral hypoglycemic agent group. No significant difference was found, however, in the number of spiral ganglion cells between the diabetic and control groups. This study suggests that hearing loss in patients with type 2 diabetes mellitus may result from cochlear microangiopathy, degeneration of the stria vascularis, and loss of cochlear OHCs.

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Author Contributions: All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Cureoglu, Schachern, and Paparella. Acquisition of data: Fukushima and Oktay. Analysis and interpretation of data: Harada. Drafting of the manuscript: Cureoglu, Fukushima, and Oktay. Critical revision of the manuscript for important intellectual content: Schachern, Paparella, and Harada. Statistical analysis: Fukushima and Oktay. Obtained funding: Paparella. Administrative, technical, and material support: Oktay. Study supervision: Cureoglu, Schachern, and Harada.

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CONCLUSION

This article demonstrates that type 2 diabetes mellitus results in changes of the cochlea, such as significant atrophy of the stria vascularis and OHC loss in the basal turn, that are likely to result in hearing loss. Significant wall thickening of the VBM and VSV was observed in diabetic patients. In addition, these pathologic changes of the insulin group showed a tendency to increase when compared with the oral hypoglycemic agent group. No significant difference was found, however, in the number of spiral ganglion cells between the diabetic and control groups. This study suggests that hearing loss in patients with type 2 diabetes mellitus may result from cochlear microangiopathy, degeneration of the stria vascularis, and loss of cochlear OHCs.

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