Impairment in the Sensorineural Elements of the Cochlea and Vasculature in Diabetes Mellitus : a Human Temporal Bone Study

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ABSTRACT. Aims/hypothesis : To evaluate impairment in the sensorineural elements of the cochlea in patients with type 2 diabetes mellitus histopathologically.
Methods : Temporal bones from 18 patients (mean age, 52.8 years) with type 2 diabetes mellitus and ones from 26 age-matched normal subjects (mean age, 52.9) were examined by light microscopy. Thickening of the walls of the cochlear vessels, the percentage of loss of cochlear hair cells and the number of spiral ganglion cells were compared between the groups.
Results : In the diabetes group, the walls of the vessels of the basilar membrane in all turns ($P < 0.01$) were significantly thicker than those in controls. Loss of cochlear outer hair cells was significantly greater in the lower basal ($P < 0.001$), upper basal and lower middle turns ($P < 0.05$) in the diabetic group. There was no significant difference in the number of spiral ganglion cells or inner hair cells between the groups.
Conclusion/interpretation : This study demonstrates that there is cochlear microangiopathy and degeneration of cochlear outer hair cells in patients with type 2 diabetes mellitus.

Key words : diabetes — human temporal bone — pathology — microangiopathy — cochlear hair cells — spiral ganglion cells

The relationship between diabetes mellitus and sensorineural hearing loss (SNHL) has been studied for more than a century. Many authors agree that diabetes mellitus can lead to SNHL.[1,2] Most audiometric studies of hearing loss in patients with diabetes have shown a gradually progressive, bilateral, high frequency SNHL, especially in elderly patients.[1]

Histopathological cochlear studies in diabetic animal models have shown thickening of the basement membranes of capillaries,[3] loss of outer hair cells (OHCs),[4-6] loss of inner hair cells,[6] atrophy of spiral ganglion cells,[5,7] and atrophy of the stria vascularis.[6-8] On the other hand, Nageris *et al* reported no statistical difference in inner and outer hair cells between diabetic rats and control subjects.[9] In human studies, there have been a few histopathologic case reports of human temporal bone from patients with diabetes mellitus,[10] but only one statistical comparative histopathologic study

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of the cochlea.[11] The percentage of histologically normal hair cells and normal stria vascularis of non-diabetics patients was compared with that of patients with diabetes mellitus. No significant differences were noted in the mean estimated percentage of hair cells and stria vascularis cells between the groups.[11] The study, however, included many elderly patients and did not differentiate diabetes mellitus into type 1 and 2, nor distinguish cochlear hair cells into outer or inner cells, nor analyze the cochlea by each cochlear turn. Thus there has been no comparative study reporting on histopathologic changes in cochlear elements in a large series of patients with type 2 diabetes mellitus. In this report, we intend to describe cochlear changes in patients with type 2 diabetes mellitus. Because type 2 diabetes mellitus in humans often occurs in elderly patients, it can be difficult to differentiate the effect of diabetes from on hearing acuity that of presbycusis. Therefore we conducted a study of the temporal bones from patients with type 2 diabetes mellitus excluding patients over 65 years of age. To the best of our knowledge, this study is the first to quantitatively document in humans changes in the sensorineural elements in the cochlea due to type 2 diabetes mellitus.

MATERIALS AND METHODS

Subjents : Eighteen patients (10 males, 8 females) with type 2 diabetes mellitus were studied. The patients ranged in age from 44 to 65 years old with a mean of 52.8 (SD = 6.50) years old. The duration of diabetes ranged from 3 to 34 years with a mean of 13.7 years (SD = 9.97). The disease was managed in 11 patients by hypodermic injection of insulin and in the 7 other patients with oral hypoglycemic agents. For the control group, 26 normal cases (12 males and 14 females) were chosen and studied. Their ages ranged from 40 to 65 years old with a mean of 52.9 (SD = 8.87) years old. Because only one temporal bone was removed in some cases, we randomly selected only one side from those cases that included both left and right ears. Excluded were subjects who had a history of acoustic trauma, systemic autoimmune disorders, ototoxic drugs or otologic surgery, and those with any other otologic diseases, such as otosclerosis or otitis media, since all of these may contribute to changes of the inner ear. All of the temporal bones in this study had been previously 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 tenth section was stained with hematoxylin and eosin and mounted on glass slides for light microscopic observation.

Vessels of the basilar membrane (VBM) : Morphometric measurements of the thickness of the walls of the VBM were made in all turns of the cochlea at the mid-modiolar level and on the adjacent two sections. An image was acquired with a CCD camera connected to a personal computer. The calibrated image was obtained at a magnification of $\times 1000$. The thickness of the vessel walls was measured using image analysis software, Image-Pro @Plus (Media Cybernetics, Silver Springs, MD ; Version 3.0) and calculated using a modification of the method described by Robinson *et al.*[12]

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

$$VWA = T - (E + Lu)$$

VWL = length of lines delimiting VWA/2

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

Hair cells : Cochlear reconstructions and standard cytocochleograms were prepared using an oil-immersion objective according to the method of Schuknecht *et al.*[13] Hair cells were identified as present or absent. Comparisons of the percentage of loss of hair cells in each turn between the diabetes and control groups were made.

Spiral ganglion cells : Rosenthal's canal was divided into four segments described previously as : I (from base to 6 mm) ; II (6 to 15 mm) ; III (15 to 22 mm) and IV (22 mm to apex).[13] 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 were made of the numbers of spiral ganglion cells in each segment between the diabetes and control groups.

Statistical analysis : The results were presented as mean +/−SD. Statistical evaluation was carried out using the non-parametric Mann-Whitney's U test. The level of significance was $p < 0.05$. Correlations were calculated with Spearman's correlation coefficient.

RESULTS

VBM : The diabetes group had significant thickening of the walls of the VBM compared to the control group in the basal, middle and apical turns of the cochlea (P=0.0015, P=0.0050, P=0.0048, respectively) **(Fig 1)**.

Hair cells : There was a significantly greater loss OHCs in the diabetic group compared with the control group in the lower basal (P = 0.0001), upper basal (P = 0.0145) and lower middle turns (P = 0.0383) **(Fig 2, 3)**. There was no relationship between increasing thickening of the walls of the VBM and loss of OHCs, and there was no significant difference between the diabetes and control groups in the percentage of loss of inner hair cells in any turn.

Spiral ganglion cells : There was no significant difference in the total number of spiral ganglion cells between the diabetes (24199 +/− 6589) and control group (25344 +/− 5587). In addition, there was no significant difference in the number of spiral ganglion cells in Rosenthal's canal in any turn between the group **(Fig 4)**. There was no relationship between decreasing spiral ganglion cells and loss of either OHCs or inner hair cells.

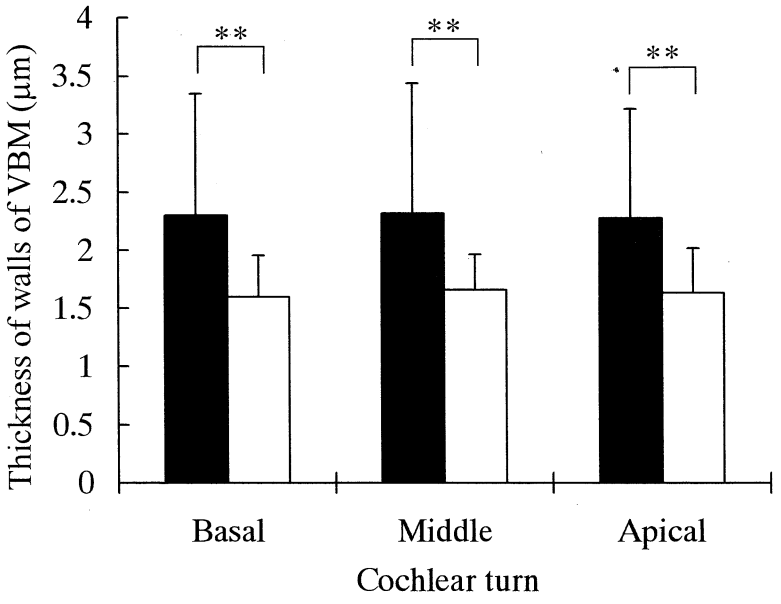

Fig 1. Graph of mean thickness of walls of vessels of the basilar membrane (VBM) in each cochlear turn for the control (white bars) and diabetic (black bars) groups. There was a significantly increased thickening of VBM in the diabetic group compared with the control group in all turns (**$P<0.01$).

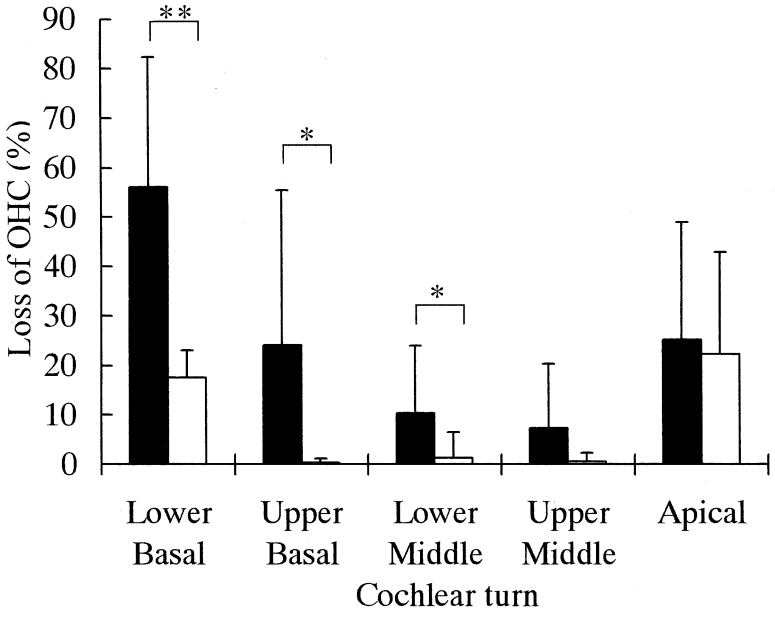

Fig 2. Graph of mean OHC loss in each cochlear turn for the control (white bars) and diabetic (black bars) groups. There was a significantly greater loss of OHCs in the diabetic group compared with the control group in the lower basal turn (**$P<0.001$) and upper basal and lower middle turns (*$P<0.05$).

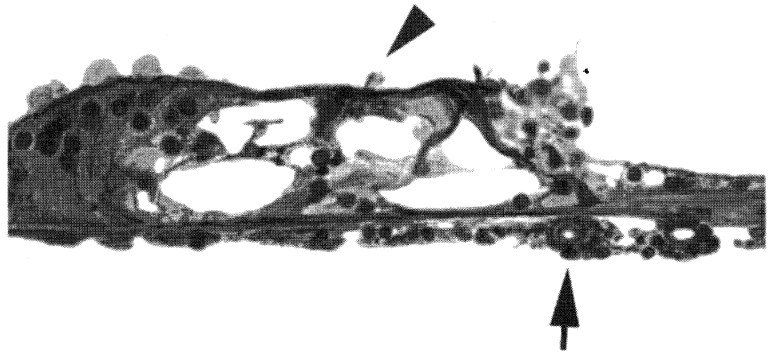

Fig 3. This temporal bone from a 56-year old female with a 16-year-history of type 2 diabetes mellitus has increased thickness of walls of vessel of the basilar membrane (arrow). IHC is identified as present. 2 OHCs are identified as absent (arrow head) in the lower middle turn. Hematoxylin, eosin, ×200.

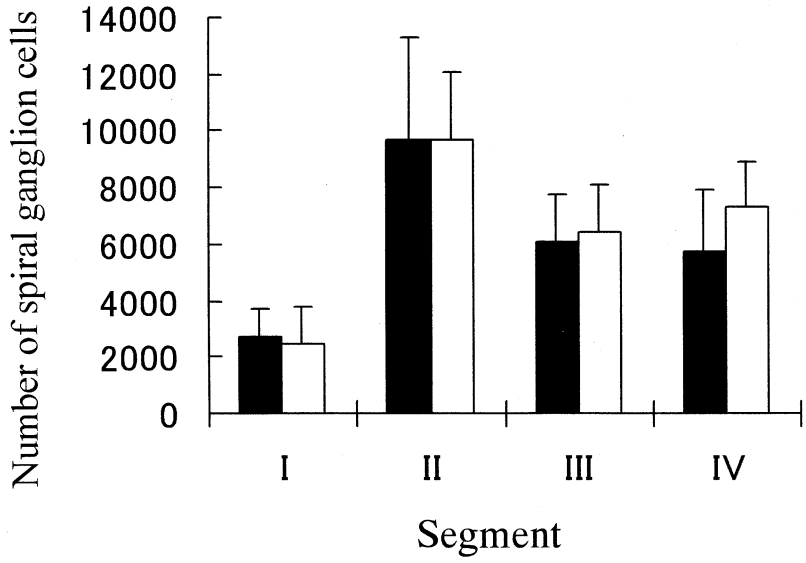

Fig 4. There is no significant difference in the number of spiral ganglion cells in any segment of Rosenthal's canal between the control (white bars) and diabetic (black bars) groups.

DISCUSSION

Thickening of the vessels of the inner ear in a diabetic animal model[3] and in patients with diabetes[11] has been reported. In the present study, we observed a significantly increased thickness of the vessel walls of the basilar membrane. Histopathologically, it has been reported that increased type IV collagen in basement membrane causes this thickening.[3] It has also been reported that these angiopathic changes occur as result of activation of the polyol pathway in the hyperglycemic state.[14]

Some animal studies have reported a significant loss of OHCs in the diabetic model.[4-6] Most of them observed damage to the OHCs mainly in the olwer turns.[5,6] In our study, a significant decrease was seen in the number of OHCs mainly in the basal turn and also in the lower middle turns of the cochlea in the type 2 diabetes group when compared to the control group. The hair cells are known to be vulnerable to hypoxia, so that loss of OHCs may be partly due to microangiopathy caused by oxidative stress as a result of activation of the polyol pathway in the hyperglycemic state. However, there was no relationship between increasing thickening of the walls of the VBM and the loss of OHCs.

Many authors have reported that noise stress[15,16] and cisplatin[17] also cause oxidative damage to the cochlear hair cells without microangiopathy. In addition, recently some authors have reported a relationship between diabetes mellitus and apoptosis.[18-21] In cochlear hair cells, apoptotic cell death induced by cisplatin,[22,23] noise stress[24] or oxidative stress[23,24] has been reported. We speculate that diabetes-induced apoptotic cell death in diabetic OHCs or oxidative damage may induce the loss of a number of cells directly without microangiopathy.

Raynor *et al* reported that loss of hair cells in diabetic animals with noise exposure was significantly greater than in diabetic animals without it.[25] Another study reported a highly significant outer hair cell loss in hypertensive diabetic rats.[26] In both studies, most of the damage was also localized to the basal and middle turns of the cochlea.[25,26] Although microangiopathy is an important factor in cochlear pathology in diabetes patients,[11] other precipitating factors such oxidative stress and apoptosis due to the hyperglycemic state, noise and hypertension can work synergistically to cause the observed pathological cochlear change in OHCs.

Loss of spiral ganglion cells has been reported in some animal models of diabetes.[5,7] No study has reported statistically comparable histological findings in human diabetic spiral ganglion cells. Accumulation of polyols leads to a decrease in myo-inositol and Na^+/K^+ ATPase in nerves, contributing to diabetic neuropathy.[27] However, we did not observe a significant decrease in the number of spiral ganglion cells in diabetic temporal bones compared with those of controls. As reported in our study, there was no correlation between the loss of hair cells and the number of spiral ganglion cells. This may be because there are two types of ganglion cells. The larger type I cells appear to constitute approximately 90-95% of all spiral ganglion cells and exclusively innervate inner hair cells, whereas the rest, the small number of smaller type II cells, innervate OHCs exclusively. In this study, we did not observe a significant loss of spiral ganglion cells in any turn, even though there was a significant decrease in the number of OHCs in the lower and upper basal turns of the cochlea in the diabetes group compared to the control group. This is because there was no significant decrease in the number of inner hair cells in the diabetes group.

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