study: acute meningitis (1 case), acute otitis media (2 cases), and Wegener granulomatosis in the middle ear (2 cases).

MRI examination

MRI was performed using a three-tesla scanner (Trio, Siemens, Erlangen, Germany). 3D-FLAIR was performed before and after the intravenous administration of a single dose of gadolinium. To delineate the anatomy of the CSF space, we performed heavily T2-weighted 3D constructive interference imaging in the steady state prior to the administration of contrast material. High signal on 3D-FLAIR after contrast enhancement was scored depending by the degree of the enhancement as follows: weak = 1, moderate = 2, and strong = 3.

Results

The clinical observation and the MRI findings in the five cases are summarized in Tables I and II. 3D-FLAIR images in five cases showed high pre-contrast signal in the cochlea, the vestibule, and the semicircular canals in the inner ear, while the post-contrast images demonstrated enhancement in the inner ear of the affected ears. High post-contrast signals examined after treatment were decreased in all cases except one (Case 2), which were accompanied by improvement in symptoms of vertigo (Cases 1 and 4), and acute SNHL (Cases 3, 4, and 5). The post-contrast images were not available in one patient because of complete ossification of the cochlea (Case 1). Correlation between clinical symptoms and FLAIR abnormalities was observed in some cases. Representative cases from different condition were described below.

Acute meningitis (Case 1)

A 42-year-old man was hospitalized because of impaired consciousness, the cause of which was diagnosed as acute meningitis. He had had severe vertigo and profound SNHL on the left side. Six months later the patient again suffered acute meningitis and was referred to our department to investigate the possibility of otitis media-induced meningitis. The patient had chronic otitis media with a perforated tympanic membrane on the left side; however, he had had no experience of ear discharge for years. A computerized tomography (CT) examination demonstrated partial ossification in the cochlea on the left side, which was considered to be due to preceding meningitis. 3D-FLAIR revealed high signal intensity in the cochlea of the inner ear on the left side and strongly increased signal in this area after the administration of gadolinium (Figure 1A,B). Enhancement was also observed in the area of the cochlear aqueduct (Figure 1C) and in the fundus of the internal auditory canal. Otitis media was ruled out as a cause of meningitis in this case; however, during follow-up it was revealed that he had had fine spontaneous nystagmus with fast phase beating contralateral to the affected ear for one year.

Acute otitis media (Case 2)

A 35-year-old man who had been hospitalized at another hospital because of ear discharge and acute SNHL on his left side was referred to our department two months after the onset of the disease. The patient had nystagmus to the right and profound SNHL on the left side, and his disease was diagnosed as severe labyrinthitis caused by acute otitis media. 3D-FLAIR revealed high signal intensity in the cochlea, the vestibule, and the semicircular canals of inner ear on the left side and strongly increased signals in these areas after the administration of gadolinium (Figure 2A, B). This enhancement was also observed one year after onset; at that time he still had fine nystagmus with fast phase beating contralateral to the affected ear.

Wegener granulomatosis (Case 4)

A 69-year-old man was referred to our department with a history of bilateral acute SNHL for three months and facial palsy on his right side for two weeks. The patient's hearing level was worse in the right ear than the left, and he noticed vertigo. The patient had been treated for bilateral otitis media

Table I. Clinical observation in five cases.

	Age (years)/gender	Symptom of inner ear disturbance	Diagnosis (cause)
Case 1	42/M	Vertigo, acute SNHL	Bacterial meningitis
Case 2	35/M	Vertigo, acute SNHL	Acute otitis media
Case 3	79/M	Acute SNHL	Acute otitis media
Case 4	69M	Vertigo, acute SNHL	Wegener granulomatosis
Case 5	45/F	Acute SNHL	Wegener granulomatosis

SNHL, sensorineural hearing loss.

Table II. 3D-flair MRI findings in five cases.

	High signal (at the initial visit)		High signal (post-treatment)
	Pre-contrast	Post-contrast	Post contrast
Case 1	Cochlea, CA	Cochlea (3), fundus (3), CA (3)	NA
Case 2	Cochlea, vestibule, Scc	Cochlea (3), vestibule (2), Scc (1)	Cochlea (3), vestibule (2), Scc (1)
Case 3	Cochlea	Cochlea (1)	None
Case 4	Cochlea, vestibule, Scc	Cochlea (3), vestibule (2), Scc (2)	Cochlea (2)
Case 5	Cochlea, Scc	Cochlea (1), Scc (1)	Cochlea (1)

CA, cochlear aqueduct; Scc, semicircular canal; Fundus, fundus of the internal auditory canal; NA, not available.

with effusion for one year. CT examination revealed widespread inflammation in the bilateral middle ear cavities; however, the inner ear appeared normal. 3D-FLAIR revealed high signal intensity in the cochlea, the vestibule, and the semicircular canals of the inner ear on both sides and increased signal in these areas after the administration of gadolinium, especially in the right ear (Figure 3A, B). Final diagnosis was Wegener granulomatosis, which was treated by chemotherapy with prednisolone and cyclophosphamide. 3D-FLAIR MRI performed after six times of the chemotherapy showed decreased post-contrast signals in the inner ear.

Discussion

3D-FLAIR images in five cases showed high pre-contrast signal in the cochlea, the vestibule, and the semicircular canals of the inner ear, while the post-contrast images demonstrated enhancement in the affected areas. This post-contrast enhancement is considered the result of a breakdown in the BLB [6,8,9,11]. The BLB supports inner ear homeostasis by maintaining constant composition of the inner ear fluid [10]; this inner ear system is concentrated in the cochlear lateral wall. Animal studies have shown breakdown of the BLB in the cochlear lateral wall following acute middle ear inflammation and an associated decrease in lateral blood flow [5,16]. Breakdown of the BLB also occurs in meningogenic suppurative labyrinthitis [17]. The findings of the 3D-FLAIR images in the present study support the explanation that etiologies similar to those in the animal studies might have occurred in the present cases.

The 3D-FLAIR images in Case 1 (acute meningitis) showed strongly increased signal in the cochlear aqueduct on the post-contrast images. This finding supports that of a previous study that the cochlear aqueduct could serve as potential pathway for the spread of infection from the meninges to the inner ear [18]. Contrast enhancement observed in

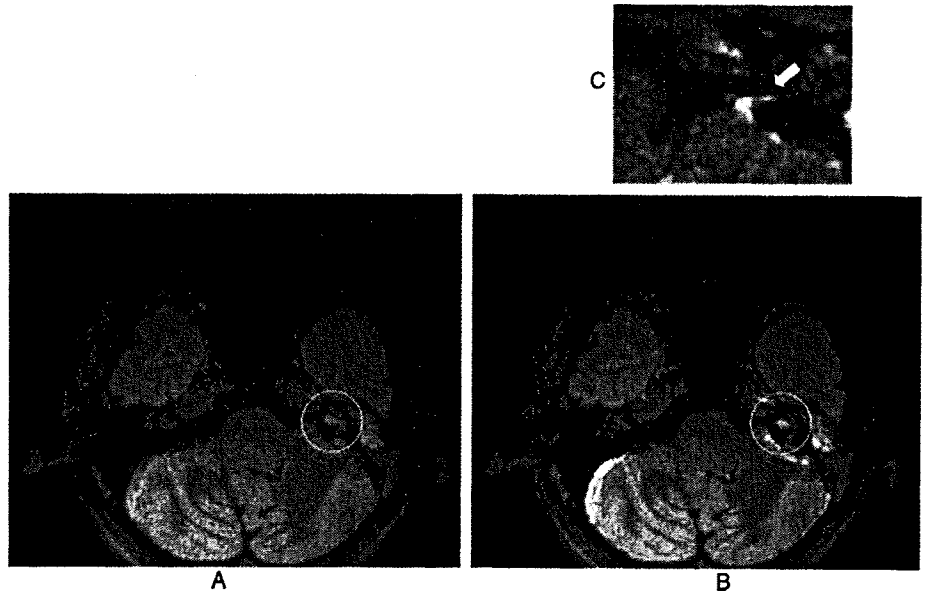

Figure 1. 3D-FLAIR images of Case 1. A, B. Pre-contrast (A) and post-contrast (B) inner ear lesions (circled). The inner ear is morphologically abnormal because of partial ossification. C. High-magnification image of enhancement in the cochlear aqueduct (arrow).

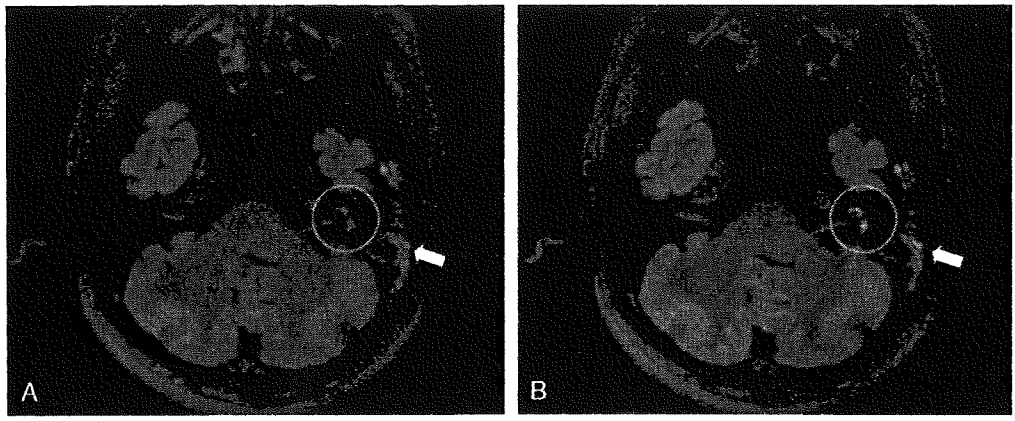

Figure 2. 3D-FLAIR images of Case 2. A, B. Pre-contrast (A) and post-contrast (B) middle ear inflammation (arrows) and inner ear lesions (circled).

the fundus of the internal auditory canal suggests a meningeal rather than otitis media origin as the cause of inner ear disturbance [15].

We have applied 3D-FLAIR to cholesteatoma cases with labyrinthine fistula and usefulness of images of the inner ear has been reported for evaluation of labyrinthine fistula in patients with cholesteatoma [13]. Case 4 with Wegener granulomatosis revealed high signal intensity in the bilateral inner ears. Increased signal of the cochlea in the post-contrast images was stronger on the side with the worse hearing level. SNHL is a significant finding in Wegener granulomatosis, and its detection is important for appropriate patient management [19]. 3D-FLAIR assessment can be a useful method in evaluating the inner ear disturbances caused by Wegener granulomatosis.

Another important finding was the duration over which the high post-contrast signal was observed. Enhancement in inflammatory conditions usually resolves over several months [7]. Decreased signal in the 3D-FLAIR was found in case 3 with mild acute otitis media and cases 4 and 5 with Wegener granulomatosis following treatment with chemotherapy. Hearing improvement was observed in these cases, however, in Cases 1 and 2, in which the

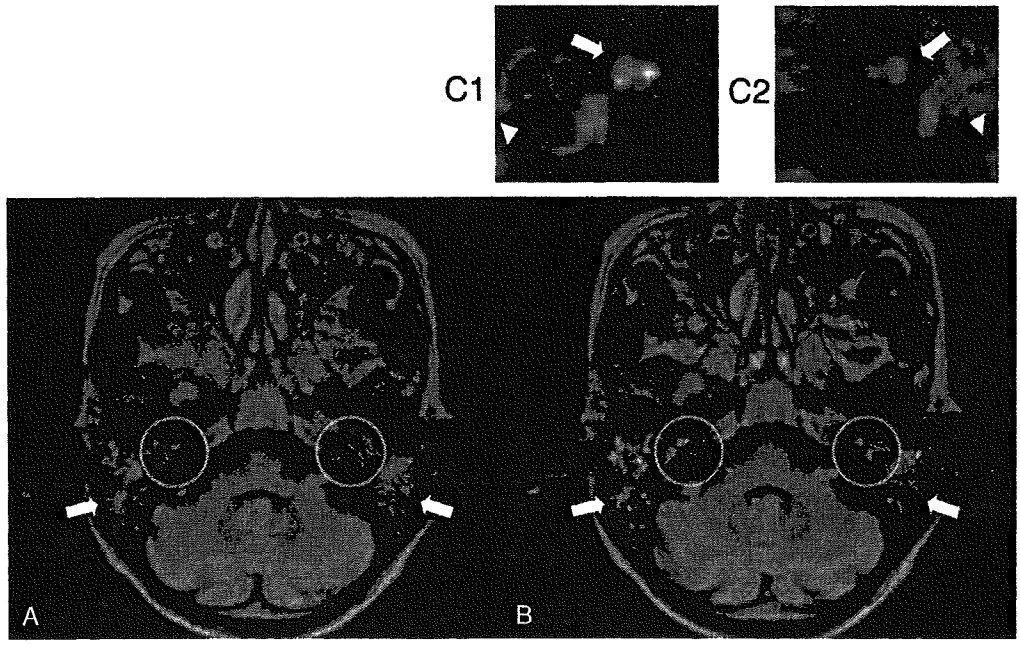

Figure 3. 3D-FLAIR images of Case 4. A, B. Pre-contrast (A) and post-contrast (B) bilateral middle ear inflammation (arrows) and inner ear lesions (circled). A stronger signal is observed in the inner ear on the side with the worse hearing level. C. Higher-magnification image of the lesions circled in B. The cochleas (arrows) and the semicircular canals (arrowheads) are indicated in the right (C1) and left (C2) ears.

patients had continuing nystagmus, high signal was observed in the inner ear over a period of several months. The severity of inner ear disturbances and clinical symptoms may correlate with 3D-FLAIR findings; breakdown of the BLB might last for a longer period in cases with aggressive disease.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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OTOLOGY

Detection of cytomegalovirus DNA in preserved umbilical cords from patients with sensorineural hearing loss

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Abstract We performed a retrospective diagnostic study of congenital cytomegalovirus (CMV) infection in patients with sensorineural hearing loss (SNHL). CMV DNA in preserved umbilical cords was analyzed using real-time polymerase chain reaction analysis. Of 45 analyzable patients with SNHL, CMV DNA was detected in the preserved umbilical cords of 3 patients, all of whom had bilateral SNHL that lacked a clear onset period. CMV DNA was not detected in any of the patients with sudden SNHL or enlarged vestibular aqueduct-associated SNHL. The features of CMV-associated SNHL were more asymmetric than those of CMV-negative bilateral SNHL.

Keywords Cytomegalovirus · Hearing loss · Enlarged vestibular aqueduct · Real-time polymerase chain reaction

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Introduction

Cytomegalovirus (CMV) infection is the most frequent congenital infection in humans, and congenital CMV infection has been identified as one of the leading causes of sensorineural hearing loss (SNHL) in children [1]. Most infected newborns are asymptomatic, although they are at risk for developing SNHL [2, 3]. Delayed onset SNHL in patients with congenital CMV infection is characterized by fluctuating, progressive, and asymmetric hearing loss [4]. These clinical characteristics are similar to those of enlarged vestibular aqueduct (EVA)-associated SNHL. Studies investigating the relationship between EVA-associated SNHL and congenital CMV infection have been performed [5, 6], but the etiology of sudden SNHL is still not known. Moreover, the relationship between sudden SNHL and congenital CMV infection has not been investigated. Currently, in most asymptomatic infants, the diagnosis of congenital CMV infection is missed during the neonatal period, and the possibility exists that CMV infection is an etiologic factor in some cases of SNHL that lack an obvious cause. However, retrospective diagnosis of congenital CMV infection has been difficult.

Obstetric hospitals in Japan have traditionally provided the dried umbilical cord to every parent as a symbol of the bond between mother and child. These dried cords are stored forever in the home. Recently, the feasibility of using dried umbilical cords for retrospective diagnosis of congenital CMV infection was reported [7–11]. In the present study, we performed a retrospective diagnosis of congenital CMV infection using preserved umbilical cords from patients with various kinds of SNHL to clarify the relationship between SNHL and congenital CMV infection.

Materials and methods

Study population

Fifty patients with SNHL were enrolled in this study. Ten patients had sudden SNHL (group A), seven patients had EVAs (group B), 16 patients had unilateral SNHL without a clear onset period (group C), and 17 patients had bilateral SNHL without a clear onset period (group D). All the patients had uneventful births, and their neonatal courses were unremarkable. Patients with apparent congenital CMV infection-associated SNHL were excluded from the study. The respective age ranges of each group at entry were as follows: Group A, 6–52 years (mean 28.9 years); B, 6–44 years (mean 16.5 years); C, 1–18 years (mean 8.6 years); and D, 2 months to 42 years (mean 12.9 years).

As positive controls, three infants with previously known congenital CMV infections were investigated. At enrollment, their ages were 36, 19, and 20 months. All three infants had bilateral SNHL and impaired motor or intellectual development. An association with CMV was confirmed by serology and typical radiological images of the central nervous system. As negative controls, 10 healthy individuals with normal hearing levels were also examined. They consisted of four male and six female subjects, and their age range was from 1 to 33 years (mean 10.8 years).

Audiometric evaluation

Audiometric evaluation was performed for each patient by pure tone audiometry using the average of 0.5, 1, and 2 kHz. Hearing levels were measured by an experienced audiologist using a clinical audiometer (RION-AA79S; RION Co., Ltd, Tokyo, Japan). When patients were too young to evaluate their hearing ability by pure tone audiometry, auditory brain stem response (ABR) was used. Otoscopic examination and tympanometry were performed before ABR assessment. Children were tested in a quiet room while sleeping. CYN-AX2100 (NEC, Tokyo, Japan) was used to record ABR. The stimuli were clicks at a repetition rate of 9.5/s with 10 dB step increases in intensity. Responses to 1,000 clicks were averaged. The threshold of wave *V* was measured as the hearing level of a patient. If the wave *V* could not be measured by the stimulation of 100 dB nHL, the hearing level was assumed to be 110 dB nHL.

Sample preparation

Preserved umbilical cords were collected from patients and controls. Umbilical cords had been preserved for 2 months to 52 years (mean 16.7 years). For the polymerase chain reaction (PCR) assay, about 5 mg of tissue was cut from the dried cords. DNA was then extracted using a QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany), eluted in 200 μL of distilled water, and stored at −30°C until analysis.

Real-time quantitative PCR assay

Real-time PCR was conducted using a TaqMan PCR kit and Model 7700 sequence detector (Applied Biosystems, Foster City, CA, USA), as described previously [8, 12, 13]. The PCR primers and fluorogenic probe for CMV DNA were from the immediate early gene [14]. A standard curve was constructed using the threshold cycle values obtained from positive control plasmids containing the target sequences. The threshold cycle values from the clinical sample were plotted on the standard curve, and the copy number was calculated automatically [13, 15]. Samples were defined as negative when the threshold value exceeded 50 cycles.

To examine whether analyzable DNA existed in the preserved umbilical cords, human β-actin DNA was concurrently quantified by real-time PCR using the TaqMan β-actin Control Reagent Kit (Applied Biosystems) [16]. When the amount of human β-actin DNA was too small (less than 4,000 copies/μg DNA), the samples were excluded from the study. The number of analyzable cells in each sample was estimated from the amount of human β-actin DNA, and the amount of CMV DNA was adjusted and expressed per 10^6 cells [16].

As controls, Epstein–Barr virus and human herpesvirus 6 DNA were also quantified by the real-time PCR assay. Sequences of primers and probes were as described elsewhere [15, 16].

Statistical analysis

The software package Stat View J 5.0 (Abacus Concepts Inc., Berkeley, CA, USA) was used for data analysis. Student's *t*-test was applied to compare the mean difference of hearing level between right and left ears.

Ethics approval

This study was approved by the local ethics committee and institutional review board of our hospital. Written informed consent was obtained from either the patients or their guardians.

Results

From the 50 umbilical cords of patients with SNHL, no or little β-actin DNA was detected in 5 patients, who were subsequently excluded from further study. Forty-five patients were thus analyzed, consisting of 9 patients from

Group A, six from Group B, 15 from Group C, and 15 from Group D. The clinical characteristics of each group are summarized in Table 1.

CMV DNA was detected in three cords, all of which were from patients in Group D, who had bilateral SNHL without a clear onset period. The serological examinations indicated that the three patients were seropositive for CMV. Details of the clinical characteristics and quantitative CMV DNA data of the three positive patients are shown in Table 2. All the three patients had asymmetric bilateral SNHL and also had neurological findings as listed in Table 2.

We then compared the difference in the hearing level between the right and left ears in the patients with bilateral hearing loss (group D). The mean difference for the patients with CMV-positive bilateral SNHL was 45.7 dB, while those with CMV-negative bilateral SNHL showed a mean difference of 10.8 dB. The difference between the right and left ears was significantly larger in the patients who were CMV-positive than those who were CMV-negative ($P < 0.01$).

CMV DNA was also detected in the preserved umbilical cords from the three positive control patients with congenital CMV infection, but was not detected in the 10 negative controls. Epstein–Barr virus and human herpesvirus 6 DNA were not detected in any of the umbilical cords.

Discussion

Virological diagnosis of congenital CMV infection is not difficult when an infant has characteristic symptoms at birth, such as petechiae, hepatosplenomegaly, jaundice, and microcephaly with calcification. Virological confirmation is made by isolating CMV from urine or detecting CMV DNA in urine or blood of infants within 2 weeks after birth. However, only 10% of congenitally infected babies have signs of infection at birth [17], and most asymptomatic infants are missed during the neonatal period. Retrospective diagnosis of CMV infection in children and adults has been difficult because CMV is ubiquitous. However, several studies have been performed on the retrospective diagnosis of congenital CMV infection by the detection of CMV DNA in umbilical cords or blood stored on Guthrie cards [7, 8, 18, 19]. Blood spots on Guthrie cards are useful to screen for genetic defects or congenital infection, but the cards are not kept long enough or are not always available for retrospective diagnosis of congenital CMV infection. In Japan, the dried umbilical cords of children are traditionally stored in the home as a symbol of the connection between mother and child. In this study, we demonstrated that the DNA in the preserved cord was analyzable for up to 52 years. The detection of CMV DNA from the umbilical cord means that the cells derived from the fetus were infected with CMV. Therefore, the detection directly indicates congenital CMV infection. However, the possibility of contamination from the birth canal does exist, as CMV is detected from genital tract secretions of pregnant women as well as HHV-6. In this study, we concurrently examined whether HHV-6 DNA could be detected from the cords. HHV-6 DNA was not detected in any of the 45 cords, which suggests that contamination was negligible. Our results imply that detecting CMV DNA from preserved

Table 1 Clinical characteristics of each group with SNHL

Groups	No.	Sex		Mean age (years)	No. of affected ears		Mean hearing levels (dB)	
		Male	Female		Right	Left	Affected ears	Difference between right and left ears
A: Sudden SNHL	9	3	6	28.1	4	5	74	65.2
B: EVAs	6	2	4	18.2	6	6	92	11.7
C: Unilateral SNHL	15	9	6	8.6	10	5	88	73.4
D: Bilateral SNHL	15	6	9	10.0	15	15	66	17.8

SNHL sensorineural hearing loss, *EVAs* enlarged vestibular aqueducts

Table 2 Clinical and virologic characteristics of CMV-positive patients with bilateral sensorineural hearing loss

Age (years)	Sex	Hearing level (dB)			Clinical findings	Radiological findings of the inner ear	CMV copy numbers (copies/10^6 cells)
		Right ear	Left ear	Difference			
5	Male	58	115	57	Developmental Delay in Infancy	Normal (CT)	9.6×10^5
6	Female	47	35	12	Mental Retardation	Normal (MRI)	2.8×10^5
9	Female	27	95	68	Hypotonic Muscles	Normal (MRI)	2×10^5

CMV cytomegalovirus, *CT* computed tomography, *MRI* magnetic resonance imaging

umbilical cords is useful for the retrospective diagnosis of congenital CMV infection. We detected CMV DNA in the preserved umbilical cords of three asymmetric SNHL patients. These three patients were missed during the neonatal periods. None of them had typical symptoms for congenital CMV infection at that time. Their developmental delay or hypotonic muscles, and SNHL appeared gradually later. Note that the difference in the hearing level between the right and left ears was larger in patients who were CMV-positive. Asymmetric SNHL is one of the features of congenital CMV infection [4].

Histopathological studies of temporal bones from patients with idiopathic sudden SNHL have suggested viral infection, a vascular origin, or the activation of cellular stress pathways [20–22]. We speculated that cases might exist in which fragility of the inner ear induced by congenital CMV infection was associated with idiopathic sudden SNHL. However, CMV DNA was not detected in any of the patients with idiopathic sudden SNHL or EVA-associated SNHL. These results suggest that a relationship between congenital CMV infection and sudden SNHL and EVA-associated SNHL does not exist.

Ganciclovir is widely used for the treatment of immunosuppressed patients with symptomatic CMV infection, particularly solid organ and bone marrow transplant recipients and AIDS patients. Ganciclovir is also used for the treatment of symptomatic congenital CMV infection. To improve the prognosis of congenital CMV infection, the efficacy of ganciclovir treatment has been evaluated, and a recent phase II clinical study demonstrated that ganciclovir treatment could reduce or stabilize hearing impairment [23, 24]. Other studies have also suggested that ganciclovir is potentially effective for CMV-associated SNHL [25, 26]. We previously reported that CMV DNA was detected in perilymph specimens obtained from two patients who had been diagnosed with congenitally symptomatic CMV infection [27]. The two patients were 2 and 3 years old at the time of the investigation. This result indicates that CMV replicates in the inner ear for several years in patients with symptomatic congenital CMV infection. These facts suggest that late ganciclovir therapy could be effective for patients with SNHL associated with congenital CMV infection. Therefore, retrospective diagnosis of congenital CMV infection is particularly important for SNHL having an unknown etiology.

Conclusion

Using real-time PCR, CMV DNA was detected in the umbilical cords of three patients with bilateral SNHL without a clear onset period. This method is useful for the retrospective diagnosis of congenital CMV infection. Moreover, a relationship between congenital CMV infection and sudden SNHL or EVA-associated SNHL does not appear to exist. The hearing loss of CMV-associated SNHL was more asymmetric than CMV-negative bilateral SNHL.

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ORIGINAL ARTICLE

3D-FLAIR MRI in facial nerve paralysis with and without audio-vestibular disorder

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Abstract

Conclusion: Among patients with facial nerve paralysis, significant difference was observed on three-dimensional fluid-attenuated inversion recovery magnetic resonance imaging (3D-FLAIR MRI) between those with and without audio-vestibular disturbance. This MRI technique may contribute to elucidation of the pathology of Ramsay Hunt syndrome and Bell's palsy. *Objective:* To evaluate the 3D-FLAIR MRI findings in patients who have facial nerve paralysis with and without audio-vestibular disturbance. *Methods:* 3D-FLAIR MRI was performed with and without gadolinium enhancement in 15 patients (5 men and 10 women) with unilateral facial nerve paralysis: 3 patients with Ramsay Hunt syndrome, 3 patients having facial nerve paralysis with hearing loss or vertigo without vesicles, and 9 patients with Bell's palsy. *Results:* Five of the six patients with audio-vestibular disturbance showed high signals in the inner ear on precontrast 3D-FLAIR. In comparison, among nine patients with Bell's palsy, only one patient showed high signals in the inner ear on precontrast 3D-FLAIR ($p < 0.05$).

Keywords: *Bell's palsy, Ramsay Hunt syndrome, inner ear*

Introduction

Facial nerve paralysis can be caused by a variety of unknown pathophysiological mechanisms, although many cases have been associated with a varicella-zoster virus (VZV) or herpes simplex virus infection. In some cases, it is very difficult to distinguish Ramsay Hunt syndrome from Bell's palsy, even by immunological examinations or imaging studies, such as magnetic resonance imaging (MRI) [1].

Three-dimensional fluid-attenuated inversion recovery (3D-FLAIR) MRI has been developed to detect haemorrhage or high concentrations of proteins, which are difficult to detect by T1- or T2-weighted MRI. Subtle high-signal areas in the cerebrospinal fluid (CSF) can be an indicator of subarachnoid haemorrhage, meningitis, or acute infarction. We have reported previously that CSF-related flow artefacts are significantly lower on 3D-FLAIR images than on 2D-FLAIR images [2]. Using 3D-FLAIR MRI at 3 Tesla (T), high signals in the inner ear have been shown in patients with various inner ear diseases, such as idiopathic sudden sensorineural hearing loss [3,4], cochlear otosclerosis [5], mumps deafness [6] and cholesteatoma with labyrinthine fistula [7]. These signals may reflect minor haemorrhage or an increased concentration of protein in the inner ear, which has passed through blood vessels with increased permeability.

Findings of high signal in the affected ear of a patient with Ramsay Hunt syndrome examined with 3D-FLAIR were reported for the first time by

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Sugiura et al. [8]. However, the differences in the 3D-FLAIR MRI findings between Ramsay Hunt syndrome and Bell's palsy have not been clarified. Accordingly, we investigated the 3D-FLAIR MRI findings in facial nerve paralysis patients with audio-vestibular disorder, who were classified as having Ramsay Hunt syndrome and atypical Bell's palsy, and in those without audio-vestibular disorder who were classified as having Bell's palsy in the present study.

Material and methods

Subjects

We evaluated 15 patients (5 men and 10 women; mean age ± SD, 47.3 ± 16.6 years) with unilateral facial nerve paralysis, who visited our hospital between February 2006 and February 2008. We diagnosed the patients as having Ramsay Hunt syndrome when they had symptoms of eighth nerve dysfunction (tinnitus, hearing loss and vertigo) and a vesicle on an external auditory meatus or auricle including symptoms of seventh nerve dysfunction. We diagnosed the patients as having Bell's palsy when they initially had only symptoms of seventh nerve dysfunction with no vesicle on an external auditory meatus or auricle [9]. Moreover, we diagnosed the patients as having atypical Bell's palsy when they had symptoms of eighth nerve dysfunction (tinnitus, hearing loss and vertigo) with no vesicle on an external auditory meatus or auricle including symptoms of seventh nerve dysfunction.

The mean ages of the three patients with Ramsay Hunt syndrome (three women), the three atypical Bell's palsy patients (two men and a woman) and the nine patients with Bell's palsy (three men and six women) were 50.3 ± 20.7, 40.7 ± 13.6 and 46.2 ± 17.7 years, respectively. Then, we reclassified these patients into two categories of facial nerve paralysis, those with and those without audio-vestibular disorder. Both Ramsay Hunt syndrome and atypical Bell's palsy were classified as facial nerve paralysis with audio-vestibular disorder, while Bell's palsy patients were classified as having facial nerve paralysis without audio-vestibular disorder. The initial House-Brackmann scale [10] score of the patients with facial nerve paralysis was IV in 4 cases, V in 10 cases, and VI in 1 case (Table I). In all patients, hydrocortisone was administered intravenously at 200 mg per day for 4 consecutive days, and then at 100 mg per day for 3 consecutive days, with adenosine triphosphate (80 mg per day). All the patients were treated with valacyclovir (3000 mg per day for 7 days) to preclude infection by VZV.

MRI

All MRI scans were performed using a 3 T magnet (Trio, Siemens, Erlangen, Germany) and a receive-only

Table I. Characteristics of patients with facial nerve paralysis.

Patient no.	Age (years)	Gender	Diagnosis	Grade of palsy (onset)	Serological VZV reactivation
1	28	F	Ramsay Hunt syndrome	IV	–
2	54	F	Ramsay Hunt syndrome	VI	–
3	69	F	Ramsay Hunt syndrome	V	+
4	56	F	Atypical Bell's palsy	V	+
5	36	M	Atypical Bell's palsy	V	+
6	30	M	Atypical Bell's palsy	IV	–
7	58	F	Bell's palsy	V	–
8	38	F	Bell's palsy	V	–
9	49	M	Bell's palsy	V	–
10	27	F	Bell's palsy	IV	–
11	78	M	Bell's palsy	V	–
12	24	F	Bell's palsy	V	–
13	31	F	Bell's palsy	IV	–
14	57	F	Bell's palsy	V	–
15	54	M	Bell's palsy	V	–

Patient nos 1, 7, 9, 11 and 14 underwent serology only at their initial visit (IgM negative).

eight-channel phased-array coil. 3D-FLAIR imaging was performed before and after the intravenous administration of a single dose of gadolinium diethylenetriaminepentaacetic acid *bis*-methyl amide (Gd-DTPA-BMA; Omniscan, Daiichi Pharmaceutical Co., Tokyo, Japan) at 0.1 mmol/kg. Heavily T2-weighted 3D constructive interference in the steady-state imaging was performed before the contrast material was administered, to delineate the anatomy of the CSF space. Contrast-enhanced 3D-FLAIR was initiated 7 min after the gadolinium was administered, so that the contrast 3D-FLAIR images were determined approximately 10 min after the administration of the gadolinium. Positive postcontrast imaging implies an increase of signal intensity after intravenous gadolinium administration. These methods have been described in detail in previous reports [3,4,11,12]. The mean period from the time of onset of symptoms to the MRI examination was 11.8 ± 11.4 days. All patients were also examined using T1-weighted MRI.

MR findings

The MRI findings for the inner ear or CSF in patients with facial nerve paralysis and idiopathic sudden sensorineural hearing loss were evaluated using the methods described in previous reports [12,13].

The MRI findings were ranked as follows: none (no signal in the affected inner ear or CSF) or high (the signals in the affected inner ear or CSF were as high as or higher than those of the cerebellar white matter).

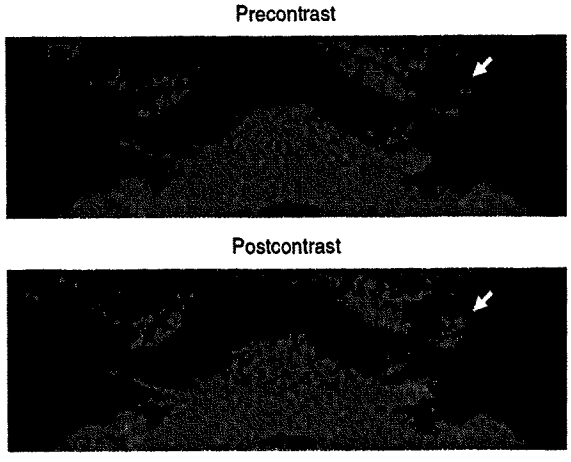

Figure 1. Patient no. 5, a case of atypical Bell's palsy (see Tables I and II). This patient had a mild hearing loss, tinnitus and dizziness with facial paralysis on his left side. He had no vesicle in his left ear. This patient showed high signals (arrow) in his left cochlea on both precontrast and postcontrast 3D-FLAIR.

These MRI findings were inspected by a neuroradiologist experienced in inner ear MRI, who was blinded to the clinical manifestations using the criteria as described above.

Statistics

We used a χ^2 test with Yates' correction for 2 × 2 tables to compare the differences in the high precontrast and postcontrast signals in the cochlea or vestibule between patients with facial nerve paralysis with and without audio-vestibular disorder. For all the above statistical analyses, a p value of <0.05 was considered significant.

Results

The characteristics of the 15 patients with facial nerve paralysis are summarized in Table I. 3D-FLAIR findings of the inner ear in these patients are summarized in Table II. Figure 1 shows an example of positive precontrast and postcontrast 3D-FLAIR images in the cochlea.

Five of the six patients with facial nerve paralysis with audio-vestibular disorder showed high signals in the inner ear on precontrast 3D-FLAIR. In contrast, only one of the nine patients without audio-vestibular disorder showed high signals in the inner ear on precontrast 3D-FLAIR ($p<0.05$), while two patients with facial nerve paralysis with audio-vestibular disorder also showed gadolinium enhancement in the inner ear. Only one of the nine patients without audio-vestibular disorder showed gadolinium enhancement in the inner ear. The high-signal areas observed on 3D-FLAIR were not detected by T1- or heavily T2-weighted MRI in any of these patients. No patient had signals in the unaffected inner ear.

Six patients had hearing loss or vertigo, as shown in Table II. Of five patients with hearing loss, three had high signals in the cochlea. Of five patients with vertigo, two had high signals in the vestibule (Table II). After treatment, all patients recovered from facial nerve paralysis.

We tested 10 patients for paired antibody formations with a serological examination. The remaining five patients underwent serology only at their initial visit. VZV reactivation was indicated serologically in 60% of patients with Ramsay Hunt syndrome or atypical Bell's palsy by either anti-VZV IgM detection or a significant change (greater than twofold) in anti-VZV IgG response [14] while VZV reactivation was indicated serologically in none of five patients (0%) with Bell's palsy by the same criteria (Table I).

Table II. 3-D FLAIR findings of the inner ear in patients with facial nerve paralysis.

Patient no.	Hearing loss	Vertigo	Period from onset to MRI (days)	Precontrast	Postcontrast enhancement
1	Yes	Yes	7	CO	No
2	Yes	Yes	4	VE	No
3	Yes	No	7	No	No
4	Yes	Yes	6	CO, VE	CO, VE
5	Yes	Yes	7	CO	CO
6	No	Yes	6	CO	No
7	No	No	15	CO	No
8	No	No	8	No	CO
9	No	No	19	No	No
10	No	No	33	No	No
11	No	No	3	No	No
12	No	No	5	No	No
13	No	No	41	No	No
14	No	No	4	No	No
15	No	No	4	No	No

No, no finding with precontrast or postcontrast 3D-FLAIR images; CO, cochlea; VE, vestibule.

Discussion

Since the study by Daniels et al. in 1989 [15], many investigators have reported the enhancement on MRI of paralysed facial nerves after Gd-DTPA. Jonsson et al. reported that ipsilateral facial nerve contrast enhancement is frequently observed in cases of acute peripheral facial paralysis [1]. It has been reported that no difference in gadolinium enhancement of the facial nerves was observed between patients with Bell's palsy and those with Ramsay Hunt syndrome [1]. However, in some cases of Ramsay Hunt syndrome, the vestibular and cochlear nerves, the labyrinth and some parts of the internal and external ear canals are enhanced [1].

The present study revealed that high concentrations of protein in the inner ear are associated with hearing deterioration or dizziness in patients with facial nerve paralysis with audio-vestibular disorder. Precontrast high signals on 3D-FLAIR may reflect minor haemorrhage or an increased concentration of protein in the inner ear, which has passed through blood vessels with increased permeability or has originated in disrupted cells in the inner ear [4]. A patient with Ramsay Hunt syndrome examined with 3D-FLAIR, previously reported to have high signals in the affected ear [8], showed gadolinium enhancement in the affected inner ear on 3D-FLAIR. We demonstrate here that 3D-FLAIR is a useful tool with which to detect the precise abnormality in the inner ear.

In conclusion, 3D-FLAIR MRI revealed differences in the precontrast and postcontrast signals in the inner ear between facial nerve paralysis patients with audio-vestibular disorder, including Ramsay Hunt syndrome and atypical Bell's palsy, and those without audio-vestibular disorder, having Bell's palsy. This suggests an abnormal elevation of blood vessel permeability in this area in Ramsay Hunt syndrome and atypical Bell's palsy.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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ORIGINAL ARTICLE

Endolymphatic space imaging in patients with delayed endolymphatic hydrops

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Abstract

Conclusion: Magnetic resonance imaging (MRI) after intratympanic gadolinium injection can reveal endolymphatic hydrops (ELH) in patients with delayed ELH (DELH). Patients with contralateral DELH may have bilateral ELH. *Objective:* DELH has previously been diagnosed based on clinical history, hearing and vestibular examinations. DELH is classified into three types: ipsilateral, contralateral and bilateral indicate the side with the longstanding hearing loss. Ipsilateral DELH occurs in the ear with a profound hearing loss, contralateral DELH in the better hearing ear and bilateral DELH in both ears. Imaging diagnosis of the endolymphatic space may add a new dimension to the diagnosis and treatment of DELH. *Patients and methods:* Gadodiamide hydrate was diluted eightfold with saline. The diluted gadodiamide hydrate was injected intratympanically through the tympanic membrane in two patients with ipsilateral DELH and five patients with contralateral DELH. One day after the injection, 3 Tesla MRI was performed to evaluate the endolymphatic space. *Results:* ELH was observed in all patients. In three patients who underwent bilateral intratympanic injection of gadolinium and were diagnosed with contralateral DELH, ELH was observed bilaterally. In one of these three patients, ELH was observed in the cochlea on the left and in the vestibule on the right.

Keywords: *Endolymphatic hydrops, 3D-FLAIR MRI, inner ear*

Introduction

Delayed endolymphatic hydrops (DELH) is a clinical entity that can be differentiated from Ménière's disease and is typically observed in patients who suffer from longstanding unilateral profound inner ear hearing loss [1]. The disease is characterized by a profound sensorineural hearing loss in one ear, found to have been present in most cases from early childhood, due to an unknown cause, trauma or viral infection. ELH is probably the most important factor. After a prolonged period (usually many years) patients with DELH experience the onset of episodic vertigo from the deaf ear (ipsilateral DELH) or develop a fluctuating hearing loss and/or episodic vertigo in the opposite ear that previously had normal hearing (contralateral DELH). ELH is the most important underlying pathology that causes the hearing loss and the vestibular symptoms both in the better ear and in the ear with profound hearing loss [2]. When ipsilateral and contralateral hydrops exist simultaneously, this is called bilateral hydrops. In bilateral hydrops, it is often difficult to judge whether hydrops exists in the ear with profound hearing loss.

Recently, we have succeeded in visualizing ELH by injecting gadolinium enhancement intratympanically and taking three-dimensional (3D) FLAIR magnetic resonance (MR) images with a 3 T MR unit. The development of 3D real inversion recovery (IR) has improved the MRI of ELH. Using 3D real IR, ELH can be observed in all cochlear turns [3].

With this new imaging method, we attempted to visualize the endolymphatic space in patients clinically diagnosed with DELH.

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Patients and methods

Patients

Seven patients were enrolled in this study. Age, sex, average hearing level at 500 Hz, 1 kHz and 2 kHz, the presence or absence of vertigo, the degree of hydrops scored by radiologists and the clinical diagnosis are presented in Table I. Two patients were diagnosed with ipsilateral DELH and five patients with contralateral DELH. All patients underwent 3 T MRI at 24 h after the intratympanic injection of gadolinium. The Ethics Review Committee of Nagoya University School of Medicine approved the protocol for the study. All patients gave their informed consent to participation in this study.

Intratympanic gadolinium injection

Gadodiamide hydrate (Omniscan Daiichi Pharmaceutical Co. Ltd, Tokyo, Japan) was diluted with saline (1:7 v/v). The diluted gadodiamide hydrate was injected intratympanically through the tympanic membrane using a 23 G needle and a 1 ml syringe after the patient was placed in the supine position with his/her head turned approximately 30° away from the sagittal line toward the uninjected ear. The gadolinium was injected until a backflow of fluid into the external ear was observed under a microscope. The amount of diluted gadolinium injected was 0.4–0.5 ml. After the injection, the patient remained in the supine position for 60 min with his/her head turned approximately 60° away from the sagittal line toward the uninjected ear. Four patients (cases 1–4) underwent intratympanic injection only in one ear. Three patients (cases 5–7) underwent intratympanic injection in both ears.

MRI

MRI scans were performed 24 h after intratympanic injection of gadolinium with a 3 T MR unit (Trio, Siemens, Erlangen, Germany) using a receive-only 12-channel phased-array coil. T1-weighted 3D fast low-angle shot imaging, heavily T2-weighted 3D constructive interference in steady state imaging and 3D fluid-attenuated inversion recovery (FLAIR) imaging were performed. For this study, we performed a second 3D-FLAIR with higher in-plane spatial resolution in addition to the methods described previously [4–6].

All MRIs were attached to the electronic medical record and reviewed independently on a liquid crystal display in the Department of Radiology and the Department of Otorhinolaryngology.

Image evaluation of endolymphatic space on MRI

The size of the endolymphatic space was scored by radiologists based on the MRI reports and using the following criteria. For the vestibule, a score of 2 indicates a noticeable enlargement of the endolymphatic space occupying more than half of the vestibule. A score of 1 indicates a moderate enlargement of the endolymphatic space occupying between

Table I. Details of the patients in the study.

Patient no.	Age (years)	Sex	HL		Vertigo (period after onset)	Ear injected with gadolinium	Gadolinium distribution	
			R	L			Cochlea	Vestibule
1	26	M	88.3	48.3	Yes (1 year)	R	2	2
2	66	F	56	55	No	L	2	0
3	56	M	81.7	103.3	No	L	1	2
4	24	F	10	101.7	Yes (1 year)	L	1	2
5	38	M	20	66.7	Yes (9 years)	Both	R1L2	R2L2
6	33	M	88.3	18.3	No	Both	R0L2	R2L0
7	66	M	18.3	103.3	No	Both	R1L1	R2L1

HL, average of hearing level of 500 Hz,1 kHz and 2 kHz (dB); M, male; F, female; R, right; L, left; Cochlea, hydrops in cochlea; Vestibule, hydrops in vestibule. The degree of hydrops determined by radiologists was: 0, none; 1, mild; 2, significant. Details of hearing level in each patient are as follows. Patient no. 1: severe hearing loss in the right ear was noticed from birth. Audiogram of the better hearing ear (left ear) showed no hearing change during our observation (ipsilateral type). Patient no. 2: fixed hearing loss was seen in the right ear, the timing of its occurrence is unknown. Fluctuation of hearing loss was observed in the left ear for 1.5 years (contralateral type). Patient no. 3: profound, fixed hearing loss was seen in the left ear after sudden deafness 14 years ago. Fluctuation of hearing loss was observed in the right ear for 2 months (contralateral type). Patient no. 4: profound, fixed hearing loss was seen in the left year since childhood. Right ear shows normal hearing (ipsilateral type). Patient no. 5: fixed hearing loss was observed in the left ear after sudden deafness 10 years ago. Fluctuation of hearing loss in the right ear with vertigo occurred 9 years ago (contralateral type). Patient no. 6: severe hearing loss was observed in the right ear after sudden deafness 8 years ago. Fluctuation of hearing loss was observed in the left ear for 1 month (contralateral type). Patient no. 7: profound hearing loss was observed in the left ear after sudden deafness 30 years ago. Fluctuation of hearing loss was observed in the right ear for 20 years (contralateral type).

one-third (33.3%) and 50% of the vestibule. A score of 0 indicates no or very mild, if any, enlargement of the endolymphatic space occupying less than one-third (33.3%) of the vestibule. For the cochlea, a score of 2 indicates a noticeable enlargement of the endolymphatic space to a size at least as large as the scala vestibuli. A score of 1 indicates a moderate enlargement of the endolymphatic space with Reissner's membrane bulging toward the scala vestibuli, although the endolymphatic space is smaller than the perilymphatic space of the scala vestibuli. A score of 0 indicates no or very mild, if any, enlargement of the endolymphatic space, with no bulging of Reissner's membrane or no endolymphatic space observed. These tentative criteria were established according to previous histological research in humans and animals [7,8].

Pure tone audiometry, DPOAE, VEMP

Functional tests such as pure tone audiometry (PTA), distortion product otoacoustic emission (DPOAE) and vestibular evoked myogenic potential (VEMP) were performed in all patients. DPOAE ($2f_1$-f_2) were collected bilaterally using Otodynamics ILO 92 version 2.04. VEMP was measured by surface myogenic potentials in the sternocleidomastoid muscle, which were added 150 times with a reference electrode over the sternum while clicks (105 dB) were presented to the ipsilateral ear and white noise (75 dB) was presented to the contralateral ear (Synax 2100, NEC Medical Systems, Tokyo, Japan). The ground electrode was on the forehead.

Results

Clinical and imaging results are summarized in Table I (7 cases, 10 ears). ELH was observed in all seven cases clinically diagnosed as DELH. The two patients with ipsilateral DELH showed ELH in both the cochlea and vestibule. They also experienced vertigo spells and exhibited remarkable ELH in the vestibule. Two MR images are presented (patient no. 1 in Figure 1, patient no. 5 in Figure 2). Patient no. 1, who had right profound perceptive deafness at birth and a moderate degree of left perceptive deafness, had suffered from rotatory vertigo for a year. Noticeable ELH was observed in the vestibule of the right ear with intratympanic administration of gadolinium. Patient no. 5 had a moderate degree of left perceptive deafness for 10 years and had suffered over that time from tinnitus and fluctuating hearing loss in the right ear and vertigo spells. A noticeable ELH was observed in the cochlea of the left ear and in the vestibules of both ears. Moderate ELH was revealed in the cochlea of the right ear.

Gadolinium injection into the asymptomatic ear with normal hearing is not approved by the Ethics Review Committee of our university. Therefore, we did not perform intratympanic injection of gadolinium in the right ear of patient no. 4. Because patient nos 1 and 2 were not hoping for the injection, we did not perform it on the left ear of patient no. 1 or the right ear of patient no. 2, both of which showed no hearing fluctuation and had moderate hearing loss. Patient no. 3 did not hope for the injection in the better hearing ear, so we did not perform it in the right ear in this patient. In patient no. 1, DPOAE responses were absent and VEMP responses were present in both ears. In patient no. 2, DPOAE response was present only at 1 kHz for the right ear and only at 3 kHz for the left ear. VEMP responses were within noise level for both ears. In patient no. 3, DPOAE responses were absent in both ears and VEMP response was present in the right ear and absent in the left ear. In patient no. 4, DPOAE showed normal response in the right ear and no response in the left ear, while VEMP responses were present in both ears.

Discussion

We have previously reported that intratympanically injected gadolinium moves quickly into the scala tympani of the basal turn of the cochlea and the perilymphatic space of the vestibule, and that 1 day after intratympanic administration of gadolinium, it appears in almost all parts of the perilymphatic space inside the inner ear. We also showed that with the use of 3 T 3D-FLAIR MRI, the size of the endolymphatic space can be clearly evaluated. In addition, with the use of 3D real IR MRI we can separately visualize endolymph, perilymph and bone, and in the cochlea can distinguish hydrops of the basal turn from hydrops of the upper turn.

This study included all clinically diagnosed cases (7 cases, 10 ears) of DELH. The most common cause of hearing loss preceding DELH is juvenile-onset unilateral profound deafness (early childhood unilateral profound sensorineural hearing loss of unknown aetiology), followed by labyrinthitis from various causes, and physical and acoustic traumas to the inner ear. Three types of DELH exist: 1) ipsilateral DELH in which the ear with profound hearing loss suffers progressive ELH; 2) contralateral DELH in which the formation of progressive ELH occurs in the ear opposite to the previously deafened ear; and 3) bilateral DELH in which both ipsilateral and contralateral DELH occurs simultaneously.

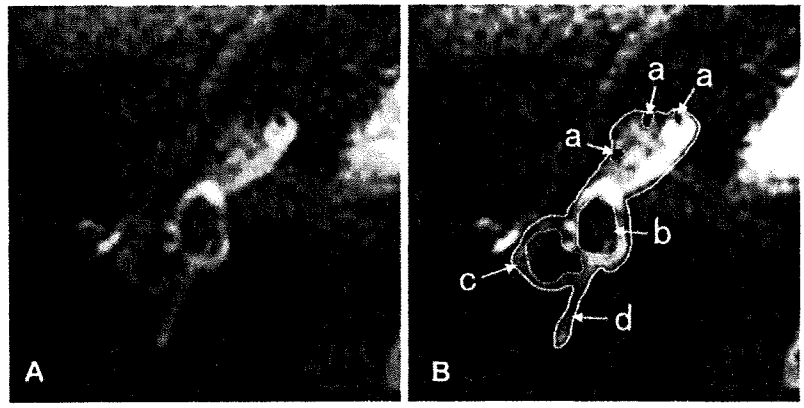

Figure 1. (A) Three-dimensional fluid-attenuated inversion recovery (3D-FLAIR) MR image obtained 1 day after intratympanic injection of gadolinium (right ear of patient no. 1 in Table I). (B) Line diagram of (A). Arrows: (a) endolymphatic space in the cochlea; (b) endolymphatic space in the vestibule; (c) lateral semicircular canal; (d) posterior semicircular canal. Gadolinium is visible in all turns of the cochlea, vestibule and semicircular canals. The black area indicated by arrows (a, b) is surrounded by gadolinium-filled perilymph. Endolymphatic hydrops (ELH) in the vestibule (b) is prominent.

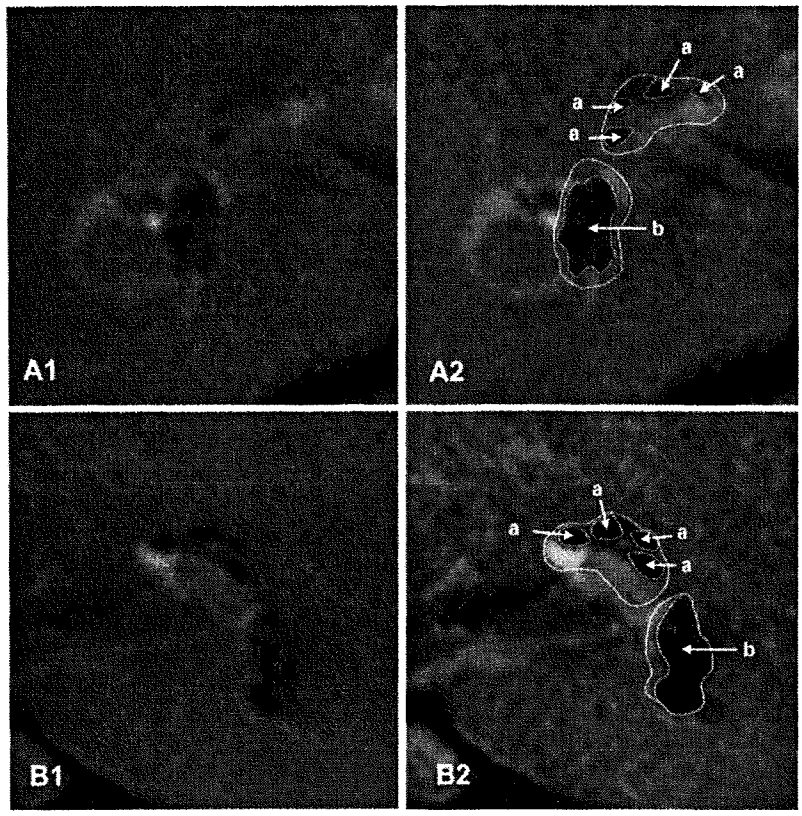

Figure 2. (A1) Three-dimensional inversion recovery utilizing real reconstruction (3D real IR) MRI obtained 1 day after intratympanic injection of gadolinium (right ear of patient no. 5 in Table I). (A2) Line diagram of (A1). Arrows: (a) endolymphatic space in the cochlea; (b) endolymphatic space in the vestibule. Gadolinium is visible in the cochlea, vestibule and semicircular canals. Black areas indicated by arrow (a) represent endolymphatic hydrops (ELH) in the basal and upper turns of cochlea. Large black areas indicated by arrows (b) represent noticeable vestibular ELH. (B1) Three-dimensional inversion recovery utilizing real reconstruction (3D real IR) MRI obtained 1 day after intratympanic injection of gadolinium (left ear of patient no. 5 in Table I). (B2) Line diagram of (B1). Arrows: (a) endolymphatic space in the cochlea; (b) endolymphatic space in the vestibule. Gadolinium is visible in basal and upper turns of cochlea and vestibule. Black areas indicated by arrows (a, b) are surrounded by gadolinium-filled perilymph. Noticeable ELH in basal and second turns of cochlea and vestibule, respectively, are visible.

The aetiology of DELH is not adequately proven. Bachor et al. proved pathologically that ELH could derive from juvenile-onset unilateral profound deafness and from congenital deafness [9,10]. However, most labyrinthitis caused by virus infection, for example by measles, rubella or mumps, causes collapse of the inner ear [11,12]. There is not sufficient histological evidence for the vertigo induced by unilateral profound deafness resulting from mumps.

Schuknecht et al. demonstrated that ELH exists in contralateral DELH because asymptomatic virus infection exists in the opposite inner ear. The damage to the opposite inner ear is negligible, so it takes a long time for ELH to be clinically apparent [13]. Alternatively, Harris and Aframian suggested that it results from an autoimmune response in the inner ear [14].

Intratympanically administered gadolinium moves first into the perilymphatic space of the inner ear. Precise MR images obtained 1 day after intratympanic administration can reveal the border between the endolymphatic and perilymphatic spaces. Injection of gadolinium into the tympanic cavity with the use of 3 T 3D-FLAIR is effective in the evaluation of the ear affected. If the endolymphatic space cannot be detected, this may be because the endolymph has collapsed, or because an extremely large ELH may prevent the intratympanically administered contrast enhancement material from moving into either or both of the cochlea and labyrinth. When recurrent episodic vertigo is intractable and cannot be cured through conservative treatment, intratympanic gentamicin administration [15], labyrinthectomy and vestibular neurectomy of the responsible ear are clinically indicated. Injection of gadolinium into the tympanic cavity with the use of 3 T 3D-FLAIR is effective in the evaluation of the ear affected. This should be taken into consideration for administration of intratympanic gentamicin therapy in patients with DELH, especially in cases with poor contrast of the vestibule and semicircular canal [16].

Patient no. 5 was diagnosed clinically with contralateral DELH, but we demonstrated ELH in both ears and a different degree of ELH in the cochlea and vestibule. Although electrocochleography and the glycerol test, which suggest the presence of ELH, cannot be performed in profound sensorineural hearing loss, we could detect ELH in profound sensorineural hearing loss with the combined use of 3 T 3D-FLAIR MRI and 3D real IR MRI at 1 day after intratympanic administration of gadolinium.

We did not perform intratympanic injection of gadolinium in the asymptomatic ears with normal hearing for ethical reasons. We will be able to detect whether or not subclinical ELH is present in the ears if we are permitted to inject gadolinium in the unaffected side.

In conclusion, we observed ELH in all seven cases of clinically diagnosed DELH using 3 T MR at 1 day after intratympanic injection of gadolinium. The identification of the ear affected may be useful in cases of resistance to conservative therapy and in understanding the aetiology of DELH.

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