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ORIGINAL ARTICLE

Cochlin-tomoprotein (CTP) detection test identifies traumatic perilymphatic fistula due to penetrating middle ear injury

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Abstract

Conclusions: The cochlin-tomoprotein (CTP) detection test can be used to make a definite, objective diagnosis of traumatic perilymphatic fistula (PLF), and therefore offers valuable information on patient selection for surgical treatment. *Objectives:* Penetrating middle ear injury can cause traumatic PLF, which is a surgically treatable otologic emergency. Recently, we have reported on CTP, a novel perilymph-specific protein. The purpose of this study was to determine if the CTP detection test is useful for the diagnosis of traumatic PLF. *Methods:* This was a prospective study of CTP detection in penetrating middle ear injury cases with tympanic membrane perforation and hearing loss. *Results:* A total of seven individuals were included in this study. CTP was detected in three of four cases with posterosuperior quadrant perforation of the tympanic membrane. In one of these three cases, even though the high resolution CT scan was not suggestive of PLF and the perilymph leakage could not be visualized intraoperatively, the CTP detection test was able to detect PLF. In two cases, the preoperative positive test results enabled us to make a diagnosis of PLF and a decision for surgical treatment. CTP was not detected in the cases with anterior or inferior tympanic membrane perforation.

Keywords: *Middle ear trauma, hearing loss, COCH gene, cochlin isoform*

Introduction

Penetrating trauma of the middle ear can occur as a result of the introduction of a variety of foreign bodies, for example, a knitting needle, hairpin, bullet, twig of a tree, ear pick, cotton-tipped applicators, stone, and iatrogenic damage [1 9]. Trauma associated with the ossicular chain is a frequent finding in these cases and carries a good prognosis. Inner ear damage, however, while less frequent, exposes the patient to permanent disabilities in hearing and vestibular function.

Etiologies of inner ear damage due to penetrating middle ear injury include labyrinthine concussion, acoustic trauma, and perilymphatic fistula (PLF) [10 12]. Among these conditions, PLF is an otologic emergency and is surgically correctable by sealing the fistula. Appropriate recognition and treatment of PLF can improve hearing and balance in the afflicted patients; otherwise, permanent deafness may result [2]. In the absence of high resolution CT scan (HRCT) findings suggestive of PLF, such as otic capsule fracture, stapes luxation or pneumolabyrinth, it is difficult to make a definite, objective diagnosis of PLF.

Recently, we reported our findings on cochlin-tomoprotein (CTP), a novel perilymph-specific protein. By proteomic analysis of inner ear proteins, certain unique properties of cochlin isoforms were discovered. We detected three cochlin isoforms, p63s, p44s, and p40s, in the inner ear tissue and a short

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16 kDa isoform, named cochlin-tomoprotein (CTP), in the perilymph [13,14]. CTP was selectively expressed only in the perilymph, not in cerebrospinal fluid (CSF), saliva or serum on testing 65, 60, 29, and 28 samples, respectively [14 16].

We have reported a standardized CTP detection test for the diagnosis of PLF, using a spiked standard of recombinant human (rh)CTP on Western blot [17].

Using this diagnostic method, we detected CTP in three of seven cases of penetrating middle ear injury. To the best of our knowledge this is the first definite diagnosis of traumatic PLF using CTP as the diagnostic marker.

Material and methods

Study design and participants

The participants in this investigation were derived from the patients who had been consecutively referred to the first author's outpatient clinic. Enrolment took place from June 2003 to December 2007, and patients were included based on a history of insertion of an object into the middle ear, tympanic membrane perforation, and accompanying hearing loss with or without balance disorders. A total of seven patients were enrolled in this study. An ear pick caused injury while removing cerumen (a common practice in Japan) in cases 1 5 and 7. A screwdriver was accidentally inserted into the ear of case 6.

We performed physiological testing by pure tone audiometry and nystagmoscopy with infrared radiation. HRCT was performed in all cases. In this study, HRCT findings suggestive of PLF are defined as otic capsule fracture, pneumolabyrinth (vestibulum or cochlea), ossicular dislocation of the stapes or incudostapedial joint. Fistula test (applying positive and negative pressure to the middle ear) was not performed to avoid further damage of the inner ear.

Standardized CTP detection test by Western blot

Samples were tested by the standardized CTP detection test [17] with minor modifications. For Western blot analysis, the rabbit polyclonal anti-CTP antibody (formerly anti-LCCL-C Ab) was prepared as described previously. In brief, a 14-mer peptide (LSRWSASFTVTKGK) corresponding to residues 114 127 in the LCCL domain was used as an antigenic peptide to generate antibody. The rhCTP was used as a spiked standard on Western blot. A putative CTP sequence predicted from our previous study, the 101 to 403 positions of the cDNA corresponding to amino acid residues 32 132, was amplified by PCR from a human expressed sequence tag clone, IMAGE ID 27789 (Kurabo, Japan). rhCTP was produced using pCR/T7/TOPO/TA Expression Kits (Invitrogen).

Samples were loaded onto 15% polyacrylamide gels and transferred onto PVDF membranes. Membranes were blocked overnight at 4°C in 5% skim milk and 0.2% polyoxyethylenesorbitan (Tween-20) dissolved in phosphate-bufferd saline (PBS; pH 7.5). Membranes were then incubated in PBS containing 1% skim milk and 0.1% Tween-20 for 2 h at room temperature with the primary antibody (anti-CTP antibody) diluted at 1:2000. After washing with 0.05% Tween-20 in PBS, membranes were incubated for 1 h at room temperature with horseradish peroxidase-labeled goat anti-rabbit IgG antibody (Dako, Japan) diluted at 1:10 000 in the same buffer as used for the primary antibody reaction. They were washed again and the reaction was developed with a chemiluminescence reaction kit (ECL Advance, Amersham), and then analyzed by an image analyzer LAS-3000 (Fuji Film, Japan). Tests were performed and analyzed by well-trained personnel who did not have any information on the clinical background of the patients.

The detection limit of the serially diluted rhCTP was between 0.27 and 0.13 ng/well. These two amounts of rhCTP were set as the high and low spiked standards, respectively, and were the amounts electrophoresed each time when we tested the samples. When the intensity of the band in the samples tested was below the high standard signal, the result was considered to be negative. The low spiked standard was used to estimate the protein transfer efficiency.

Test results were expressed qualitatively (positive or negative) by the presence or absence of the anti-CTP antibody reacting protein with the molecular weight, which exactly matched the molecular weight of native CTP (16 kDa) on Western blot. The result of the CTP detection test was provided to the clinic within 4 days of sampling.

Performance of the CTP detection test

The performance of the CTP detection test was described previously [17]. In brief, the detection limit of perilymph was 0.161 µl per lane in an average of five samples. This means that the test can detect CTP if there is 3.3 µl of perilymph in 0.3 ml of middle ear lavage (MEL) (explained in detail by Ikezono et al. [17]). We also studied the stability of CTP protein when samples were stored at room temperature (25°C) or 4°C for as long as 55 days. The effect of repeated freezing and thawing was also evaluated. The

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results showed that CTP is a stable protein and detection is not affected by the storage condition or repeated freezing and thawing [16].

We also reported the specificity of the CTP detection test by testing non-PLF cases [17]. We defined 'non-PLF' as those cases with otosclerosis (that had undergone stapedectomy), profound deafness (cochlear implant surgery), or conductive hearing loss (exploratory tympanotomy) without any sign of inflammation or infection. We took MEL before the stapedectomy or cochleostomy, or before surgical treatment of conductive hearing loss. The MEL in 54 of 55 non-PLF cases was negative with the CTP detection test, i.e. the specificity of the test was found to be 98.2%. To further elucidate the limitations to this test, we analyzed the MEL collected from patients with middle ear infections, which can give a false-positive result. The MEL in 43 of 46 cases with chronic suppurative otitis media or middle ear cholesteatoma was negative for CTP. The specificity of the CTP detection test decreases to 93.5% when applied to inflamed and infected ears. The high protein concentration of the thick pus present with infection was the most likely cause. In the present investigation inflamed ears were studied, so the specificity is thought to be due to the latter. These findings indicate that CTP can be considered a marker for the diagnosis of PLF.

Sampling method

We aimed to establish an easy-to-perform sampling method. Samples were collected by lavaging the middle ear cavity four times with the same bolus of 0.3 ml of saline and recovering the fluid. This was defined as middle ear lavage (MEL). In the outpatient clinic, MEL was collected through a perforated tympanic membrane (cases 3 7), or at myringotomy in case 2 whose traumatic tympanic membrane perforation had been closed during the 27 day period between the onset and the first visit to our clinic. MEL was also collected during surgery in cases 1 3. Hence, in cases 2 and 3, MEL was collected twice (before and during operation) and we could test whether the leakage continued during these two sampling time points. Samples were centrifuged at 1250 g for 1 min and the supernatants were frozen and stored at 80°C until use. Sixteen µl of MEL were mixed with 8 µl of three times concentrated sample buffer (150 mM Tris-HCl (pH 6.8), 6% SDS, 30% glycerol, 0.3% bromophenol blue, 300 mM DTT) for Western blot analysis. All patients gave their full informed consent and the study was approved by the Ethics Committee of Nippon Medical School.

Therapeutic procedure

All cases except for cases 4 and 5 received tapering doses of oral prednisolone (60 30 mg) down to 5 mg in an 8 14 day period depending on the patient's general condition and any other systemic illnesses. Surgery (exploratory tympanotomy and PLF repair surgery) was performed under general anesthesia in cases 1 3, in order to inspect and seal the potential perilymph leak from the fistula and to reconstruct the ossicular chain (explained in detail in the Results section). Conservative treatment (medication and bedrest) was selected in cases 4 7 and tympanic membrane perforation was closed with patching.

Evaluation of the therapeutic outcome

Pure tone audiogram was obtained before and after treatment. The pure tone average (PTA) hearing thresholds of bone conduction and air conduction were determined by four frequencies (500, 1000, 2000, and 4000 Hz). The patients were followed up regularly and PTA was performed until complete recovery, or for more than 2 months (average 8 months). Hearing improvement after treatment was gauged by PTA of air conduction using the following criteria; CR, complete recovery (final audiogram ≤20 dB HL, or improvement to the same degree of hearing as the unaffected ear); RI, remarkable improvement (improvement >30 dB HL); I, improvement (improvement of 10 30 dB HL); NC, no change or deterioration (improvement <10 dB HL) (modified from Kanzaki et al. [18]). Successful hearing improvement was defined as complete recovery or remarkable improvement.

Results

The patient information and test results (age, sex, affected side, HRCT suggestive of PLF, CTP test results, onset to MEL sampling (days), site of perforation, pre- and post-treatment PTA, air bone gap, hearing improvement, nystagmus) are summarized in Table I. Representative Western blot analysis of CTP detection in MEL from cases 2, 3, and 7 are shown in Figures 1 and 2. None of these cases presented with infection in the middle ear. The contralateral ear exhibited normal hearing in all cases. Successful hearing improvement (complete recovery or remarkable improvement) was achieved in all cases except in case 7. Vertigo, as a sign of inner ear damage, was observed in cases 1 4, and disappeared in all cases during the follow-up period. In cases 5 7, perforation was not in the posterosuperior quadrant (PSQ) and CTP was negative (Figure 2), suggesting

Table I. Summary of patient information and test results.

Parameter	Case no.						
	1	2	3	4	5	6	7
Age	54	29	7	27	28	39	48
Sex	F	F	M	F	F	M	M
Affected side	L	L	R	L	R	L	L
HRCT suggestive of PLF	+	+					
CTP test results	+	+, +	+, +				
Onset to MEL sampling (days)	8	27, 35	2, 9	1	4	1	1
Site of perforation	PSQ	PSQ	PSQ	PSQ	Inferior	Anterior	Anterior
Pretreatment bone PTA	33	20	5	35	14	20	35
Pretreatment air PTA	65	70	20	56	40	58	56
Pretreatment ABG	32	50	15	21	26	38	21
Post-treatment Bone PTA	20	19	3	24	11	15	28
Post-treatment air PTA	23	33	10	36	15	19	36
Post-treatment ABG	3	14	8	13	4	4	9
Hearing improvement	RI	RI	CR	CR	CR	CR	I
Nystagmus	S	S, P	S	S, P, BPPV		P	S

Middle ear lavage (MEL) was collected only once except in cases 2 and 3, where MEL was taken twice preoperatively and during the operation. +, +, CTP detection test was performed twice and both results were positive. ABG, air bone gap; BPPV, benign paroxysmal positional vertigo; CR, complete recovery; CTP, cochlin-tomoprotein; HRCT, high resolution CT scan; I, improvement; MEL, middle ear lavage; NC, no change or deterioration; P, rotatory and horizontal, paretic; PLF, perilymphatic fistula; PSQ, posterosuperior quadrant; PTA, pure tone average; RI, remarkable improvement; S, rotatory and horizontal, stimulatory.

that the inner ear damage could be attributed to non-PLF causes. Therefore, in the following section we focus on discussing cases 1–4.

Case 1 had mixed hearing loss and developed severe rotatory vertigo 36 h after the traumatic event. HRCT exhibited pneumolabyrinth in the vestibulum and dislocation of the incudostapedial joint. The stapedial footplate seemed to be in an intact position. The intraoperative findings showed that the stapes annular ligament was lax with excess mobility of the stapes. The stapedial footplate was slightly elevated (probably an ear pick with a spoon-like tip lifted the stapes when the patient pulled it out after penetration, as shown by Kobayashi and Gyo [1]). Perilymph leakage was detected, the stapes was replaced after coverage of the oval window with fascia, and the ossicular chain was reconstructed. The MEL performed intraoperatively was positive for CTP. The PTA demonstrated remarkable hearing improvement after surgery (Figure 3a).

Case 2 was referred to our clinic because of sustained conductive hearing loss and vertigo for 1 month post injury. HRCT revealed stapes luxation and air in the vestibulum. MEL was taken on the 35th day and CTP was detected (Figure 1), suggesting that the perilymph had been continuously leaking out and/or had pooled in the middle ear cavity for

35 days without remarkable exacerbation of the sensorineural component of hearing loss. During surgery, perilymph leakage was detected and the stapes was smoothly taken out through the oval window. The ossicular chain was reconstructed with a Teflon piston and the oval window was covered with fascia to seal the perilymphatic leakage. MEL taken intraoperatively was positive for CTP. The PTA demonstrated remarkable hearing improvement post surgery (Figure 3b).

Case 3 had severe rotatory vertigo 10 h after the traumatic event and mild conductive hearing loss (Figure 3c). HRCT did not exhibit any findings suggestive of PLF. MEL taken on the second day after the event showed positive CTP expression (Figure 1). Due to the traumatic PLF and the possibility that the hearing might worsen, surgery was performed on the ninth day. Intraoperatively, the stapes annular ligament was lax with excess mobility of the stapes, and there was slight dislocation of the incudostapedial joint. Granulation was observed around the stapes without identifiable leakage of perilymph. MEL taken intraoperatively was positive for CTP. The oval window was sealed with fascia and fibrin glue and the incudostapedial joint was reconstructed. The audiometric findings were normal and there was no vertigo post surgery (Figure 3c). The
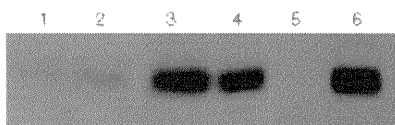
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Figure 1. The middle ear lavage (MEL) samples collected preoperatively from cases 2 and 3 were tested by Western blot using anti-cochlin-tomoprotein (CTP) antibody to detect CTP. The MEL from both cases 2 and 3 shows a positive result. Lane 1, rhCTP low spiked standard (0.13 ng/well), CTP-negative; 2, rhCTP high spiked standard (0.27 ng/well), CTP-positive; 3, 16 μl of MEL from case 3, CTP-positive; 4, 2 μl of perilymph (positive control), CTP-positive; 5, blank, CTP-negative; 6, 16 μl of MEL from case 2, CTP-positive.

MEL samples taken preoperatively and at surgery were both positive for CTP, indicating that the perilymph was still leaking out and/or had pooled in the middle ear cavity for 8 days.

In case 4, perforation was in the PSQ, but the HRCT finding was not suggestive of PLF and the CTP was negative. The vertigo and hearing loss resolved with conservative treatment.

Discussion

Penetrating middle ear injuries are reportedly commonly caused by the insertion of various kinds of foreign objects. These foreign objects result in perforation of the tympanic membrane, ossicular dislocation or, most seriously, direct damage to the stapes. In Japan, removing the cerumen by an ear pick is a common practice, and there were 20 cases of penetrating middle ear injuries reported over a 10-year period [1]. The current report comprises the largest number of middle ear injury cases (seven cases) in a single study.

We would like to emphasize that even though it is not a common trauma, penetrating middle ear injury is a very important condition for understanding the pathomechanisms of traumatic inner ear injuries. An improved understanding will enable therapeutic and

preventative strategies for acute, late onset, and debilitating neuro-otological problems of patients with similar pathomechanics, such as temporal bone fracture, pneumolabyrinth or iatrogenic injuries during otologic surgery.

Symptoms commonly associated with this type of injury include acute hearing loss, vertigo, tinnitus, and pressure sensation in the affected ear. The severity of the injury was first analyzed by the symptoms and test results such as HRCT findings, audiogram, and nystagmus. Inner ear damage can have various etiologies, such as labyrinthine concussion, acoustic trauma or PLF [10–12]. Conservative treatment is adequate for labyrinthine concussion and acoustic trauma, but traumatic PLF is an otologic emergency that may require surgical treatment. Appropriate recognition and treatment of PLF is especially important and it can improve hearing and balance, and hence the quality of life of the afflicted patients.

However, differential diagnosis among these various etiologies is not a simple task. This has led to a series of research efforts to identify an endogenous marker of perilymph [14]. Previously beta2-transferrin was thought to be a marker; however, a more recent study showed that, because of the relative amount of serum and perilymph in a mixed sample, electrophoretic separation of the transferrin variant might not be diagnostic [19]. As explained in the Methods section, we have shown that CTP was selectively detected in the perilymph and established a standardized CTP detection test for the diagnosis of PLF, using a spiked standard of rhCTP on Western blot [15–17]. We have reported the performance of the CTP detection test, including the specificity of the test and the stability of the CTP protein.

In the present study, we have shown the usefulness of the CTP detection test for the diagnosis of traumatic PLF in the clinical setting. In four middle ear trauma cases suspected to have stapes injury due to the symptoms and the site of perforation (i.e. the PSQ), three cases had a positive CTP detection test (cases 1–3). When the site of perforation was in the PSQ and HRCT detected pneumolabyrinth, a diagnosis of traumatic PLF due to the direct injury to the stapes was made, as in cases 1 and 2. Even HRCT was not clearly indicative of PLF, since it was the CTP detection test that enabled the detection of PLF in case 3. If the site of perforation and HRCT findings are not suggestive of PLF, a negative CTP result can thus help to exclude PLF, as in cases 5–7.

The conventional gold standard for PLF detection is the intraoperative microscopic visualization of perilymph leakage and fistula [17]. By this intraoperative microscopic visualization, a definitive diagnosis of
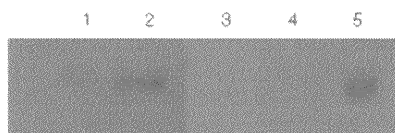

Figure 2. The middle ear lavage (MEL) sample from case 7 was tested by Western blot using anti-cochlin-tomoprotein (CTP) antibody to detect CTP, which shows a negative result. Lane 1, rhCTP low spiked standard (0.13 ng/well), CTP-negative; 2, rhCTP high spiked standard (0.27 ng/well), CTP-positive; 3, blank, CTP-negative; 4, 16 μl of MEL from case 7, penetrating middle ear injury, CTP-negative; 5, 0.25 μl of perilymph (positive control), CTP-positive.
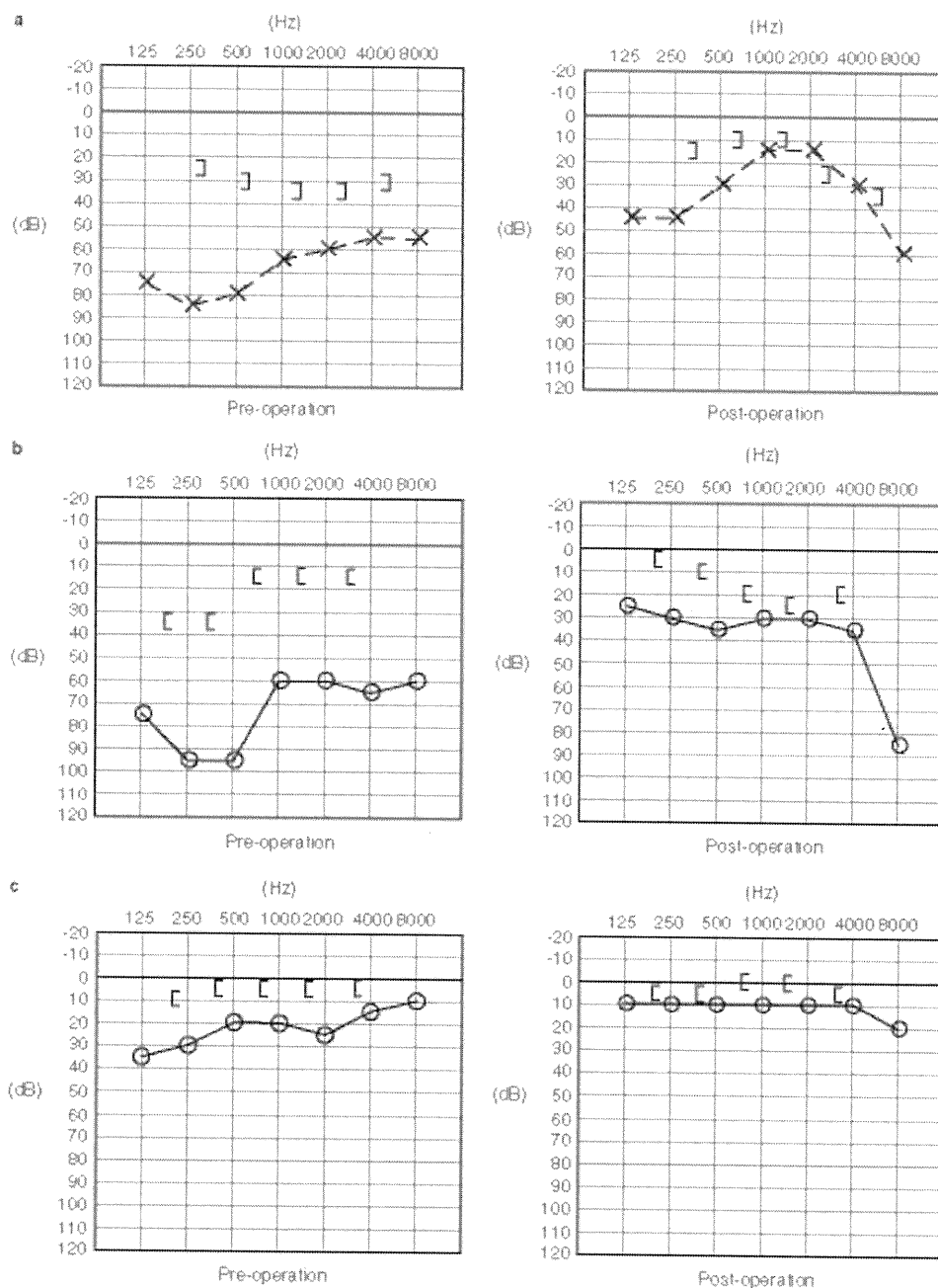
a

b

c

Figure 3. Pre- and post-treatment pure tone audiograms of case 1 (a), case 2 (b), and case 3 (c), with positive CTP detection test results.

traumatic PLF was made in both cases 1 and 2, and the CTP positive result confirmed this. In case 3, the oval window fistula and leakage were not apparent, even though the annular ligament was identified as lax with excess mobility. Even in this type of ambiguous condition, the CTP detection test was able to clearly identify PLF. In these cases with a positive CTP test result, severe rotatory vertigo was one of the main complaints. Even if the hearing loss was stable or had not deteriorated remarkably, the presence of

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vestibular symptoms is important for an accurate diagnosis.

The preoperative CTP detection test was valuable for the decision as to the need for surgery in cases 2 and 3. In chronic cases such as case 2, the key question is whether the perilymph leakage persists or not due to spontaneous healing with granulation formation in the oval window. When the leakage ceases, conservative treatment is indicated. When the leakage persists, surgical treatment (sealing the fistula and reconstruction of ossicular chain) is needed to prevent inner ear infection, meningitis or further deterioration of the inner ear dysfunction. On the other hand, with mild traumatic PLF as in case 3, sealing the damaged annular ligament can be performed without serious complications.

We were able to achieve improvement in both hearing and vestibular symptoms in cases 1-3. However, surgical treatment is still a controversial issue in these types of stapes injury. Hearing and vestibular function vary after surgical treatment due to the initial degree of inner ear damage caused by the injury, and the additional effect caused by the surgical procedure itself, which may further damage the inner ear.

Conclusion

The CTP detection test enabled a definite diagnosis of traumatic PLF among penetrating middle ear injury cases. These PLF cases, which were definitely diagnosed with the positive CTP test results, revealed the patient symptoms and physiological test results to be widely variable. The inner ear damage may be dependent upon the rapidity of onset, duration of the perilymph leakage, the site of the leakage, and other biological factors. Using this CTP detection test, a definitive diagnosis of PLF can be made and appropriate therapeutic options for this surgically correctable disease can be taken into consideration. Further studies will be needed to provide insight into the etiology and pathomechanisms, and such insight may lead to the development of therapeutic and preventative strategies for acute, late onset, and debilitating neuro-otological problems.

Acknowledgments

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

CTP (Cochlin-tomoprotein) detection in the profuse fluid leakage (gusher) from cochleostomy

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Abstract

Conclusions: By testing 125 samples, we confirmed that Cochlin-tomoprotein (CTP) is present in the perilymph, not in cerebrospinal fluid (CSF). Perilymph and CSF exist in two distinct compartments, even in the case of a malformed inner ear with a bony defect in the lamina cribrosa, as described here. Cochleostomy might have suddenly decreased the perilymph pressure, allowing the influx of CSF into the inner ear resulting in profuse fluid leakage, first perilymph then CSF. *Objectives:* The first purpose of this study was to further confirm the specificity of the perilymph-specific protein CTP that we reported recently. Secondly, we assessed the nature of the fluid leakage from the cochleostomy using the CTP detection test. *Methods:* A standardized CTP detection test was performed on 65 perilymph and 60 CSF samples. Samples of profuse fluid leakage collected from cochleostomy during cochlear implantation surgery of one patient with branchio-oto-renal (BOR) syndrome were also tested by the CTP detection test. *Results:* CTP was detected in 60 of 65 perilymph samples but not in any of the CSF samples. The leaked fluid was shown to contain CTP, i.e. perilymph, at the outset, and then the CTP detection signals gradually disappeared as time elapsed.

Keywords: *Perilymph, CSF, cochlear implant, COCH gene, Cochlin isoform, branchio-oto-renal syndrome, sensorineural hearing loss*

Introduction

Normally the cerebrospinal fluid (CSF) in the subarachnoid space extends laterally into the internal auditory canal (IAC) as far as the lateral fundus, where it is separated from the perilymph by the bony plate of the lamina cribrosa, the nerves that pass through the lamina cribrosa and the spiral ganglion. Profuse leakage of perilymph and CSF is the result of an abnormal bony defect in the lamina cribrosa, rather than enlargement of the cochlear aqueduct. In some congenitally dysplastic ears there is a deficiency in this barrier, allowing direct confluence between the CSF and perilymph [1,2].

In a large series of cochlear implants, the incidence of this profuse leakage of perilymph and CSF, i.e. a 'gusher', was approximately 1% of all cases. The term gusher is widely used in the literature to describe egress of clear fluid from the cochleostomy site. However, the term is variably applied and bears no explicit relation to the amount of fluid leaking, time elapsed until the flow ceases, pressure of the fluid column or origin of the fluid. The fluid may be CSF, perilymph, or a mixture of the two [3]. Even though the current level of diagnostic imaging technology is considerably advanced, at present it is unclear whether the perilymph and CSF spaces are separate and function as two distinct compartments or not. The fragile membranous tissue at the fundus of the IAC can be perforated after the fenestration of the cochlea due to a pressure decrease in the perilymphatic space resulting from the cochleostomy.

Alternatively, these liquid compartments may be mixed preoperatively because of a defect in the lamina cribrosa. The confluence of these two fluids could be one of the causes of the deterioration of hearing [2].

Recently, we reported the features of Cochlin-tomoprotein (CTP), a novel perilymph-specific protein. By proteomic analysis of inner ear proteins, we found certain unique properties of the cochlin isoforms. We detected three cochlin isoforms, p63s, p44s and p40s, in the inner ear tissue, and a short 16 kDa isoform, named Cochlin-tomoprotein (CTP), in the perilymph [4,5]. Since COCH gene/cochlin is known to be highly specific to the inner ear, we examined CTP expression in body fluid. CTP was selectively expressed in the perilymph of all 20 peri-lymph samples tested, but not in 77 samples of the body fluids, i.e. serum (28 samples), CSF (20 samples) or saliva (29 samples) [6]. Using CTP as a biomarker, we have established a standardized CTP detection test for the diagnosis of perilymphatic fistula (PLF), using a spiked standard of recombinant human (rh)CTP in Western blotting [7].

In the present study, we further analysed the specificity of CTP expression in body fluids, testing 65 perilymph and 60 CSF samples. The results confirm that CTP is present in the perilymph, but not in the CSF. Perilymph and CSF are distinguished by the presence or absence of a particular protein, CTP. Then we tested the samples of the profuse fluid leakage collected temporally from one case of cochleostomy during cochlear implantation surgery. The leaked fluid at the beginning of the cochleostomy contained CTP, i.e. perilymph, and the CTP signal gradually disappeared as time elapsed. These results indicate that even in the malformed inner ear of the case presented here, with a defect in the bony barrier at the fundus, the perilymph and CSF fluid spaces function as two separate compartments. Cochleo-stomy might have suddenly decreased the perilymph pressure and allowed CSF influx into the inner ear, resulting in profuse fluid leakage (perilymph then CSF) from the cochleostomy site. This is the first report of a temporal analysis of the CTP protein in gusher fluid.

Material and methods

Analysis of CTP expression in CSF and perilymph: collection and processing of body fluid samples

For the assessment of the specificity of CTP expression in body fluids, we collected perilymph during stapedectomy for otosclerosis or during cochleostomy for cochlear implant surgery. CSF was purchased from Biotech (Valley Center, CA, USA). The CSF was collected from consenting donors at an FDA-licensed and registered facility. No adverse events were observed during sample collection. The samples were centrifuged at 1250 g for 1 min and the supernatants were frozen and stored at 80°C until use. All patients gave their full informed consent and the study was approved by the ethics committee of Nippon Medical School.

Samples of perilymph or CSF (4 µg) were mixed with 5 µl of sample buffer (150 mM Tris-HCl (pH 6.8), 6% SDS, 30% glycerol, 0.3% bromophenol blue, 300 mM DTT) after normalization per average protein concentration (perilymph 200 mg/dl, CSF 40 mg/dl) [8], then analysed by Western blot.

Analysis of CTP expression in the profuse fluid leakage from cochleostomy

We performed cochlear implantation in a 50-year-old male patient who was diagnosed as having branchio-oto-renal (BOR) syndrome (without renal manifestation) based upon the diagnostic criteria [9]. The patient started to use a hearing aid at the age of 8 years, and his hearing had progressively deteriorated. At the time of his first visit to our office, he had profound bilateral sensorineural hearing loss, right preauricular sinus and a slightly malformed left auricle. The tympanic membranes showed no anatomical abnormalities. High resolution computed tomography (HRCT) of the temporal bones (Fig. 1) presented findings typically seen in BOR cases; hypoplastic apical turn of the cochlea, a funnel-shaped IAC. The fundus of the IAC was wide, the lamina cribrosa was hypoplastic and no bony structure was seen [10]. Echogram and HRCT revealed no renal malformation. An 18-year-old daughter of the patient had bilateral profound sensorineural hearing loss and a history of surgical removal of bilateral cervical fistulas. She had similar HRCT findings. The patient's father also had bilateral progressive hearing loss.

During cochlear implantation in this case, profuse fluid leakage from the cochleostomy (gusher) was observed. We collected the leakage samples in a temporal manner at the following time points: 0 (immediately after fenestration), 0.5, 2, 3, 6, 15, 25, 35 and 40 min. It is the policy of this department to wait until the flow of the leakage weakens and then seal the cochleostomy with fascia and fibrin glue to avoid lumbar drainage. As a control, middle ear lavage (MEL), i.e. lavaging the middle ear cavity three to four times with the same bolus of 0.3 ml of saline and recovering the fluid, was
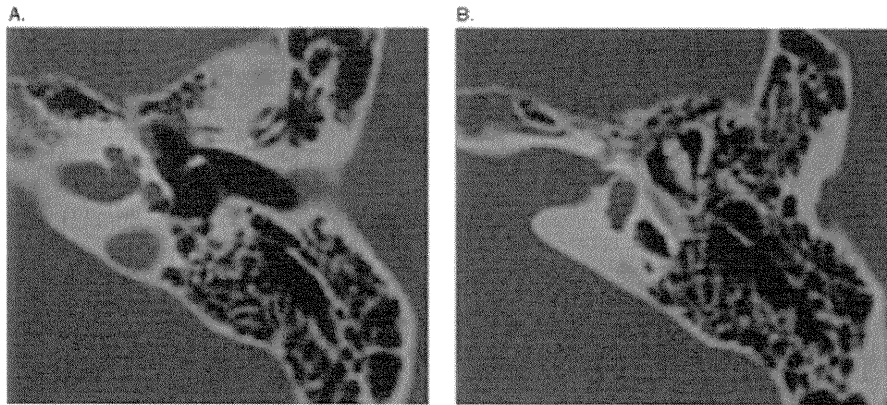
Figure 1. HRCT of the right ear in the axial plane. Both ears had findings typically seen in BOR cases: (a) the hypoplastic apical turn of the cochlea, the fundus of the internal auditory canal was wide, the lamina cribrosa was hypoplastic and no bony structure was seen; (b) the funnel-shaped internal auditory canal (IAC).

taken from the middle ear before cochleostomy. MEL (16 μl) was mixed with 8 μl of 3× concentrated sample buffer (150 mM Tris-HCl (pH 6.8), 6% SDS, 30% glycerol, 0.3% bromophenol blue, 300 mM DTT) for Western blot analysis.

Standardized CTP detection test by Western blot

Samples were tested by the standardized CTP detection test [6,7], with minor modifications. For Western blot analysis, the rabbit polyclonal anti-CTP antibody (formerly anti-LCCL-C Ab) was prepared as described previously. In brief, a 14-mer peptide (LSRWSASFTVTKGK) corresponding to residues 114–127 in the LCCL domain was used as an antigenic peptide to generate antibodies.

Samples were loaded onto 15% polyacrylamide gels and transferred onto PVDF membranes. Membranes were blocked overnight at 4°C in 5% skim milk and 0.2% polyoxyethylenesorbitan (Tween-20) dissolved in PBS (pH 7.5). Membranes were then incubated in PBS containing 1% skim milk and 0.1% Tween-20 for 2 h at room temperature with the primary antibody (anti-CTP antibody) diluted to 1:2000. After washing with 0.05% Tween-20 in PBS, membranes were incubated for 1 h at room temperature with horseradish peroxidase-labelled goat anti-rabbit IgG antibody (Dako, Tokyo, Japan) diluted to 1:10 000 in the same buffer as used for the primary antibody reaction. They were washed again and the reaction was developed with a chemiluminescence reaction kit (ECL Advance, Amersham) and then analysed with an image analyser LAS-3000 (Fuji Film, Tokyo, Japan). Tests were performed and analysed

by well-trained personnel who did not have any information on the clinical background of the patients, to avoid any biased judgments. Test results were expressed qualitatively (positive or negative) by the presence or absence of the anti-CTP antibody reactive protein, with a molecular weight that exactly matched the molecular weight of native CTP (16 kDa) on Western blot.

rhCTP was used as a spiked standard on the Western blot. A putative CTP sequence predicted from our previous study, the 101–403 positions of the cDNA corresponding to amino acid residues 32–132, was amplified by PCR from a human expressed sequence tag clone, IMAGE ID 27789 (Kurabo, Japan). rhCTP was produced using pCR/T7/TOPO/TA expression kits (Invitrogen). To establish a clinical test for the diagnosis of perilymph leakage and avoid test variability, we standardized the CTP detection test using high (0.27 ng) and low (0.13 ng) spiked standard levels of rhCTP on Western blot. When the intensity of the band in the samples tested was below the high-level standard signal, the result was considered to be negative. The low spiked standard was used to estimate the protein transfer efficiency.

Diagnostic performance of the CTP detection test

To evaluate the specificity of the CTP detection test in the clinical setting, we previously reported the test results of samples from non-PLF cases. In that study, we defined non-PLF as those cases with otosclerosis (that had undergone stapedectomy), profound deafness (cochlear implant surgery), or conductive hearing loss (exploratory tympanotomy), without

Table I. CTP expression in perilymph and CSF.

Sample	Total	CTP positive	CTP negative
Perilymph	65	60	5
Stapedectomy	36	34	2
Cochlear implant	29	26	3
CSF	60	0	60

any sign of inflammation or infection. The MEL in 54 of 55 non-PLF cases was negative for the CTP detection test, i.e. the specificity of the test was found to be 98.2%. To further elucidate the limitations of this test, we analysed the MEL collected from patients with middle ear infections, which can give a false positive result. The MEL in 43 of 46 cases with chronic suppurative otitis media or middle ear cholesteatoma was negative for CTP. The specificity of the CTP detection test decreases to 93.5% when applied to infected ears [7]. The high protein concentration of the thick pus present with infection was the most likely cause. In the present study we studied a non-infected ear with BOR syndrome, so the specificity is thought to be the former.

Results

CTP expression in perilymph and CSF (Tables I and II, Fig. 2)

In all, 34 perilymph samples from 36 stapedectomy and 26 samples from 29 cochlear implant patients were positive for CTP. In total, 60 of 65 perilymph samples were positive for CTP. However, CTP was not detected in any of the 60 CSF samples.

Analysis of profuse fluid leakage from cochleostomy site (Table III, Fig. 3)

As a control, MEL was taken from the middle ear before cochleostomy. The MEL described here contains middle ear mucosal secretions and other substances normally expressed in the middle ear cavity. These substances may cause false positive reactions to the antibody. The MEL taken before the fenestration of cochlea was negative for CTP.

Immediately after the fenestration of the cochlea, fluid leaked excessively from the cochleostomy site. The leakage collected at 0 min showed a CTP signal above the high-level standard signal and at 0.5 3 min

Table II. Western blot analysis of CTP expression in perilymph and CSF.

Lane	Sample	Amount of sample per lane	Result
(a) Perilymph and MEL			
1	High-level standard	rhCTP 0.27 ng	+
2	Low-level standard	rhCTP 0.13 ng	
3	Case A: perilymph stapedectomy	2 μl	+
4	Case A: MEL before stapedectomy	16 μl of MEL	
5	Case B: perilymph cochleostomy	2 μl	+
6	Blank	...	...
7	Case B: MEL before cochleostomy	16 μl of MEL	
8	Case C: perilymph stapedectomy	2 μl	
9	Case C: MEL before stapedectomy	16 μl of MEL	
10	Perilymph (positive control)	1 μl	+
(b) CSF			
1	High-level standard	rhCTP 0.27 ng	+
2	Low-level standard	rhCTP 0.13 ng	
3	CSF	10 μl	
4	CSF	10 μl	
5	CSF	10 μl	
6	CSF	10 μl	
7	CSF	10 μl	
8	CSF	10 μl	

MEL, middle ear lavage.

A.

B.

Figure 2. Western blot analysis of CTP expression in perilymph and CSF. The expression of CTP was analysed by Western blot using the anti-CTP antibody. CTP expression (16 kDa) was only detected in the perilymph (cases A and B), not in the CSF. The perilymph sample from case C was negative for CTP. Further details are shown in Table II.

showed a negative result, with a faint signal below the high-level standard signal, and the signal disappeared at 6 min and thereafter.

Discussion

In the present study we have further tested the specific expression of CTP in the perilymph. Sixty of 65 perilymph samples were positive for CTP. However, CTP was not detected in any of the 60 CSF samples. In the previous study, we tested 20 perilymph and 20 CSF samples [6], and the results showed that CTP was detected in all the perilymph samples and was negative in all the CSF samples. Therefore, the sum total is that 80 of 85 perilymph samples were positive for CTP and all 80 SCF samples were negative for CTP. These results further confirm that CTP is a perilymph-specific protein.

CTP was not detected in five of the perilymph samples, and this may be attributed to the low CTP protein concentrations because of dilution by blood and seepage in the surgical field. Alternatively, especially in the three CTP-negative cases of cochlear implantation with profound deafness, abnormal cochlin isoform processing might have resulted in an undetectable level of CTP production due to

mutations in COCH or related genes. No genetic testing to confirm this theory has been performed in these cases as yet.

Using CTP as a marker to detect perilymph, we tested the nature of the profuse leakage from cochleostomy in an anomalous cochlea case with BOR syndrome. The fluid that leaked at the beginning of the cochleostomy was proved to contain CTP, i.e. perilymph, and the CTP detection signals gradually disappeared as time elapsed. Even though the CTP signal was below the high-level standard signal and was evaluated as negative by standardization, faint CTP signals were detected from 0.5 to 3 min (Fig. 3). The total volume of leakage was approximately 10 ml over 3 min. Since the volume of the human perilymph is estimated to be 150 μl by MRI [11], we consider the perilymph to have been washed out from the cochlea immediately after the leakage started. The faint signals observed here might be derived from the perilymph pooled in the middle ear and mastoid cavity.

Perilymph is thought to be derived from both CSF and the vascular supply of blood plasma [12]. Protein analysis revealed the perilymph to be different from blood plasma and CSF, supporting the dual origin theory [13,14]. The average protein concentration is 40 mg/dl in the CSF and 200 mg/dl in the perilymph of human samples, and recent proteomic analysis of mouse samples revealed a 2.8 times higher amount of protein in the perilymph. The exclusive expression of CTP in the perilymph presented in this study also shows that these three human body fluids are discrete in nature.

Table III. Results of CTP detection test by Western blot of the leakage from cochleostomy.

Lane	Sample	Amount of sample per lane	Result
1	High-level standard	rhCTP 0.27 ng	+
2	Low-level standard	rhCTP 0.13 ng	–
3	Pre-cochleostomy	16 μl of MEL	–
4	Leakage at 0 min	2 μl of fluid	+
5	Leakage at 0.5 min	2 μl of fluid	–
6	Leakage at 1 min	2 μl of fluid	–
7	Leakage at 2 min	2 μl of fluid	–
8	Leakage at 3 min	2 μl of fluid	–
9	Leakage at 6 min	2 μl of fluid	–
10	Perilymph (control)	2 μl of fluid	+

Note that leakage collected at 25, 35 and 45 min was negative for CTP (data not shown). MEL, middle ear lavage.
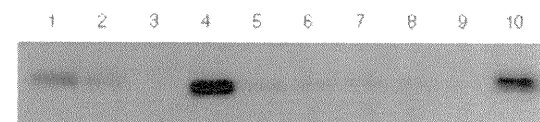

Figure 3. The results of the CTP detection test by Western blotting of the leakage from cochleostomy. MEL obtained before the fenestration of the cochlea was negative for CTP (lane 3). The leakage collected at 0 min showed a CTP signal above the high-level standard signal (lane 4) and the samples collected at 0.5–3 min showed negative results with a faint signal below the high-level standard signal, and the signal disappeared at 6 min and thereafter. Further details are shown in Table III.

It has been reported that there is communication between the labyrinthine perilymph and the CSF space. Histological study revealed that the cochlear modiolus is highly porous [14]. The porous structure in the surface of the modiolus allows communication between perilymph and the perivascular and perineural space in the modiolus. A recent MRI study in humans using intratympanic injection of gadolinium diethylenetriaminepentaacetic acid revealed the permeability of the modiolus [15]. In terms of pathology, this communication is important as a potential route for the spread of infection and subarachnoid haemorrhage. In addition, an extremely wide communication channel can result in a gusher during cochlear implantation [2,3]. In evaluating the pathology of an anomalous inner ear, it is helpful to check for two possible pathological conditions, i.e. whether a congenital defect of the bony barrier to CSF at the lateral end of the IAC caused CSF leakage into the perilymphatic space preoperatively, or whether a sudden decrease of perilymphatic pressure induced by the cochleostomy resulted in the rupture of the weak boundary of these two spaces and thereby caused CSF influx. As discussed above, the CSF and perilymph are different body fluids, not only based on the protein constituents, but also other characteristics, such as their electrolyte concentrations and pressure [8,12 14]. The potassium gradient from the CSF, perilymph and endolymph is 2.8, 10.7 and 144.2 (mEq/l), respectively, on average in human samples [16 18]. Mixture of these two fluids abruptly changes the homeostasis of the inner ear and may cause functional disturbances such as hearing loss.

In a review of congenital malformations of the cochlea by Graham et al. [2], a large defect in the IAC fundus was found to be one of the causes of the profound deafness, and gradual or intermittent mixture of these two fluids resulted in fluctuations and progressive hearing loss. The pulsatile perilymph often found at cochleostomy would be more compatible with a small direct communication between CSF and perilymph, of the kind found in the Mondini and common cavity deformities. Lemmerling et al. [19] reported evidence that temporal bones with the isolated finding of a wide vestibular aqueduct also had modiolar defects. In patients with Mondini deformities who start life with relatively good hearing, sudden rises in CSF pressure caused by changes in posture or in intra-abdominal and/or thoracic pressure can result in fluctuation and deterioration in the auditory threshold.

We have tested samples of profuse fluid leakage from only one patient and further study will be necessary to understand the pathology of this disease entity. In the case of cochlear implantation, it would be interesting to record the presence of a gusher at the time of cochleostomy, thus providing evidence for the increased pressure of the perilymph and the temporal CTP detection test result reported in this study.

Conclusion

This report has confirmed that CTP is exclusively expressed in the perilymph. Furthermore, the CTP detection test revealed the nature of the profuse leakage from cochleostomy in an anomalous cochlea of a case with BOR syndrome. The initial egress of CTP-positive fluid (perilymph) changed to CTP-negative CSF as time elapsed, indicating that the membranous boundary between these two spaces had ruptured intraoperatively. We have previously reported CTP as a specific diagnostic marker of perilymph leakage. This marker will help shed light on the mechanism of perilymph production and the pathology of anomalous cochlea.

Acknowledgments

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シンポジウム「めまいの新しい疾患概念」

外リンパ瘻

池園　哲郎

Perilymphatic fistula and vestibular symptoms

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Purpose: Perilymphatic fistula (PLF), defined as an abnormal communication between the inner and middle ear, presents with a symptomatology of hearing loss and vestibular disorder that is indistinguishable from a number of other inner ear diseases. Methods of diagnosis remain controversial. We previously showed that CTP (Cochlin-tomoprotein) was selectively detected in the perilymph. We also established a definite diagnostic test for PLF using CTP as a biochemical marker. Here, we examined the diagnostic performance of the CTP detection test to determine the usefulness of this test in a clinical setting.

Methods: The CTP detection test was performed using a western blot analysis with recombinant human (rh)CTP as a spiked standard. We evaluated the specificity of the CTP detection test by also testing non-PLF cases. To describe the limitations of the test, we tested samples from patients with middle ear infection. Serially diluted perilymph was tested to determine the detection limit of the CTP test. We then applied the CTP detection test in cases of spontaneous, traumatic and iatrogenic (surgical) PLF.

Findings: We established a standardized CTP detection test using high (0.27 ng) and low (0.13 ng) spiked standards of rhCTP and a western blot analysis. MEL (middle ear lavage) samples from 54 of the 55 non-PLF cases tested negative for CTP, i.e., the specificity of the test was 98.2%. MEL samples from 43 out of 46 cases with chronic suppurative otitis media or middle ear cholesteatoma tested negative for CTP. The detection limit in perilymph was 0.161 uL/lane for an average of 5 samples. We elucidated the clinical characteristics of the PLF cases in each category.

Interpretation: CTP is a stable perilymph specific protein, and this CTP detection may be the first clinically established diagnostic tool for the detection of PLF with a high specificity. PLF is surgically correctable by sealing the fistula. The appropriate recognition and treatment of PLF can improve hearing and balance in afflicted patients.

Key words: Perilymphatic fistula (PLF), CTP (Cochlin-tomoprotein), specificity, detection limit

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はじめに

外リンパ瘻は今まで考えられてきたよりも，様々な原因で発症し，多彩な臨床像を呈する（表1）。外リンパ瘻の定義は「外リンパ腔が骨迷路の異常な交通路を介して外腔と交通している状態」である。この定義に従えば最近報告が多い半規管裂隙症候群は「漏出がない外リンパ瘻」の代表である。一方本邦で外リンパ瘻といえば，原因不明もしくは鼻かみや飛行機搭乗など介達外力による外リンパ瘻が想起される。海外ではこのタイプの外リンパ瘻は特発性外リンパ瘻と分類されているが，特に北米では，その疾患カテゴリーの存在は常に議論の的となり，現在ではこのカテゴリー自体存在しないものとされている。また，アブミ骨外傷による外傷性外リンパ瘻は，耳かき習慣のある本邦からの報告がほとんどであるなど，そのカテゴリーごとに特徴がみられる。世界的にみると「外リンパ瘻」という疾患名を用いた論文は徐々にその数を減少しつつあり，その理由は客観的確定診断法が確立しなかった事にある。

最近報告された外リンパ漏出の生化学的確定診断マーカー CTP（cochlin-tomoprotein）を用いた報告では，外リンパ瘻の特徴が明らかになりつつある。例えば，特発性外リンパ瘻は確かに存在し，めまいを訴える頻度が高く，眼振が認められる症例が多い。アブミ骨外傷症例やアブミ骨術後症例では難聴よりもむしろめまいを主訴として受診す

る，など前庭系の症候が診断の鍵となる。この点についてもフォーカスを当てながら，本稿では外リンパ瘻の診断・治療を論ずる。

1. 外リンパ瘻の概念

外リンパ瘻の概念は今まで混乱してきた。これは80〜90年代に主に北米で生じた外リンパ瘻に関する激しい論争に由来する。当時外リンパ瘻はmyth（神話，作り話）と呼ばれついには耳鼻咽喉科の癌とまで呼ばれた。（PLF is a Mith（絵空事）（Schuknecht[1]），PLF is the Cancer eating at the credibility of otology（Shea[2]））これは主に特発性外リンパ瘻に対する論争であったにもかかわらず，その他の外傷性，奇形に伴うものなどのカテゴリーも一緒に否定され，嫌われる用語となった[3]。一方国内においては，外リンパ瘻の存在は否定されることなく，優れた研究が行われ，主に突発性難聴の鑑別診断として常に念頭におかれる疾患となっている[4]。ちなみに，David Zee, M.D.（Professor of Neurology, Johns Hopkins Hospital）の2008年京都バラニー学会での講演ではAnybody who has valsalva induced nystagmus or vertigo has Chiari Syndrome or a fistula, typically a superior canal dehiscence syndrome（口頭発表原文そのまま）と述べており，日本の外リンパ瘻の概念とは大きく異なる。

2. 外リンパ瘻の分類

外リンパ瘻では外リンパの漏出を伴う場合，伴

表1　外リンパ瘻の原因

〈後天性 acquired〉
　・真珠腫，医原性（アブミ骨手術，中耳手術），梅毒，腫瘍
　・外傷
　　直達外力：頭部外傷，側頭骨骨折，中耳（耳小骨）外傷

> 　　介達外力＊2：implosive　中耳圧変化，圧外傷
> 　　　　　　　　explosive　脳脊髄圧の変化
> 　　　　　　　　音響外傷
> 　　狭義の特発性（idiopathic/spontaneous）全く誘因のみつからないもの

〈先天性 congenital〉明らかな内耳奇形から微細な中耳奇形までを含む
　・Mondini dysplasia などの内耳奇形
　・中耳奇形に伴うもの

〈先天・後天いずれか不明〉
　・上半規管裂隙症候群
　＊1　上記□枠部分が広義の特発性で本邦で用いられている診断基準はこれに該当する
　＊2　微細な内因性，外因性圧外傷のこと。鼻かみ，力み，飛行機搭乗などによるもの。

表2　本稿で扱う外リンパ瘻の分類

分類	臨床像・病態
A．特発性	急性感音難聴（突発性難聴様），メニエール病様めまい
B．頭部外傷	頭部外傷性外リンパ瘻
C．鼓膜・中耳外傷	鼓膜損傷　中耳直達外傷　アブミ骨損傷
D．医原性	耳科手術後の難聴・めまい

表3　外リンパ瘻の診断基準平成2年度（案）
（文献11より一部抜粋）

1．確実例
　手術（鼓室開放術），内視鏡等により前庭窓，蝸牛窓のいずれかまたは両者より外リンパあるいは髄液の漏出を確認できた例，又は瘻孔の確認できた例
2．疑い例
　髄液圧，鼓室圧の急激な変動を起こすような誘因の後に，耳閉塞感，難聴，耳鳴，めまい，平衡障害などが生じた例
注；報告書には「海外では外傷や内耳奇形によるものも含めた報告が多いが，本研究班の対象は原因不明なものである」すなわち "特発性" を対象とすると記載されている。

わない場合がある。外リンパの漏出を通常伴わない外リンパ瘻で最も頻度が高いのは真珠腫による内耳（蝸牛・半規管）瘻孔で，真珠母膜により骨迷路が破壊されて生じる。また近年報告された新しい疾患である半規管裂隙症候群は外リンパ腔が硬膜外腔と交通しており，前庭窓，蝸牛窓以外に生じた第三の窓（third mobile window）がその疾患の本態である[5][6]。

外リンパの漏出を伴う外リンパ瘻には，奇形，外傷（アブミ骨，頭部）に伴うもの，さらに本邦診断基準が対象とする「特発性外リンパ瘻」などが挙げられ，正円窓，卵円窓，microfissure（fistula ante fenestram）から漏出する。外傷性の場合は内耳骨折部位，中耳直達外傷ではアブミ骨底板が漏出部位となる。

3．診断・治療総論

診断のポイントは，瘻孔又は外リンパ漏出の有無を判断することにある。瘻孔の診断には高分解能 thin slice-CT が有用で 0.9–0.5 mm スライスが推奨されている。試験的鼓室開放術により，瘻孔が確認できる症例もある。内耳奇形，中耳奇形，外傷による内耳骨折，耳小骨骨折を診断する。

瘻孔に外リンパ漏出を伴えば術中診断は容易である。たとえば，外傷によるアブミ骨底板嵌頓，真珠腫による内耳瘻孔で手術的に病変を剥離した場合などである。しかし，瘻孔が小さく「外リンパ漏出のみ」の術中診断はむずかしい。顕微鏡下に正円窓，卵円窓を観察しても，そもそも 150 ul しかない外リンパの漏出は判断しずらい。さらに，内耳窓窩は窪んでおり，そこに周囲から手術操作にともなう液体（組織液，局所麻酔薬，洗浄液）が流入する。このため漏出の有無の判断はどうしても主観的となる[7]。

そこで，客観的な外リンパ漏出の診断マーカー

が研究された。髄液中と血清中の存在比率が有意に前者で高い β2 transferrin は，外リンパ瘻の診断に用いられて多くの論文が発表された。外リンパは髄液由来という説もあることから，外リンパ瘻マーカーとして流用されたわけである。しかしその後の研究により，β2 transferrin の診断マーカーとしての価値は否定的となった[8]。

最近，新しい生化学的診断マーカーとして CTP（cochlin-tomoprotein）が報告された。CTP は外リンパ瘻の生化学的診断マーカーとして十分な外リンパ発現特異性を兼ね備えていることが判明し，実地臨床レベルで実用化される可能性が非常に高い外リンパ瘻診断マーカーである[9][10]。

CTP 検出による外リンパ瘻診断では中耳洗浄液（Middle Ear Lavage；MEL）を検査する。手術中もしくは外来で鼓膜切開を行い，鼓室を 0.3 ml 生理食塩水で3〜4回洗浄し回収した MEL をウェスタンブロットで検査する。

実際の診療にこの検査を応用するために，その精度管理を目的として下記3ポイントを実施している。

1．recombinant human CTP を作製し，内部標準（Spiked Standard）として使用
2．最新式イメージアナライザー（LAS 300）で最適な SN 比をもつ結果画像を選定
3．臨床経過を知らない第3者的立場の担当者が結果判定

この方法で検査の精度は，recombinant human CTP の検出下限 0.27 ng/lane，ヒト外リンパの検出下限 0.161 ul/lane であった。これを換算する

と，中耳腔に数μlの外リンパが存在すれば検出できることになる。本検査の特異度は非感染耳で98.2%，感染耳では93.5%である。今後はエライザによるCTP検出法を開発し，各病院で検査を施行できる体制を目指している。

この検査を用いて判明した外リンパ瘻の臨床像の特徴には下記が挙げられる。

1：内耳障害の程度は極めて軽度のものから廃絶に近いものまで多様である

2：時に症状の変動や進行が見られる症例がある

3：高度障害例であっても，自然経過や手術治療により治癒する症例がある

臨床像があまりに多彩であることは外リンパ瘻という疾患カテゴリーが否定されてきた一つの理由でもある。例えば，我々が経験したCTP陽性例（外リンパ瘻確実例）での聴力図は，低音障害型，高音障害型，水平型など一定の傾向は無く，その聴力型から他疾患との鑑別はできない。外リンパ瘻の4つのカテゴリー（表2）に焦点をしぼり解説する。

4．診断各論

A．特発性外リンパ瘻

「特発性」外リンパ瘻（Spontaneous PLF）という診断名は，全く誘因が見あたらない症例（狭義の特発性）でも用いられるし，介達外力に伴う外リンパ瘻（広義の特発性；Goodhillが提唱した労作時の脳脊髄圧の上昇 [explosive route]，もしくは中耳圧の急激な変化 [implosive route]）にも用いられる[1]。本邦でひろく用いられている外リンパ瘻診断基準（表3）は後者の広義の「特発性」を対象にしている[11]。

今まで特発性外リンパ瘻は否定され，非難されてきた疾患であるが，我々の検査結果はこのカテゴリーが実在することを示した。特発性外リンパ瘻疑い症例200例以上にCTP検出検査を施行したところ，CTP陽性例は，約8％であった。92％はCTP陰性であったが，これは外リンパ漏出自然停止，間欠的又は微量漏出などの可能性があり外リンパ瘻を否定するものではない。陽性例を検討したところ，内因性の誘因として咳，鼻かみ，いきみ，外因性誘因として飛行機，ダイビング，水上スキーがあり，誘因無し（狭義の特発性）が7例あった。臨床症状，検査所見は多様であり，聴力型，眼振めまいの有無などの所見は，診断の

「決め手」にはならなかった。しかしながら，眼振が6割，めまいが7割の症例にみられ，通常の突発性難聴症例400例での我々の過去のデーター（眼振が4割，めまいが3割）と比較すると，多い傾向がみられた。すなわち，診断の決め手にはならないが，前庭症候がより多いのは間違い無いと思われる。流水性耳鳴，瘻孔症状などの診断感度・特異度は現在検討中であるが，必ずしもその割合は高くない。

CTP陽性例の聴力を詳しくみてみると進行性・変動性に悪化したものが6割，突発性難聴様が3割，再発が1割であった。突発性難聴の非典型例，すなわち変動性難聴，変動しながら悪化する，改善した難聴が再度悪化する，などの病歴は外リンパ瘻の可能性を検討すべきである。狭義の特発性が7例あったことは，通常我々が突発性難聴と診断している症例の中に，外リンパ瘻が含まれていることを示唆している。

B．外傷性外リンパ瘻

セシル内科書最新版（23版）には，"PLF may be congenital or may follow stapes surgery or head trauma" と記載されている。北米で後天性外リンパ瘻といえば特発性ではなく「外傷性」を指し示す。北米では頭部外傷後の外リンパ瘻はメニエール病と症状が似通っているとする報告が多く，[12]メニエール病を疑う症例では，外傷の既往の問診が必要とされている。また，北米の報告[13]では外リンパ瘻平均罹病期間が数ヶ月から数年と長く，ほとんどの症例が外傷後の遅発性または慢性例である。外傷性内耳障害は報告が多いため鑑別診断を挙げながら解説する。

(1)頭部外傷による内耳障害の概論

頭部外傷による内耳障害の原因としてもっとも診断しやすいのは側頭骨骨折である。側頭骨骨折は迷路骨包保存型（otic capsule sparing），迷路骨包骨折型（otic capsule violating）の2種類に分けると，内耳障害の程度を推測しやすい。迷路骨包が傷害されている場合には，顔面神経麻痺のリスクが2倍，脳脊髄液漏が4倍，高度難聴が7倍のリスクを有することが報告されている[14]。迷路骨包骨折型では，外傷性外リンパ瘻が生じる。迷路骨包保存型では，内耳振盪，外傷性良性発作性頭位めまい症，内耳窓やminor fissureから外リンパが漏出する外リンパ瘻が鑑別診断となる。

(192)