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.

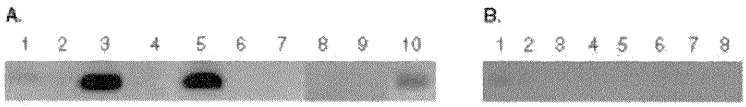

Figure 2. Western blot analysis of CTP expression in perilymph and CSF. The expression of CTP was analysed by Western blot using the anti-CTP antibody. CTP expression (16 kDa) was only detected in the perilymph (cases A and B), not in the CSF. The perilymph sample from case C was negative for CTP. Further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

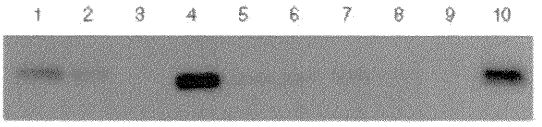

Figure 3. The results of the CTP detection test by Western blotting of the leakage from cochleostomy. MEL obtained before the fenestration of the cochlea was negative for CTP (lane 3). The leakage collected at 0 min showed a CTP signal above the high-level standard signal (lane 4) and the samples collected 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.

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References

[1] Jackler RK, Hwang PH. Enlargement of the cochlear aqueduct: fact or fiction? Otolaryngol Head Neck Surg 1993;109:14–25.

[2] Graham JM, Phelps PD, Michaels L. Congenital malformation of the ear and cochlear implantation in children: review and temporal bone report of common cavity. J Laryngol Otol Suppl 2000;25:1–14.

[3] Papsin BC. Cochlear implantation in children with anomalous cochleovestibular anatomy. Laryngoscope 2005;115 (1 Pt 2 Suppl 106):1–26.

[4] Ikezono T, Omori A, Ichinose S, Pawankar R, Watanabe A, Yagi T. Identification of the protein product of the Coch gene hereditary deafness gene as the major component of bovine inner ear protein. Biochim Biophys Acta 2001;1535:258–65.

[5] Ikezono T, Shindo S, Li L, Omori A, Ichinose S, Watanabe A, et al. Identification of a novel Cochlin isoform

in the perilymph: insights to Cochlin function and the pathogenesis of DFNA9. Biochem Biophys Res Commun 2004;314:440–6.

[6] Ikezono T, Shindo S, Sekiguchi S, Hamprasertpong C, Li L, Pawankar R, et al. Cochlin-tomoprotein (CTP), a novel perilymph-specific protein and a potential marker for the diagnosis of perilymphatic fistula. Audiol Neurootol 2009;14:338–44.

[7] Ikezono T, Shindo S, Sekiguchi S, Morizane T, Pawankar R, Watanabe A, et al. The performance of cochlin-tomoprotein detection test in the diagnosis of perilymphatic fistula. Audiol Neurootol 2009;15:168–74.

[8] Thalmann I, Kohut RI, Ryu J, Comegys TH, Senarita M, Thalmann R. Protein profile of human perilymph: in search of markers for the diagnosis of perilymph fistula and other inner ear disease. Otolaryngol Head Neck Surg 1994;111:273–80.

[9] Kochhar A, Fischer SM, Kimberling WJ, Smith RJ. Branchio-oto-renal syndrome. Am J Med Genet 2007;143A:1671–8.

[10] Propst EJ, Blaser S, Gordon KA, Harrison RV, Papsin BC. Temporal bone findings on computed tomography imaging in branchio-oto-renal syndrome. Laryngoscope 2005;115:1855–62.

[11] Buckingham RA, Valvassori GE. Inner ear fluid volumes and the resolving power of magnetic resonance imaging: can it differentiate endolymphatic structures? Ann Otol Rhinol Laryngol 2001;110:113–17.

[12] Zou J, Pyykkö I, Counter SA, Klason T, Bretlau P, Bjelke B. In vivo observation of dynamic perilymph formation using 4.7 T MRI with gadolinium as a tracer. Acta Otolaryngol 2003;123:910–15.

[13] Hara A, Salt AN, Thalmann R. Perilymph composition in scala tympani of the cochlea: influence of cerebrospinal fluid. Hear Res 1989;42:265–71.

[14] Rask-Andersen H, Schrott-Fischer A, Pfaller K, Glueckert R. Perilymph/modiolar communication routes in the human cochlea. Ear Hear 2006;27:457–65.

[15] Naganawa S, Satake H, Iwano S, Sone M, Nakashima T. Communication between cochlear perilymph and cerebrospinal fluid through the cochlear modiolus visualized after intratympanic administration of Gd-DTPA. Radiat Med 2008;26:597–602.

[16] Schielke GP, Betz AL. 1992. Electrolyte transport. In: Bradbury MWB, editor. Physiology and pharmacology of the blood-brain barrier. Heidelberg: Springer-Verlag. p 221–43.

[17] Anniko M, Wróblewski R. Ionic environment of cochlear hair cells. Hear Res 1986;22:279–93.

[18] Swan EE, Peppi M, Chen Z, Green KM, Evans JE, McKenna MJ, et al. Proteomics analysis of perilymph and cerebrospinal fluid in mouse. Laryngoscope 2009;119:953–8.

[19] Lemmerling MM, Mancuso AA, Antonelli PJ, Kubilis PS. Normal modiolus: CT appearance in patients with a large vestibular aqueduct. Radiology 1997;204:213–19.

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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

Tetsuo Ikezono

Department of Otolaryngology, Saitama Medical University

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

埼玉医科大学耳鼻咽喉科

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年京都パ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[9]。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。

表4　試験的鼓室開放術における手術所見の解釈（文献2を改変）

漏出		診断
あり	外リンパか脳脊髄液と証明された場合	外リンパ瘻
あり	組織液	他疾患
なし		他疾患
なし	自然治癒	外リンパ瘻
なし	間欠的漏出	外リンパ瘻
なし	迷路内破綻（collapse inside labyrinth）	外リンパ瘻

慢性に経過し，遅発性にメニエール病様症状を呈する場合には外傷性内リンパ水腫の可能性も考慮する。

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（外傷性BPPV），内耳窓やminor fissureから外リンパが漏出する外リンパ瘻が考えられる。慢性に経過し，遅発性にメニエール病様症状を呈する場合には外傷性内リンパ水腫が原因と考えられる。これらについて以下に解説する。

⑵頭部外傷性外リンパ瘻

頭部外傷後に難聴，めまいを主訴に当科へ紹介された症例を検討したところ，5割に末梢性眼振が観察され，そのうち半数にBPPV様めまいと眼振を認めた。また，全体の3割の症例がCTP陽性であった。このCTP陽性外リンパ瘻確実例を検討したところ，めまい，難聴の程度は様々であったため，一定の傾向をつかむことは難しい。しかし，内耳骨包は保存され，乳突蜂巣に微細な側頭骨骨折しか認められないにも関わらず，受傷直後から患側聾となった症例がCTP陽性だったことは特筆すべき所見であった。恐らく全身打撲による脳脊髄圧上昇により外リンパ漏出をきたしたものと推測された。このような症例が的確に診断され内耳窓閉鎖術が施行されれば，後遺症軽減に役立つと考えられる。

⑶特に外リンパ瘻と鑑別が必要な疾患

・内耳振盪症

骨折が無い場合でも頭部打撲による衝撃で蝸牛，前庭，半規管が損傷を受ける。これを内耳振盪と呼ぶ。そのメカニズムはいまだ完全には明らかにされていないが，急速な加速度外傷（速度の急激な上昇と下降に伴う加速度変化）により前庭末梢器の剪断（shearing）や，感覚細胞の障害を生じると言われている[15]。軽症の場合の内耳機能障害は可逆的であることが多い。剪断により前庭の微小血管から出血し，これが迷路内の結合組織，瘢痕，骨の増成をもたらすと慢性機能障害を呈すると考えられている[16]。

内耳振盪の臨床診断の定義は曖昧である。一般には外傷直後に発症し，明らかな外傷性BPPV所見を呈さない内耳性めまい症例に用いられる事が多いが，文献によっては外傷後の難聴のことを示すこともある。

・外傷性BPPV

頭部打撲は耳石器に剪断性の外力を与え，はがれ落ちた耳石が内リンパ腔へ入り込む。これが原因となり，種々のタイプの前庭機能障害が生ずる。典型的なBPPVの症状（頭位変換時の発作性の回転性めまい）に合致しないめまい症状がみられることが多く，合併する耳石機能障害によるものと考えられる。外傷性BPPVが頭部外傷後に生じる頻度は8～20%程度と言われている。

外傷後のBPPVと通常の特発性BPPVを比較するとその病態が異なることが推察される[17]。外傷性では両側性が多いことも知られており14～19%と高率だが，特発性BPPVでは2～6%である[18]。特発性は通常女性に多いと言われるが外傷性では性差が無い。外傷性は治療に抵抗性があり，67%の症例で耳石置換法による治療を複数回必要としたが，特発性では14%であった。再発率も外傷性で57%，特発性では19%であった。外傷性BPPVが治療抵抗性である理由として，内耳における出血や組織が剪断され生化学的な変化を

きたした細かな半規管内浮遊物が塊を形成しやすいことが考えられている。

・外傷性遅発性内リンパ水腫

外傷後，数ヶ月から数年の経過後に発症するメニエール病様症状（耳閉感，耳鳴，変動する難聴，発作性のめまい）を呈する疾患のことである[19]。側頭骨骨折に伴い前庭水管に骨折が及んでいる症例では閉塞や狭窄により内リンパドレナージ機能が傷害されるため内リンパ水腫をきたすと推測されている[20][21]。

C．鼓膜・中耳外傷

本邦では耳かきが日常習慣的に行われており，中耳(鼓膜，耳小骨)外傷の最も多い原因である。湿性耳垢の多い白人社会では，耳垢は点耳薬で洗い流すものと教育されており，耳かき外傷は少ない。後上象限，すなわちアブミ骨付近に鼓膜穿孔をきたした症例では，アブミ骨外傷性外リンパ瘻を念頭において診療する。さらに骨導の悪化，末梢性眼振，めまい（回転性，浮動性）があれば外リンパ瘻の可能性が高くなる。CTでアブミ骨底の骨折や陥入を確認できれば確定診断となる。平手打ちによる鼓膜損傷も日頃よく経験するが，この受傷機転でCTP陽性だった症例は今のところ経験していない。

我々の経験では，耳かきによる鼓膜穿孔症例の90％に末梢性眼振が認められたが，めまいを自覚したのは後上象限穿孔例（全体の65％）にほぼ限られていた。後上象限穿孔例の80％からCTPが検出されており，アブミ骨外傷は外リンパ瘻をきたしやすいことが確認された。通常はアブミ外傷で外リンパ漏出が持続すれば，混合難聴が次第に増悪し聾になると予測される。しかし興味深いことに，CTP陽性例でも，骨導が悪化しない症例がみられた。これらの症例では，めまいの増悪や，末梢性眼振の方向が数時間から数日で変化する（麻痺性から，刺激性へ，さらに麻痺性へなど）ことが特徴的で，卵円窓の破綻においては前庭系の所見が病態の進行を鋭敏に反映していた。

ある一症例は受傷直後と安静治療後数日経過してから，２回サンプルを採取，両方ともCTPが陽性であった。持続的漏出が客観的に証明された本症例の骨導の悪化をきたさず軽度伝音難聴のみ，めまいが主訴であったことは，特筆に値する。以前から，難聴を伴わない，めまいのみの外リンパ瘻が存在することが提唱され[22][23]，通常の外リンパ瘻の概念を超えるものであり受け入れがたいとする意見もあったが，本症例はこの疾患カテゴリーが実在することを示唆している。

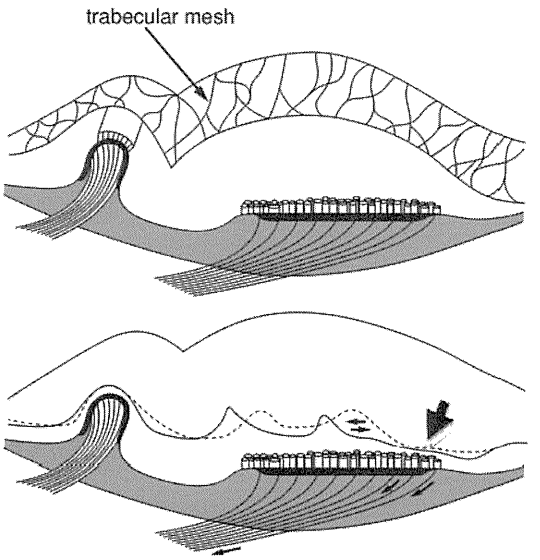

図１　外リンパ瘻の病態説明モデル Floating Labyrinth（文献24を改変）
部分的な虚脱を起こした膜迷路が耳石・半規管の感覚細胞を刺激する（矢印）

アブミ骨付近に鼓膜外傷があってもCTP陰性例もあった。外リンパ瘻以外にも中耳に外力が加わって骨導の悪化をきたすことは以前より知られており，内耳への物理的刺激による内耳振盪や音響外傷と診断される。

D．医原性外リンパ瘻（耳科手術）

アブミ骨手術後に感音成分増悪をきたした場合に，術後性外リンパ瘻の可能性を考慮する。発症時期により早期，晩期型の２つに分類される。変動する感音難聴，耳鳴，耳閉感に伴うめまいが特徴である。北米では，プロテーゼならびに卵円窓の閉鎖手技によるアブミ骨術後性外リンパ瘻頻度が報告されており，ゼルフォーム3.5％，脂肪1.9％，筋膜0.6％であった。

本邦では耳硬化症の頻度が少ないことからアブミ骨術後のめまいも報告が少ないが，最近筆者らは，術後10年以上経過してめまいを主訴に受診した症例でCTP陽性例を経験した。

5．外リンパ瘻と前庭症状

既述のように，極めて特徴に富み，多彩な臨床所見を呈する外リンパ瘻の病態を探るべく，以前から基礎的研究が行われてきた。外リンパ瘻の前庭症状の原因として“Floating” labyrinth 説がある。モルモットを用いた研究で，くも膜下腔に急速に 1-2 ml の液体を注入，もしくは正円窓から 4 μl の外リンパを吸引して作成した外リンパ瘻モデルが報告されている。Pars superior（半規管，球形嚢）には trabecular mesh があり膜迷路を支えているが，上記実験操作により mesh が様々な程度に破綻し，膜迷路が落ち込み，半規管膨大部，球形嚢の感覚細胞を刺激する病態が組織学的に観察された（図１）。外リンパ瘻における多様な前庭症状，検査所見を説明しやすい所見である[20]。前庭では虚脱のみが観察されたが，さらに蝸牛をみるとライスネル膜の病変は様々で，虚脱，破綻，膨隆いずれもが生じていた。これは前庭膜迷路の虚脱により内リンパが pars inferior へ入り込んだため生じた変化と考えられる。

6．治療法

A．保存的療法

自然閉鎖の可能性があるので，入院の上，脳脊髄圧を下げる目的で頭を30度挙上した状態で安静を保ちながら突発性難聴に準じた処方を行う。めまいで嘔吐が強いときには制吐薬を用いる。頻回に純音聴力検査を行い聴力の推移をみる。重量物の運搬や強い鼻かみ，過度のいきみを禁止する。

B．外科的療法

保存治療に反応しない例や，聴平衡機能の悪化，変動を示す例，安静解除で再び症状が出現する場合は，外科治療の適応となる。特発性外リンパ瘻 CTP 陽性例の経験では，術後，めまいは大多数の症例で消失するが難聴は約４割に治癒・著明改善，４割に改善，２割に不変・悪化がみられた。特に発症後14日以内に手術を行った症例で聴力改善が良好であるが，受傷後１月以上経過して手術した症例で治癒した症例もある。

手術は全身麻酔科，局所麻酔いずれでも可能である。耳内法で内耳窓を明視下に観察する。瘻孔と外リンパ瘻漏出を確認するように努める。特に正円窓では偽膜との鑑別を要する。内耳窓閉鎖は，自己組織の筋膜，軟骨膜，結合組織を用いて，瘻孔・漏出の確認の有無にかかわらず，前庭窓，蝸牛窓，fistula ante fenestram をそれぞれ覆い生体糊で固定する[25]。再発予防のため術後は頭部を30度上げ３日間ベッド上安静とする。術後の日常生活では３か月間は鼓室圧，髄液圧が高まるような動作を避けるよう指導する。術中所見から外リンパの漏出の有無を判断することは容易ではなく，客観的な判断指針が報告されている（表４）[9]。

アブミ骨外傷・手術後の外リンパ瘻疑い症例の治療方針決定の上でもっとも重要なポイントは，外リンパ漏出が持続しているか否かである。漏出が持続していれば，いずれ内耳機能が廃絶することが予想され手術的な修復が必要となる。軽度輪状靱帯損傷に伴う外リンパ漏出であれば筋膜による被覆のみで治療できるので，手術のリスクは低い。しかしアブミ骨底が陥入している場合にはアブミ骨を引き上げ整復するため，手術操作自体が内耳障害を増悪させる可能性がある。聴覚・前庭所見，その変動の様子を参考にしながら慎重に判断する。保存的に様子をみる方法と，再手術による瘻孔閉鎖術を比較した報告では，治癒率に明らかな有意差が無いという報告もあり，明らかに外リンパ瘻と考えられた場合にのみ手術を行うのが適切である[26]。

最後に

多彩な臨床像を呈する外リンパ瘻診療においては，前庭系の症候に特に注意が必要で，症例に応じた対応が必要である。

文　献

1）Schuknecht HF: Myths in neurotology. Am J Otol 13: 124-126, 1992

2）Shea JJ: The myth of spontaneous perilymph fistula. Otolaryngol Head Neck Surg 107: 613-616, 1992

3）Meyerhoff WL: Spontaneous perilymphatic fistula: myth or fact. Am J Otol 14: 478-481, 1993

4）Nomura Y: Perilymph fistula: concept, diagnosis and management. Acta Otolaryngol Suppl 514: 52-54, 1994

5）Minor LB: Labyrinthine fistulae: pathobiology and management. Curr Opin Otolaryngol Head Neck Surg 11: 340-346, 2003

6）Merchant SN, Rosowski JJ: Conductive hearing loss caused by third-window lesions of

the inner ear. Otol Neurotol 29: 282-289, 2008
7) Friedland DR, Wackym PA: A critical appraisal of spontaneous perilymphatic fistulas of the inner ear. Am J Otol 20: 261-276, 1999
8) Rauch SD: Transferrin microheterogeneity in human perilymph. Laryngoscope 110: 545-552, 2000
9) Ikezono T, Shindo S, Sekiguchi S, et al: Cochlin-tomoprotein (CTP), a novel perilymph-specific protein and a potential marker for the diagnosis of perilymphatic fistula. Audiol Neurootol 14: 338-344, 2009
10) Ikezono T, Shindo S, Sekiguchi S, et al.: The performance of CTP detection test for the diagnosis of perilymphatic fistula. Audiol Neurootol 15: 168-174, 2009
11) 厚生省特定疾患急性高度難聴調査研究班平成2年度研究業績報告書：外リンパ瘻の診断基準　平成2年度（案），20頁，1990
12) Fitzgerald DC: Perilymphatic fistula and Meniere's disease. Clinical series and literature review. Ann Otol Rhinol Laryngol 110: 430-436, 2001
13) House JW, Morris MS, Kramer SJ, et al.: Perilymphatic fistula: surgical experience in the United States. Otolaryngol Head Neck Surg 105: 51-61, 1991
14) Dahiya R, Keller JD, Litofsky NS, et al.: Temporal bone fractures: otic capsule sparing versus otic capsule violating clinical and radiographic considerations. J Trauma 47: 1079-1083, 1999
15) Schuknecht HF : Mechanism of inner ear injury from blows to the head. Ann Otol Rhinol Laryngol 78: 253-262, 1969
16) Weissman JL, Curtin HD, Hirsch BE, et al.: High signal from the otic labyrinth on unenhanced magnetic resonance imaging. Am J Neuroradiol 13: 1183-1187, 1992
17) Gordon CR, Levite R, Joffe V, et al.: Is post-traumatic benign paroxysmal positional vertigo different from the idiopathic form? Arch Neurol 61: 1590-1593, 2004
18) Katsarkas A : Benign paroxysmal positional vertigo (BPPV): idiopathic versus post-traumatic. Acta Otolaryngol 119: 745-749, 1999
19) DiBiase P, Arriaga MA :Post-traumatic hydrops. Otolaryngol Clin North Am 30: 1117-1122, 1997
20) Rizvi SS, Gibbin KP: Effect of transverse temporal bone fracture on the fluid compartment of the inner ear. Ann Otol Rhinol Laryngol 88: 741-748, 1979
21) Paparella MM, Mancini F: Trauma and Meniere's syndrome. Laryngoscope 93: 1004-1012, 1983
22) Brandt T: Otolithic Vertigo. eds by Huy B, Toupet M. Karger AF Barsel. In Otolith function and disorders. Advances in Oto Rhino Laryngology 58: 34-47, 2001
23) 深谷　卓，野村恭也：聴力障害を欠く外リンパ瘻症例　試験鼓室開放術の適応拡大についての提案．耳鼻臨床　78：1398-1392，1985
24) Nomura Y, Okuno T, Hara M, et al.: "Floating" labyrinth. Pathophysiology and treatment of perilymph fistula. Acta Otolaryngol 112: 186-191, 1992
25) 池園哲郎：聴力改善手術　外リンパ瘻．耳喉頭頸　77：162-173，2005
26) Yamasoba T, Amagai N, Karino S et al.: Traumatic luxation of the stapes into the vestibule. Otolaryngol Head Neck Surg 129: 287-290, 2003

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Various levels of plasma brain-derived neurotrophic factor in patients with tinnitus

Fumiyuki Goto [a,b,*], Juri Saruta [c], Sho Kanzaki [b], Masahiro To [c], Tomoko Tsutsumi [a], Keiichi Tsukinoki [c], Kaoru Ogawa [b]

[a] Department of Otolaryngology, Hino Municipal Hospital, Japan
[b] Department of Otolaryngology, Keio University, Japan
[c] Department of Maxillofacial Diagnostic Science, Division of Pathology, Kanagawa Dental College, Japan

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ABSTRACT

Objective: Thus far, no objective measure has been developed to evaluate tinnitus severity. There is a close relationship between tinnitus and depression, in which brain-derived neurotrophic factor (BDNF) has a pathophysiological role. To determine whether BDNF levels could be used to evaluate tinnitus severity, we evaluated plasma BDNF levels in patients with tinnitus.
Methods: Plasma BDNF levels were measured in 43 tinnitus patients and 30 healthy control patients. The severities of tinnitus, depression, and anxiety were measured using the tinnitus handicap inventory (THI) and the hospital anxiety and depression scale (HADS), respectively. Patients with tinnitus were divided into 2 groups depending on their THI scores: mildly handicapped (<36) and severely handicapped (>38). We also divided our subjects into 2 groups depending on the HADS score, which represents patient mood, including depression and anxiety.
Results: Plasma BDNF levels were significantly higher in the mildly handicapped group than in the severely handicapped and control groups ($P < 0.01$). Patients with HADS scores of ≤14 had significantly lower THI scores ($P < 0.05$) and higher BDNF levels ($P < 0.01$).
Conclusions: Our findings show for the first time that plasma BDNF levels vary with the severity of tinnitus, suggesting that plasma BDNF level is a useful tool for objective evaluation of tinnitus.

1. Introduction

Tinnitus is the perception of sounds in the absence of external noise. Subjective tinnitus is defined as the perception of phantom sounds. Tinnitus can affect the entire life of an individual, preventing intellectual work and generally impairing quality of life. In some cases, tinnitus can cause suicidal behavior [2]. Severe tinnitus is often accompanied by affective disorders such as depression. Psychiatric comorbidity occurs especially in individuals with severe tinnitus and adds considerably to patient suffering [2]. Major depressive disorder and anxiety disorder occur most frequently in individuals with chronic disabling tinnitus, with a prevalence of 60% or more [11,34]. Several studies have shown that tinnitus severity and tinnitus-related distress are correlated with depression [32].

In general, subjective tinnitus has no physical signs, and there are no objective clinical diagnostic tests to evaluate its severity. Currently, only patient descriptions can serve as a basis for clinical evaluation. It is therefore very important to develop objective tools for evaluation of tinnitus. The tinnitus handicap inventory (THI) is a very useful test to evaluate the handicap caused by tinnitus [21]. In a consensus meeting (additional) use of the THI was recommended for clinical studies in order to facilitate comparison of results from different studies [17].

Brain-derived neurotrophic factor (BDNF) is a member of the "neurotrophin" protein family of growth factors, which are related to the prototypical "nerve growth factor" NGF [3]. Neurotrophic factors are found in the brain and the periphery. BDNF acts on certain neurons of the central nervous system (CNS) and peripheral nervous system, supporting the growth, differentiation, and survival of neurons and synapses [3]. BDNF plays a central role in synaptic plasticity and neurogenesis in general. It was reported that brain BDNF levels correlate with serum BDNF concentrations [8];

Abbreviations: BBB, blood–brain barrier; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; HADS, hospital anxiety and depression scale; HRP, horseradish peroxidase; NGF, nerve growth factor; NT-3, neurotrophin-3; NT-4, neurotrophin-4; THI, tinnitus handicap inventory; TMB, tetramethylbenzidine; BDI, Beck Depression Inventory.

* Corresponding author at: Department of Otolaryngology, Hino Municipal Hospital, 4-3-1 Tamadaira Hino-shi, Tokyo 191-0062, Japan. Tel.: +81 42 581 2677; fax: +81 42 581 2923.
E-mail address: amifumi@bc5.so-net.ne.jp (F. Goto).

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therefore, blood levels of BDNF may serve as an indirect measure of brain BDNF levels. According to Lommatzsch et al. [19], changes in plasma BDNF levels reflect BDNF concentration changes in the brain. It was recently reported that increased plasma BDNF concentrations observed during physical exercise in humans were due to the enhanced release of BDNF from the brain. These results indicate that serum BDNF is a non-specific trait marker of depression [25], whereas plasma BDNF is a state marker [8].

Strong evidence suggests that serum BDNF levels are abnormally low in patients suffering from major depressive disorder [29]. In addition, close relationships between BDNF gene expression levels and tinnitus have been reported [12,28,31]. Because tinnitus and depression tend to coexist, we reasoned that plasma BDNF levels may serve as tools for the evaluation of tinnitus.

BDNF levels may serve as markers for activity changes in the auditory system. Increased expression of BDNF and gamma-aminobutyric acid (GABA) in the inferior colliculus [24] as well as a reduction in local field potentials in the auditory cortex [30] were reported to be associated with tinnitus. BDNF-induced changes in glutamatergic signaling suggest that BDNF exerts modulatory effects on spontaneous neuronal firing rates in the auditory cortex [13].

In the present study, we examined plasma levels of BDNF in patients with tinnitus and in healthy controls. We tested for any correlations between plasma BDNF levels and clinical characteristics, including tinnitus severity. The objective of the study was to investigate the plasma levels of BDNF in patients with tinnitus.

2. Materials and methods

To investigate whether alterations in neurotrophin levels can be detected in subjects with tinnitus, we determined the peripheral levels (plasma) of BDNF in patients with tinnitus ($N = 43$; 14 male and 29 female) and healthy controls ($N = 30$; 15 male and 15 female). Our subjects included tinnitus patients without other inner ear disorders like sudden deafness or otitis interna. We carefully excluded subjects with tinnitus due to acute inner ear disorder by asking patients about recent history of hearing deterioration. The average age was 57.1 ± 15.2 years (average ± SD) and 50.7 ± 10.1 years, respectively. The severity of tinnitus was evaluated by the THI score. The proposed severity according to THI score was as follows: no handicap (0–16), mild handicap (18–36), moderate handicap (38–56), severe handicap (58–76) and catastrophic handicap (78–100) [22]. Patients with THI scores of less than 36 were classified as having mild tinnitus. Patients were classified as having severe tinnitus if the THI score was more than 38. Subjects were first-visit patients complaining of tinnitus at the Hino Municipal hospital. None of the patients had psychiatric disorders at the time of the first visit, and none reported taking drugs for the treatment of psychiatric disorders, including antidepressants and anxiolytics. Conventional audiological evaluation was conducted by pure tone audiometry. Severity of tinnitus was evaluated by the THI score. Mood, including anxiety and depression, was evaluated using the hospital anxiety and depression scale (HADS) [33]. We divided our subjects into 2 groups depending on the HADS score. In our previous study, we determined that a total score of 15 or more is indicative of mood disorder [10]. We classified patients with total scores of 14 or below as HADS ≤ 14 and those with scores of 15 or above as HADS ≥ 15. In addition we collected data on hearing threshold, site of tinnitus (left ear, right ear, bilateral, or intracranial), and duration of tinnitus from the initial onset. The hearing threshold was calculated as the average of 4 consecutive frequencies of 500, 1000, 2000, and 4000 Hz.

2.1. Plasma BDNF measurements

Blood samples from all subjects were drawn between 0900 and 1000 h. Approximately 4 mL of blood was collected in a vacuum tube with lithium heparin and immediately centrifuged at 3800 rpm for 10 min. Plasma was stored at −70 °C prior to use. Human BDNF was detected by sandwich ELISA according to the manufacturer's instructions (CYT306; Millipore Co., Billerica, MA, USA). All assays were performed in F-bottom 96-well plates (Nunc, Wiesbaden, Germany). Tertiary antibodies were conjugated to horseradish peroxidase (HRP). Color was developed with tetramethylbenzidine (TMB) and measured at 450/570 nm. BDNF content was quantified against a standard curve calibrated with known amounts of BDNF. The detection limit was <4 pg/mL. All samples were tested twice, and mean values were calculated. Cross-reactivity to related neurotrophins (NGF, NT-3, and NT-4) was less than 3%. Intra-assay and inter-assay coefficients of variation were 3.7% and 8.5%, respectively. Concentration was expressed as pg/mL. The relationship between the THI score and plasma BDNF concentration was investigated.

We tested for correlations between plasma BDNF levels, tinnitus handicap, depression, and anxiety. All experiments were carried out in accordance with the guidelines of the ethics committee of the Hino Municipal Hospital and the Declaration of Helsinki.

2.2. Statistical analysis

All data were analyzed using Microcal Origin R version 6.0 software (Microcal Software Inc., Northampton, MA, USA) and GraphPad Prism software (GraphPad Software Inc., La Jolla, CA, USA). Statistical analyses were performed using t-tests, repeated measures ANOVA, and chi-square tests. If the P value was less than 0.05, the results were considered statistically significant.

3. Results

Thirteen patients reported tinnitus in the right ear and 12, in the left ear. Eleven patients reported bilateral tinnitus, and 7 reported intracranial tinnitus. The average hearing threshold was 22.9 ± 20.3 dB for the right ear and 20.9 ± 14.8 dB for the left. THI ranged from 2 to 90 (average: 38.2 ± 23.4). The duration of tinnitus ranged from 2 days to 312 months (average duration: 25.5 ± 59.6 months). Initially, patients with tinnitus were divided into 2 groups depending on the duration of tinnitus. Tinnitus with duration of less than 2 months was defined as acute tinnitus. Tinnitus with duration of more than 3 months was defined as chronic tinnitus [20]. Twenty patients had acute tinnitus, and 22 patients had chronic tinnitus. When we compared BDNF levels, HADS scores, and THI scores between these groups, there were no significant differences. Therefore, we combined these 2 groups and treated them as 1 group for comparison purposes. The total HADS scores of tinnitus patients (14.5 ± 7.5) were significantly higher than those of controls (7.8 ± 5.4; $P < 0.0001$). Plasma BDNF levels ranged from 48.6 to 4045.4 pg/mL (average, 768.7 ± 961.4 pg/mL) in tinnitus patients and from 44.8 to 1289.9 pg/mL (average, 338.5 ± 287.7 pg/mL) in the controls (Table 1 and Fig. 1). The site of tinnitus, hearing threshold, and the duration of tinnitus did not correlate with the THI scores, HADS scores, or BDNF levels.

There were 25 patients with mild tinnitus and 18 with severe tinnitus. Fig. 1 shows that mild tinnitus patients showed significantly higher plasma levels of BDNF than severe tinnitus patients (1.321.9 ± 1266.1 vs. 385.1 ± 524.9 pg/mL; $P < 0.01$) and controls ($P < 0.01$; Fig. 1). Plasma BDNF levels were negatively correlated with HADS scores ($R = -0.35$, $P < 0.05$), while THI and HADS scores were positively correlated ($R = 0.55$ $P < 0.0001$). After adjusting for

Table 1
THI scores and plasma BDNF levels in patients with different mood statuses.

THI scores and plasma BDNF levels in patients with different mood statuses.

		Patients with tinnitus	
	controls	HADS≤14	HADS≥15
THI	N.A.	25.9 ± 16.3	49.5 ± 23.5
			**
BDNF	338.5 ± 287.7	1092.1 ± 1157.6	474.6 ± 634.1
			*
HADS	7.8 ± 5.4		14.5 ± 7.5
		***	

*P<0.05, **P<0.01, ***P<0.001

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

possible effects of HADS scores, partial correlation coefficients for BDNF levels and THI scores indicated that there was no relationship between BDNF levels and THI scores. As shown in Table 1, patients with HADS scores of ≤14 had significantly lower THI scores ($P < 0.05$) and higher BDNF values ($P < 0.01$) than patients with HADS scores of ≥15.

4. Discussion

Tinnitus is a phantom auditory perception that is associated with hearing loss and altered neuronal excitability in peripheral and central auditory neurons [24]. BDNF is affected by changes in excitability and plasticity and is involved in neuronal survival and differentiation. BDNF is a key player in the mechanism of onset and persistence of salicylate-induced tinnitus. Increased BDNF expression in spiral ganglion neurons and increased spontaneous activity in the cochlear nerve and dorsal cochlear nucleus have been reported in a salicylate-induced tinnitus model [5]. Salicylate-induced upregulation of BDNF in the spiral ganglion neurons may result in an imbalance of central auditory neuronal activity, which is associated with tinnitus.

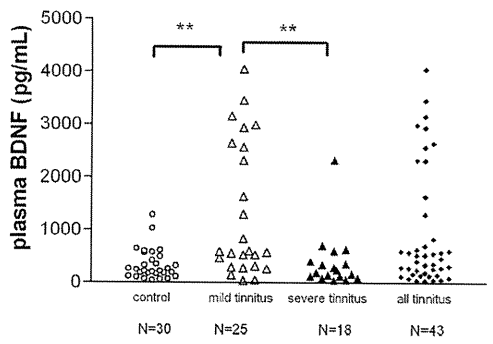

Fig. 1. Plasma BDNF levels in controls, patients with mild tinnitus, and patients with severe tinnitus. Plasma BDNF levels are significantly higher in patients with mild tinnitus. Both normal controls and patients with severe tinnitus showed low plasma BDNF levels. **$P < 0.01$.

To verify whether BDNF-related mechanisms are involved in tinnitus, we investigated whether peripheral circulating BDNF levels differ between patients with tinnitus and healthy patients. THI was developed to quantify the symptoms of tinnitus [21], so THI scores were used to evaluate tinnitus. Our results indicate that both normal controls and severely affected tinnitus patients had low plasma BDNF levels (Fig. 1). Interestingly, about 50% of the patients had low plasma BDNF levels, even mildly distressed patients (Fig. 1). Whether plasma BDNF levels are sensitive enough to serve as clinical biomarkers in tinnitus patients remains to be investigated. Future research will therefore focus on conditions that may affect BDNF levels. Since hearing levels were not correlated with the plasma BDNF levels in this study, elevated BDNF levels are unlikely to be linked to cochlear function. Tinnitus is likely associated with many parts of the central nervous system, in contrast to hearing function, which is associated with discrete CNS components.

BDNF is found in both serum and plasma in humans [9]. Human platelets contain a large amount of BDNF [9]. As a consequence, serum levels of BDNF are about 200-fold higher than plasma levels [27]. Since BDNF crosses the blood–brain barrier (BBB) in both directions, circulating BDNF might originate from neurons and glial cells in the brain [23]. Therefore, plasma BDNF may reflect circulating levels rather than BDNF stored in platelets.

Meta-analyses showed a reduction in both serum and plasma BDNF levels in major depression [4,6,29]. Previous studies have reported that serum BDNF levels are significantly lower in drug-free patients with major depression [14]. Karege et al. reported that both

serum and plasma BDNF levels were significantly lower in patients with major depression than in normal controls [14]. In addition, previous studies found lower serum BDNF levels in patients with higher Beck Depression Inventory (BDI) [1] sum scores [7].

It is unclear whether altered plasma BDNF levels are primary or secondary signs in patients with mild tinnitus. Changes in BDNF expression subsequent to the onset of tinnitus have been reported. BDNF is affected by changes in excitability, and plasticity and is one of the key players in the onset and persistence of salicylate-induced tinnitus [24]. Therefore, increased plasma BDNF levels in mild tinnitus patients may reflect increased BDNF levels in the central auditory system. In severe tinnitus patients, the relative reduction in plasma BDNF can be explained by stress-induced reduction of BDNF in the central nervous system. There was no correlation between THI scores and plasma BDNF levels after adjustment for HADS scores. BDNF levels may be more likely to be related to HADS scores than to THI scores, although our data cannot prove this. The fact that elevated BDNF levels occur only in patients with mild tinnitus is interesting. The limitation of the present study is that we evaluated tinnitus on the basis of THI scores alone. We did not measure tinnitus loudness in patients. If we had measured tinnitus loudness and tested for a correlation between tinnitus loudness and BDNF, we may have determined whether the plasma BDNF levels reflect auditory function. THI scores alone do not provide sufficient information to adequately determine the relationship between tinnitus and plasma BDNF levels. Psychiatric comorbidities, especially depression and anxiety disorders, are common phenomena in tinnitus patients. Our subjects did not have psychiatric diagnoses at the time of evaluation. However, the HADS scores indicated that the mood status in our patients differed from that of healthy subjects. Patients with HADS scores of ≥15 had lower plasma BDNF levels than those with HADS scores of ≤14 (Table 1). This result can be explained by the effect of mood of the patients. There is no simple explanation for the observed changes in plasma BDNF; however, these changes can be partly attributed to the effect of mood, including depression and anxiety.

It is unlikely that tinnitus and depression coexist by chance. Instead, these conditions represent a complex interplay between tinnitus and depression [18]. Whether altered BDNF levels are a result of depression caused by tinnitus or are due to the tinnitus alone is still not known.

We should therefore carefully consider the implications of the changes in BDNF levels in patients with mild tinnitus.

From a clinical point of view, it is important to note that in tinnitus patients, their suffering is frequently linked to concomitant depressive symptoms. Improvement of depression is paralleled by improvements in functional ability [16]. It is therefore important that clinicians who treat tinnitus patients are observant of comorbid psychiatric symptoms, especially depression and anxiety. It is especially important that these clinicians consider affective symptoms when providing treatment for tinnitus.

Whether plasma BDNF levels reflect dynamic changes in BDNF levels in the CNS is debatable. Intact BDNF in the peripheral circulation crosses the BBB using a high-capacity, saturable transport system [23]. Brain and serum BDNF levels undergo similar changes during maturation and aging processes in rats [15]. In addition, there is a significant positive correlation between plasma and cerebrospinal fluid (CSF) BDNF levels in psychiatric patients [26]. These results indicate that plasma BDNF concentrations reflect dynamic changes in the brain. Plasma BDNF levels may be used to objectively evaluate tinnitus severity and may assist in the identification of comorbid psychiatric disorders, including depression. Treatment of comorbid psychiatric disorders can substantially reduce the burden of the disease and improve the quality of life of individuals with tinnitus.

5. Conclusion

The results of our study suggest that plasma BDNF levels are higher in patients with mild tinnitus than in healthy controls. Our findings show for the first time that changes in peripheral levels of BDNF occur in patients suffering from different levels of tinnitus, suggesting a potential involvement of BDNF in the severity of tinnitus. These results suggest that differences in peripheral BDNF levels may help distinguish patients with mild tinnitus from healthy subjects. Further study is required to identify the possible physiological mechanisms responsible for altered BDNF levels in patients with tinnitus.

Conflict of interest

The authors declare no conflicting financial interests or commercial considerations related to the issues presented herein.

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References

[1] A.T. Beck, C.H. Ward, M. Mendelson, J. Mock, J. Erbaugh, An inventory for measuring depression, Arch. Gen. Psychiatry 4 (1961) 561–571.
[2] S. Belli, H. Belli, T. Bahcebasi, A. Ozcetin, E. Alpay, U. Ertem, Assessment of psychopathological aspects and psychiatric comorbidities in patients affected by tinnitus, Eur. Arch. Otorhinolaryngol. 265 (2008) 279–285.
[3] D.K Binder, H.E. Scharfman, Brain-derived neurotrophic factor, Growth Factors 22 (2004) 123–131.
[4] L. Bocchio-Chiavetto, V. Bagnardi, R. Zanardini, R. Molteni, M.G. Nielsen, A. Placentino, C. Giovannini, L. Rillosi, M. Ventriglia, M.A. Riva, M. Gennarelli, Serum and plasma BDNF levels in major depression: a replication study and meta-analyses, World J. Biol. Psychiatry 11 (2010) 763–773.
[5] T.J. Brozoski, C.A. Bauer, D.M. Caspary, Elevated fusiform cell activity in the dorsal cochlear nucleus of chinchillas with psychophysical evidence of tinnitus, J. Neurosci. 22 (2002) 2383–2390.
[6] A.R. Brunoni, M. Lopes, F. Fregni, A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression, Int. J. Neuropsychopharmacol. 11 (2008) 1169–1180.
[7] B.A. Bus, I. Tendolkar, B. Franke, J. de Graaf, M.D. Heijer, J.K. Buitelaar, R.C. Oude Voshaar, Serum brain-derived neurotrophic factor: determinants and relationship with depressive symptoms in a community population of middle-aged and elderly people, World J. Biol. Psychiatry (2011) 19.
[8] B.S. Fernandes, C.S. Gama, K. Maria Cereser, L.N. Yatham, G.R. Fries, G. Colpo, D. de Lucena, M. Kunz, F.A. Gomes, F. Kapczinski, Brain-derived neurotrophic factor as a state-marker of mood episodes in bipolar disorders: a systematic review and meta-regression analysis, J. Psychiatr. Res. 45 (2011) 995–1004.
[9] H. Fujimura, C.A. Altar, R. Chen, T. Nakamura, T. Nakahashi, J. Kambayashi, B. Sun, N.N. Tandon, Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation, Thromb. Haemost. 87 (2002) 728–734.
[10] F. Goto, T. Tsutsumi, K. Ogawa, Anxiety and depression levels measured using a hospital anxiety and depression scale in patients with an otolaryngological disorder, Pract. Otorhinolaryngol. 102 (2009) 1071–1075.
[11] J. Harrop-Griffiths, W. Katon, R. Dobie, C. Sakai, J. Russo, Chronic tinnitus: association with psychiatric diagnoses, J. Psychosom. Res. 31 (1987) 613–621.
[12] K.C. Horner, The emotional ear in stress, Neurosci. Biobehav. Rev. 27 (2003) 437–446.
[13] J.A. Kaltenbach, Neurophysiologic mechanisms of tinnitus, J. Am. Acad. Audiol. 11 (2000) 125–137.
[14] F. Karege, G. Bondolfi, N. Gervasoni, M. Schwald, J.M. Aubry, G. Bertschy, Low brain-derived neurotrophic factor (BDNF) levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity, Biol. Psychiatry 57 (2005) 1068–1072.
[15] F. Karege, M. Schwald, M. Cisse, Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets, Neurosci. Lett. 328 (2002) 261–264.
[16] W. Katon, M. Sullivan, J. Russo, R. Dobie, C. Sakai, Depressive symptoms and measures of disability: a prospective study, J. Affect Disord. 27 (1993) 245–254.
[17] B. Langguth, R. Goodey, A. Azevedo, A. Bjorne, A. Cacace, A. Crocetti, L. Del Bo, D. De Ridder, I. Diges, T. Elbert, H. Flor, C. Herraiz, T. Ganz Sanchez, P. Eichhammer, R. Figueiredo, G. Hajak, T. Kleinjung, M. Landgrebe, A. Londero, M.J. Lainez, M. Mazzoli, M.B. Meikle, J. Melcher, J.P. Rauschecker, P.G. Sand, M. Struve, P. Van de Heyning, P. Van Dijk, R. Vergara, Consensus for tinnitus patient assessment

and treatment outcome measurement: tinnitus research initiative meeting, Regensburg, July 2006, Prog. Brain Res. 166 (2007) 525–536.
[18] B. Langguth, M. Landgrebe, T. Kleinjung, G.P. Sand, G. Hajak, Tinnitus and depression, World J. Biol. Psychiatry 12 (2011) 489–500.
[19] M. Lommatzsch, D. Zingler, K. Schuhbaeck, K. Schloetcke, C. Zingler, P. Schuff-Werner, J.C. Virchow, The impact of age, weight and gender on BDNF levels in human platelets and plasma, Neurobiol. Aging 26 (2005) 115–123.
[20] A.R. Moller, B. Langguth, D. De Ridder, T. Kleinjung, Chapter 5 epidemiology of tinnitu in adults, in: A.R. Moller (Ed.), Textbook of Tinnitus, Springer, New York, 2011, pp. 29–38.
[21] C.W. Newman, G.P. Jacobson, J.B. Spitzer, Development of the tinnitus handicap inventory, Arch. Otolaryngol. Head Neck Surg. 122 (1996) 143–148.
[22] C.W. Newman, S.A. Sandridge, G.P. Jacobson, Psychometric adequacy of the Tinnitus Handicap Inventory (THI) for evaluating treatment outcome, J. Am. Acad. Audiol. 9 (1998) 153–160.
[23] W. Pan, W.A. Banks, M.B. Fasold, J. Bluth, A.J. Kastin, Transport of brain-derived neurotrophic factor across the blood–brain barrier, Neuropharmacology 37 (1998) 1553–1561.
[24] R. Panford-Walsh, W. Singer, L. Ruttiger, S. Hadjab, J. Tan, H.S. Geisler, U. Zimmermann, I. Kopschall, K. Rohbock, A. Vieljans, E. Oestreicher, M. Knipper, Midazolam reverses salicylate-induced changes in brain-derived neurotrophic factor and arg3.1 expression: implications for tinnitus perception and auditory plasticity, Mol. Pharmacol. 74 (2008) 595–604.
[25] A. Piccinni, D. Marazziti, M. Catena, L. Domenici, A. Del Debbio, C. Bianchi, C. Mannari, C. Martini, E. Da Pozzo, E. Schiavi, A. Mariotti, I. Roncaglia, A. Palla, G. Consoli, L. Giovannini, G. Massimetti, L. Dell'Osso, Plasma and serum brain-derived neurotrophic factor (BDNF) in depressed patients during 1 year of antidepressant treatments, J. Affect Disord. 105 (2008) 279–283.
[26] A. Pillai, A. Kale, S. Joshi, N. Naphade, M.S. Raju, H. Nasrallah, S.P. Mahadik, Decreased BDNF levels in CSF of drug-naive first-episode psychotic subjects: correlation with plasma BDNF and psychopathology, Int. J. Neuropsychopharmacol. 13 (2010) 535–539.
[27] R.D. Rosenfeld, L. Zeni, M. Haniu, J. Talvenheimo, S.F. Radka, L. Bennett, J.A. Miller, A.A. Welcher, H. Fujimura, C.A. Altar, R. Chen, T. Nakamura, T. Nakahashi, J. Kambayashi, B. Sun, N.N. Tandon, Purification and identification of brain-derived neurotrophic factor from human serum Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation, Protein Expr. Purif. 6 (1995) 465–471.
[28] P.G. Sand, B. Langguth, T. Kleinjung, P. Eichhammer, Genetics of chronic tinnitus, Prog. Brain Res. 166 (2007) 159–168.
[29] S. Sen, R. Duman, G. Sanacora, Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications, Biol. Psychiatry 64 (2008) 527–532.
[30] J. Tan, L. Ruttiger, R. Panford-Walsh, W. Singer, H. Schulze, S.B. Kilian, S. Hadjab, U. Zimmermann, I. Kopschall, K. Rohbock, M. Knipper, Tinnitus behavior and hearing function correlate with the reciprocal expression patterns of BDNF and Arg3.1/arc in auditory neurons following acoustic trauma, Neuroscience 145 (2007) 715–726.
[31] R.S. Tyler, C. Coelho, W. Noble, Tinnitus: standard of care, personality differences, genetic factors, ORL J. Otorhinolaryngol. Relat. Spec. 68 (2006) 14–19, discussion 20–12.
[32] J. Unterrainer, K.V. Greimel, M. Leibetseder, T. Koller, Experiencing tinnitus: which factors are important for perceived severity of the symptom? Int. Tinnitus J. 9 (2003) 130–133.
[33] A.S. Zigmond, R.P. Snaith, The hospital anxiety and depression scale, Acta Psychiatr. Scand. 67 (1983) 361–370.
[34] S. Zoger, J. Svedlund, K.M. Holgers, Psychiatric disorders in tinnitus patients without severe hearing impairment: 24 month follow-up of patients at an audiological clinic, Audiology 40 (2001) 133–140.

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Efficacy of a Vibrotactile Neurofeedback Training in Stance and Gait Conditions for the Treatment of Balance Deficits: A Double-Blind, Placebo-Controlled Multicenter Study

*Dietmar Basta, †Marcos Rossi-Izquierdo, ‡Andrés Soto-Varela, §Mario Edwin Greters, §Roseli Saraiva Bittar, ||Elisabeth Steinhagen-Thiessen, ||Rahel Eckardt, ¶Tatsuhiko Harada, #Fumiyuki Goto, **Kaoru Ogawa, and *Arne Ernst

**Department of Otolaryngology at UKB, Hospital of the University of Berlin, Charité Medical School, Berlin, Germany; †Department of Otolaryngology, University Hospital Lucus Augusti, Lugo; ‡Department of Otolaryngology, University Hospital of Santiago de Compostela, Santiago de Compostela, Spain; §Department of Otolaryngology, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil; ||Geriatrics Research Group, Charité Medical School, Berlin, Germany; ¶Department of Otolaryngology, International University of Health and Welfare, Atami; #Department of Otolaryngology, Hino Municipal Hospital; and **Department of Otolaryngology, Keio University, School of Medicine, Tokyo, Japan*

Objective: Vestibular rehabilitation strategies mostly require a long-lasting training in stance conditions, which is finally not always successful. The individualized training in everyday-life conditions with an intuitive tactile neurofeedback stimulus seems to be a more promising approach. Hence, the present study was aimed at investigating the efficacy of a new vibrotactile neurofeedback system for vestibular rehabilitation.
Study Design: Double-blinded trial.
Patients: One hundred five patients who experience one of the following balance disorders for more than 12 months were included in the study: canal paresis, otolith disorder, removal of an acoustic neuroma, microvascular compression syndrome, Parkinson's disease, and presbyvertigo.
Interventions: Vibrotactile neurofeedback training was performed daily (15 min) over 2 weeks with the Vertiguard system in those 6 tasks of the Standard Balance Deficit Test with the most prominent deviations from the normative values.
Main Outcome Measures: Trunk and ankle sway, dizziness handicap inventory, and vestibular symptom score were measured in the verum and placebo group before the training, on the last training day and 3 months later.
Results: A significant reduction in trunk and ankle sway as well as in the subjective symptom scores were observed in the verum group. Such an effect could not be found in any of the outcome parameters of the placebo group.
Conclusion: The vibrotactile neurofeedback training applied in the present study is a highly efficient method for the reduction of body sway in different balance disorders. Because the rehabilitation program is easy to perform, not exhausting, and time saving, elderly patients and those with serious, long-lasting balance problems also can participate successfully. **Key Words:** Neurofeedback—Postural control—Vestibular rehabilitation—Vibrotactile.
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Numerous diseases are accompanied by balance deficits, which are frequently characterized by an increase in body sway and a higher risk to fall. Different strategies in the conservative management of those balance deficits have been applied successfully over the last few decades to improve central compensation of the tonus imbalance within the vestibular system and to facilitate substitution (1) in different types of peripheral or central vestibular disorders (2,3). Various exercise programs (home or supervised) have been described, including physical training (4), Cawthorne-Cooksey interventions (5), and alternative strategies—such as Tai Chi (6).

Address correspondence and reprint requests to Dietmar Basta, Ph.D., Department of Otolaryngology at UKB, Warener Str. 7, 12683 Berlin, Germany; E-mail: dietmar.basta@ukb.de

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However, these vestibular rehabilitation strategies mostly require a long-lasting, intensive training approach (i.e., over several weeks or even months) which is finally not always successful. Current studies have shown that rehabilitation strategies including a sensory feedback signal could be much more effective. The first feedback applications consisted of stance tasks with visual feedback (3,7,8), galvanic feedback (9,10), or vibrotactile feedback (11,12). Because patients tend to fall mostly in dynamic (i.e., movement) conditions, those stance tasks in balance rehabilitation should be accompanied by gait (or dynamic) tasks including daily-life situations. Earlier studies showed a high effectiveness of a free-field auditory neurofeedback training to reduce the body sway in patients with different peripheral vestibular disorders (13–15). This auditory neurofeedback application, however, is limited to the laboratory situation and those patients with good hearing (which is frequently not the case in the elderly or patients with a vestibular disorder). Therefore, an intuitive tactile neurofeedback stimulus could be superior for encoding of the individual sway information during the training of everyday-life conditions.

Hence, the present study was aimed at investigating the efficacy of a newly developed method for vestibular rehabilitation with a vibrotactile neurofeedback system.

MATERIALS AND METHODS

Patients' Characteristics

Patients which chronically experienced dizziness (longer than 12 mo) were recruited within 17 months from neuro-otologic or neurologic clinics.

The inclusion criteria to participate in the neurofeedback training program was a pathologic body sway (measured at the hip in pitch and roll direction) compared with normal age- and sex-related controls as recorded by the diagnostic, mobile posturography device Vertiguard-D (Vesticure GmbH, Germany). The pathologic sway should be found in at least one of the test conditions of the Standard Balance Deficit Test (SBDT) (15) or the Geriatric Standard Balance Deficit Test (gSBDT) (manual Vertiguard, Vesticure GmbH).

The SBDT contained the following 14 tasks: standing on 2 legs with eyes open/closed, standing on 1 leg with eyes open/closed, 8 tandem steps (1 foot in front of the other) with eyes open, standing with 2 legs on a foam support surface (height, 10 cm; density, 25 kg/m^3) with eyes open/closed, standing on 1 leg on a foam support surface, 8 tandem steps on a foam support surface, walking 3 m while rotating the head, walking 3 m while vertically pitching the head in rhythm, walking 3 m forward with eyes open/closed, and walking over 4 barriers (height of 26 cm with an interbarrier distance of 1 m).

The following tasks were skipped in the gSBDT (for patients older than 59 yr): standing on 1 leg with eyes closed and standing on 1 leg on a foam support surface.

The tasks "stand up" and "sit down" were added as last conditions to the gSBDT.

The recording time was 20 seconds for all stance tasks and as long as required for gait tasks (mostly <20 s).

Exclusion criteria for the study were as follows: the use of drugs, which actively influence the vestibular system (e.g., cinnarizine, dimenhydrinate, betahistidine); sensory deficits exceeding age-related values (e.g., auditory symptoms, blurred vision, anosmia); a combination of different types of vestibular disorders in 1 patient (e.g., canal paresis and otolith disorder) because 1 important aim of the present study was to investigate a possible correlation between the efficacy of the training and a specific vestibular disorder; an acute vestibular disorder (due to World Health Organization definition); and all included patients received no other treatment (whether medical, surgical, or rehabilitative) for their balance disorder during the study period.

Of the 132 patients who experienced dizziness or instability, 27 were excluded from the study. Seven of them showed a combination of different types of vestibular disorders; 4 had sensory deficits, which exceeded the normal age-related values (auditory symptoms); and 16 patients showed no pathologic body sway within the SBDT. In total, 105 patients were included in this study. The sample contained patients with 6 different peripheral or central balance disorders, including the following (for details, see Table 1): unilateral canal paresis (semicircular canal paresis [SCC]); otolith disorder (O), that is, unilateral or bilateral loss of sacculo-utricular function; patients after removal of an acoustic neuroma (AN) with resection of the vestibular nerve; micro(neuro) vascular compression syndrome (MVCS) of the VIIIth cranial nerve; Parkinson's disease (PD); and presbyvertigo (P), that is, patients older than 59 years with no specific vestibular deficit but an increase in body sway as result of this complex disorder.

Patients with an otolith disorder showed a combined sacculo-utricular dysfunction. All the vestibular tests were applied to all patients to exclude an overlapping of group-specific pathologies. Pathologic findings in the vestibular testing of the same side as affected by an AN or a micro(neuro) vascular compression were related to the disorder of the VIIIth nerve function. The vestibular test battery contained the following procedures: caloric testing (pathologic results: side differences of more than 15% [slow phase velocity]); cervical vestibular evoked myogenic potentials (pathologic results: absence of N1/P1 even if the required tonic muscle activity was achieved); and subjective haptic vertical (pathologic results: side asymmetry or difference to the vertical of more than 10 degrees).

TABLE 1. *Characterization of treatment subgroups and classification criteria*

Subgroup	Age	n	Female	Male	Classification criteria
Semicircular canal function loss	60.2 + 13.6	25	10	15	Pathologic results during caloric irrigation
Otolith disorder	54.6 + 13.8	21	10	11	Pathologic vestibular evoked myogenic potentials or subjective haptic vertical
Acoustic neuroma removal	60.2 + 10.1	10	4	6	Surgical removal of an acoustic neuroma
Microvascular compression syndrome	52.0 + 10.8	12	6	6	Radiographic defined 8. Nerve-anterior inferior cebellar artery contact
Parkinson's disease	68.1 + 9.1	10	2	8	Idiopathic type
Presbyvertigo	73.4 + 6.0	13	6	7	Dizzy elderly patients (>59 yr) without a vestibular disease

After calculating the minimal sample size for the control (placebo) group by using the software G*Power 3.1.2 (University Kiel, Germany) (16) with an effect size of 0.9, $p = 0.05$, and a statistical power of 0.8, 14 patients were randomly selected from the initial study sample of 105 patients under consideration of the initial distribution of balance disorders and sex. Six female subjects (64.0 ± 9.6 yr) and 8 male subjects (58.8 ± 8.5 yr) were included in the control group with the following distribution of balance disorders: SCC, 28.6%; O, 21.4%; AN, 14.3%; MVCS, 14.3%; PD, 7.1%; and P, 14.3%.

All other patients were included in the treatment group. This group contained 91 patients (39 female subjects—59.1 ± 14.1 yr; 52 male subjects—61.7 ± 12.7 yr). The distribution of balance disorders and sex was similar to that of the control group. Table 1 shows the details of the treatment subgroups and the classification criteria.

Vestibular Rehabilitation Training

The training was performed using the Vertiguard training device (Vesticure GmbH). It contains a battery-driven main unit (120 × 76 × 32 mm, 190 g) which is fixed on a belt at the center of body mass (hip) and 1 vibration stimulator on the front, back, left, and right side, respectively (Fig. 1). The vibration stimulators are mounted on the same belt as the main unit. They are adjustable by sliding them over the belt into the correct position of the individual patient. The main unit continuously records the Coriolis force during body movements in pitch and roll by inbuilt gyroscopes and compares those values with individually preset thresholds for the stimulator activation in the specific direction. Preset thresholds were task specific. They were determined for the individual patient based on the maximum age- and sex-related normative sway in the specific SBDT condition and sway direction. The thresholds were stored for each training task in the main unit. Training tasks were selected automatically by analyzing the results of the SBDT or gSBDT. Only those 6 tasks with the most prominent deviations from the normative values were included in the training program. The number of training tasks was limited by the device capacity. This is related to the assumption that almost all patients show a pathologic sway in not more than 6 conditions of the SBDT. The patient was able to switch between the tasks by pressing a button on the main unit. To prevent the selection of wrong thresholds for the performed task, the chosen task was shown together with the patient's name in the display of the main unit. No vibrotactile feedback stimulus was delivered via the stimulators if the patients' sway were below the preset thresholds. In contrast to this, if the body sway exceeded the thresholds, the perceived vibration was increased with the amplitude of body sway. The patients were instructed daily by a nurse to adjust the vibratory stimulation step by step (within a scale of 10 steps) at the beginning of each selected training task by pressing the sensitivity buttons (up/down) on the main unit. During this procedure, the individual preset thresholds were similarly decreased for all sway directions of the specific training condition until the patient perceived a vibration during performing the training task.

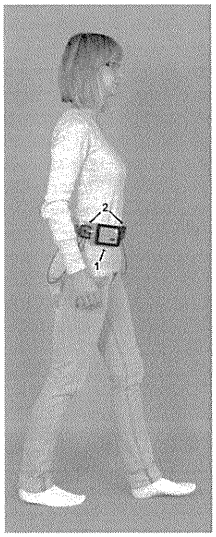

FIG. 1. Application of the vibrotactile neurofeedback system Vertiguard for the treatment of balance disorders. The system with the main unit (1) and the vibration stimulators (2) is fixed on a belt at the center of body mass. Only 2 of the 4 vibration stimulators are visible in the picture.

Vestibular rehabilitation exercises were performed daily over a 2-week period with 10 days of exercising (weekends excluded). Each session contained 5 repetitions of the selected tasks. The time limit for 1 repetition was 20 seconds for all stance tasks and as long as needed for gait tasks (similar to the recording time of the SBDT/gSBDT). The total daily training time was approximately 15 to 20 minutes.

Patients of the control group performed the similar protocol with a sham device (emitting randomly assigned signals to the vibrators). The patients as well as the supervisor did not know the group classification (double-blinded study design).

Evaluation of the Effects of the Vestibular Rehabilitation

Trunk sway of the patients was measured in pitch and roll for each exercise task (without feedback) before and after the training by means of the Vertiguard D system (Vesticure GmbH). The results were averaged across all tasks.

The composite score of the sensory organization test (SOT) of the BalanceMaster (Nicolet Biomedical, Clackamas, OR, USA) ankle-sway referenced system (platform), the dizziness handicap inventory (DHI) (17), and the vestibular symptom score (VSS) (18) were obtained before the training, on the last training day, and 3 months later.

Objective measures of trunk sway (pitch and roll) and ankle sway (SOT composite score) were used as primary end points. The SOT composite score is scored between zero (fall) and 100 (maximum stability). The results of questionnaires (DHI and VSS) were classified as secondary end points. Lowering of DHI or VSS scores indicate a decline of handicaps or symptoms. Primary and secondary end points were statistically compared in the treatment group (also for all subgroups) and placebo group before and after the rehabilitation period by the t test for dependent samples or the Wilcoxon's test (depending on data distribution). The similar tests were used for the comparison between the results of all investigated parameters before the training and after the follow-up (SPSS 11.0). A Bonferroni alpha correction was applied for multiple comparisons. In the case of missing values, the patient was excluded from the analysis of the related parameters. Data were tested for a normal distribution by the Kolmogoroff-Smirnoff test.

The statistical power and effect size was determined by post hoc calculations for each comparison with the software G*Power 3.1.2 (University Kiel, Germany) (16). Statistical power estimates