Endolymphatic sac tumours and von Hippel-Lindau disease – case report, molecular analysis and histopathological characterization

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Abstract

Endolymphatic sac tumours (ELST) are aggressive papillary tumours of the temporal bone. The name was finally determined after the endolymphatic sac was determined as the site of their origin. They should be considered in patients with tumours eroding the petrous part of the temporal bone, extending to the cerebellopontine angle or other adjacent structures. These very rare tumours in the general population have much higher prevalence in von Hippel-Lindau disease. Hence molecular analysis of the VHL gene should be performed in patients with ELST and their relatives.
The purpose of this study is to present a case report, histopathological characterization of endolymphatic sac tumours, their association with von Hippel-Lindau disease and use of molecular analysis.

Key words: *ELST, VHL, cerebellopontine tumours.*

Introduction

Endolymphatic sac tumours (ELST) are very rare neuroectodermal tumours of the temporal bone. The probable site of origin of the tumours was not clearly established until a study by Hassard et al. in 1984 [14] and two following reports of light, electron microscopic and immunohistochemical studies [15,27]. Li et al. classified them as endolymphatic sac tumours and used the acronym ELST [25]. The tumour has not been described in Polish literature to our knowledge but one can assume that some reported adenomatous and/or papillary tumours of the temporal bone described before as choroid plexus papillomas, low grade papillary adenomas, some middle ear adenomas invading the temporal bone, adenocarcinomas or other tumours of the region could actually represent ELST.

Von Hippel-Lindau (VHL) disease is an inherited tumour predisposing syndrome. It has been reported that among other tumours ELST may be one of the characteristic neoplasms of the disease [11,22,44].

This report presents ELST and describes the association of this tumour with von Hippel-Lindau disease.

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Case report

Patient's medical history and family history

A 32-year old man was invited to our centre for a consultation at the Polish VHL Registry as a member of a family with suspected von Hippel-Lindau disease (VHL). He had a history of left facial nerve palsy at the age of 19 years due to tumour of the temporal bone. The patient was operated on a few months later and a histopathological diagnosis of choroid plexus papilloma was made. Two years later he was reoperated on due to recurrence and treated with adjunctive radiotherapy. Three years later the patient developed ataxia and was treated surgically because of a cerebellar tumour that was histopathologically diagnosed as haemangioblastoma. Due to recurrence of this tumour the patient was reoperated on two years later. He also had a history of retinal capillary angioma treated by cryo- and laser therapy.

Although many of the patient's family members developed lesions in sites suggesting VHL including multiple central nervous system tumours (probable haemangioblastomas), renal tumours (probable renal cell carcinomas), pancreatic tumours (probable cysts and neuroendocrine tumours), temporal bone tumours (probable ELST), and some of the family members have lost their sight because of retinal detachment (probably in the case of retinal haemangiomas), the diagnosis of VHL has not been established in the family.

VHL was highly suspected in the patient and thus histopathological slides of temporal bone tumour were re-examined and then followed by DNA molecular testing.

Histopathological findings

Examination of the patient's histopathological slides of the temporal bone specimen revealed a tumour of mixed structure. A single layer of cuboidal to low columnar epithelial cells arranged in papillary patterns and disposed in a fibrous stroma was found in some areas (Fig. 1), whereas in others a more adenoid cytostructure with flattened cells arranged in a glandular pattern and enclosing spaces containing proteinaceous material was found (Fig. 2). The latter structure was reminiscent of thyroid follicles. The tumour was infiltrative and poorly circumscribed, no necrosis was noted and mitotic figures were very rarely present. On the basis of the above features the diagnosis of ELST was established.

Molecular testing

To detect VHL gene mutation peripheral blood leukocytes obtained from the patient were processed for DNA isolation using standard methods. PCR products were generated with pairs of primers as previously described. SSCP analysis was performed on the Phast System (Farmacia Biotech) at 4°C according to the manufacturer's protocol. Long PCR was also done as described before [5]. TaqDyeDeoxy Terminator Cycle Sequencing (ABI, USA) was performed with three sets of primers: #3 and #101, #102 and #103, #107 and #6 for exons I, II, III respectively using Ready Reaction Kit (Perkin Elmer, Part No. 402122) according to the supplier's sequencing protocol. The products of sequencing reactions were analyzed with an automated sequencer (model 373A, ABI, USA).

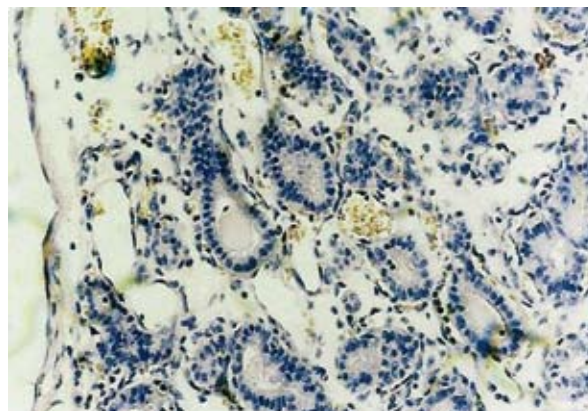**Fig. 1.** Papillary cytostructure of ELST

Fig. 2. Adenoid cytostructure of ELST

The 407 C to T VHL gene mutation was detected in the patient. The molecular analysis of blood samples of two of his children also revealed the mutation.

Discussion

Classification

Since the first report by Treiter in 1898 there have been many attempts to categorize adenomatous and papillary tumours of the temporal bone [45]. Many sites of their origin have been postulated: apocrine glands of the external auditory canal, ectopic minor salivary glands, glands of the middle ear, indigenous middle ear mucosa epithelium, choroid plexus papilloma, to mention only some of many [9,13,19,20,29,42,43]. Different names of the tumours have also been used, e.g. "ceruminoma of cerebellopontine angle", "adenoid cystic adenoma", "pleomorphic adenoma", "adenocarcinoma of temporal bone", "aggressive papillary middle ear/temporal bone tumour (APMET)", to mention some of them [10,28,33]. In the last decade it has become more widely accepted to classify the tumours into two groups: middle ear adenomas (MEA), restricted to the middle ear, and more aggressive and papillary endolymphatic sac tumours (ELST) [11,28]. After Hassard published a case of adenomatous tumour of the endolymphatic sac [14] the concept of endolymphatic sac origin of adenomatous, papillary tumours of the temporal bone was proposed by MacDougall et al. [27], which was supported by Heffner's paper [15]. In 1989 Li et al. 1993 finally classified the tumours as ELST [25]. This nomenclature has since been widely accepted and used [26,28,38,39].

Anatomical features and pathogenesis

The endolymphatic sac is part of a membranous labyrinth. It plays an important role in inner ear homeostasis probably by resorption of endolymph. The endolymphatic sac is lined by a mucus epithelium of neuroectodermal origin. It consists of two parts. The proximal portion called pars rugose is a continuation of the endolymphatic duct and lies intraosseously within the vestibular canal. The lumen of this portion consists of folded tubular structures with ducts, sinuses and multiple recesses. The distal smooth portion lies extraosseously in a dural duplication of the posterior surface of the petrous part of the temporal bone [41].

Most authors agree that ELST arise from a proximal "rugose" part of the endolymphatic sac; however, Roche et al. suggested that in one of their three cases the tumour developed from the distal part of the sac in the jugular foramen area [14,28,39]. The location of the site of origin of ELSTs and their potential infiltrative character make it possible for them to destroy the temporal bone, and extend posteriorly to the posterior cranial fossa (mainly the cerebellopontine angle), anteriorly to the middle cranial fossa, petroclival area, cavernous sinus, laterally to the mastoid, middle and external ear, and inferiorly to involve the skull base in the region of the jugular foramen [36,40].

The location and growth extension of the tumour determine the clinical course and symptoms in affected patients. Otological symptoms may include: unilateral or bilateral hearing loss, tinnitus and/or vertigo. Some patients complain of symptoms resembling Ménière's disease. Intracranial extension and temporal bone destruction can cause ataxia, disequilibrium, headache, nausea and vomiting. More extensive growth can cause nystagmus and pyramidal defects. Cranial involvement may include V, VII, IX, X or XI nerve with relevant symptoms and signs [26,30,38,39,36,40].

Histopathological features

Macroscopically the tumour is described as red hypervascular, red or bluish tissue of generally soft consistency or in parts that contain bone more firm. Microscopically as described in our case two patterns of cytoarchitecture may prevail: glandular and papillary. In the first glandular pattern cysts filled with colloid material are characteristic. They are surrounded by a single layer of epithelial cells that are arranged in follicular configurations. The cells are cuboidal, slightly flattened, mostly clear, sometimes eosinophilic with ovoid nuclei. The colloidal material filling the cysts stains strongly with PAS. The similarity to thyroid tissue is striking.

The second more solid pattern is papillary. Large areas of clear cytoplasm cells with central nuclei can resemble renal carcinoma cells. Both patterns of ELST cytoarchitecture can overlap in some areas. In both patterns stromal tissue is well vascularized. Mitotic figures are very rare, necrosis is absent and pleomorphism is minimal.

ELST can contain fragments of bone with smooth margins but with no aggressive growth or high oste-

oclastic activity. It has been postulated that bone destruction is caused mainly by bleeding within the tumour and bone foreign body cell reaction against hemosiderin and cholesterol crystals. Giant cells of foreign body type and sideroblasts have been reported in an ELST specimen [7]. Cholesterol granulomas can also develop because of tumour obstruction of petrous cells and impairment of their ventilation.

It has been reported in the literature that electron microscopy of ELST reveals microvilli whose presence is also a feature of a normal endolymphatic sac [1,11,26]. No typical cilia with 9 and 2 tubules arrangement in ELST or the endolymphatic sac can usually be detected, which is contrary to what can be found in the epithelium of mastoid cells and middle ear mucosa [16,17]. These findings support the hypothesis of endolymphatic sac origin of the tumours.

Immunochemistry

Expression of neuroectodermal markers such as neuron-specific enolase (NSE) has been reported as well as glial antigens – glial fibrillary acidic proteins (GFAP) and vimentin [8,21,24]. Other reported positive markers include cytokeratin, periodic acid Schiff's reactive material (PAS), protein S100, epithelial membrane antigen (EMA), desmin and actin [4,8,14,15,21,24,25]. One should remember that MEA can also express neuroectodermal markers. It also has to be stressed that to date immunohistochemical data regarding ELST are based on a very limited number of cases but immunochemistry can certainly help to differentiate between ELST and tumours of non-neuroectodermal origin (e.g. metastatic carcinoma of kidney or thyroid).

Association of ELST and von Hippel-Lindau disease (VHL)

Von Hippel-Lindau disease is an autosomal dominantly inherited disorder highly predisposing carriers of germline mutation in the VHL gene to develop various benign and malignant tumours. Typical tumours include: retinal and central nervous system haemangioblastomas, renal cell carcinomas, neuroendocrine tumours of pancreas (NET), pheochromocytomas and paragangliomas, and epididymal papillary cystadenomas. Pancreatic, renal and epididymal cysts are also considered to be a part of VHL syndrome. In recent years two other entities that may be a part of

VHL have been brought to attention: adnexal papillary tumour of probable mesonephric origin (APMO), and ELST. Although the first VHL patient described by von Hippel developed a tumour of the petrous bone [46] and despite some later reports of petrous bone tumours in VHL patients [3,6,7, 10,28,34,37] it was not until 1997 that ELST were recognized as part of VHL [22,30,44].

Molecular analysis

The tumour suppressor gene responsible for von Hippel-Lindau disease was mapped to chromosome 3p25 and then finally identified in 1993 [23]. Its length is about 14500 base pairs (bp) of genomic DNA and the protein-encoding region is divided into 3 exons. Inactivation of both copies of the VHL gene in a cell is required to cause development of tumour, which is consistent with Knudson's "two-hit" hypothesis. Inherited germline mutation affecting usually all or in the case of mosaicism some somatic cells is the first hit. When a second copy of the VHL gene in the cell is inactivated (second hit) the process of tumorigenesis is thought to be started.

Identification of a germline mutation in a patient with ELST can establish the diagnosis of VHL. All first-degree relatives should also be tested as identification of asymptomatic gene carriers is crucial in presymptomatic tumour diagnosis. Molecular testing can also be of great help when diagnosis of ELST is in doubt. ELST must strongly be considered if VHL mutation in a patient with tumour of the middle ear, temporal bone or cerebellopontine angle is detected.

Many molecular techniques are used to detect VHL gene mutation. Single-strand conformation polymorphism analysis (SSCP), heteroduplex and direct sequencing for point mutations, fluorescence in situ hybridization (FISH), Southern blotting or long PCR for larger deletions and partial deletions are among the most commonly used [5,47].

Differential diagnosis

Because of its rarity ELST can be easily misdiagnosed, especially as it can mimic other tumours. MEA can express most immunocytochemical markers that ELST stains positive for. MEAs are however less papillary, they do not erode bone and are limited to the middle ear. In electron microscopy of MEA ciliary features of middle ear mucosa can be found.

There have been reports of misdiagnosing ELST with metastases from follicular thyroid carcinoma [21]. Metastases of thyroid carcinoma have more features of malignant growth in histopathological examination, and they can stain positively for thyreoglobulin. Also RCC metastases have more characteristics of malignant potential. Immunohistochemistry for neuroectodermal can be useful in these instances.

Paragangliomas have a typical pattern of reticulin fibres that can be shown by silver impregnation techniques [12, 40]. True choroid plexus papillomas may be located in the cerebellopontine angle from extension via the foramen of Luschka but they remain subdural and do not erode bone. "Extradural choroid plexus papillomas" were reported [35], some in VHL patients [3, 32], but the hypothesis of their ectopic choroid plexus origin has not been proven scientifically and tumours could actually represent ELST [39]. In contrast to ELST choroid plexus papillomas express transthyretin in immunochemistry [28].

Ceruminous gland adenocarcinomas usually occur more laterally, originating from the external auditory canal. Apocrine cells can often be detected and pleomorphism, nuclear anaplasia and mitosis are prominent [25,39].

Treatment and prognosis

The treatment of choice for ELST is surgery [12, 30, 34, 37]. It is however often not possible to excise a tumour totally due to its aggressive growth pattern. Intraoperative complications such as profuse bleeding can be caused by the hypervascular nature of the tumour and preoperative embolization may be considered [31]. Adjunctive radiotherapy after surgery has been used by some authors [2]. Recurrences, as in our case, could occur many years after the primary surgery [15,28,34]. Cochlear implants have been successfully used in patients with ELST [21].

Conclusions

1. ELST is a rare entity but should be considered in patients with tumours eroding the petrous part of the temporal bone, extending to the cerebellopontine angle or other adjacent structures.
2. VHL patients are of higher risk of developing these tumours.
3. Molecular analysis of the VHL gene should be performed in patients with ELST and their relatives as it can help diagnosing people with VHL.

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