Clinicopathological characteristics of neck ganglioneuroma

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Abstract: Ganglioneuroma of the head and neck is rare. The clinical, histopathological and immunohistochemical characteristics of 6 cases of cervical ganglioneuroma were analyzed. The average age of the patients in this study was 32.8 years (6-62 years). The tumors grew slowly and the patients were asymptomatic. Grossly, they were well encapsulated. Under the microscope, the tumors consisted of primarily Schwann cells, tangled masses of neurites in bundles, and variably-distributed large ganglion cells. The ganglion cells showed positive immunohistochemical reactivity to neuron-specific enolase, neurofilament, chromogranin A, and synaptophysin but negative for S100, the same as in the controlled normal sublingual ganglion cells. All the tumors were treated with surgical excision. There was no recurrence and metastasis during a follow-up time of 3-5 years.

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Introduction

Ganglioneuroma consists of well differentiated gangliocyte and neural fibrous components. It is considered by most investigators as a benign tumor of the peripheral neural system originating from the neural crest. But some cases with malignant potential have also been reported (1-2). Ganglioneuromas often occur in children and adolescents, with a slight predominance in females. The most common tumor locations are the posterior mediastinum, the retroperitoneum neighboring the spine, and the adrenal glands, with only about 5% to 8% arising in the head and neck (3-4). The limited reports regarding neck ganglioneuroma rarely discuss the tumor's histopathological features (5-6). We investigated the clinicopathological and immunohistochemical features of 6 cases of neck ganglioneuroma.

Materials and methods

Clinical data

Clinical data such as age, affected locations, disease course, symptoms, and clinical findings from 6 cases of neck ganglioneuroma were reviewed. Histopathologic sections of postoperative tissues from the 6 patients were stained with hematoxylin and eosin and reviewed for histopathologic changes.

Immunohistochemistry

Immunohistochemical staining was performed using a standardized SP method. Formalin-fixed and paraffin embedded specimens from 6 cases of ganglioneuroma were cut into 5 µm thick sections. The sections were deparaffinised in xylene and rehydrated through a graded ethanol series. To quench endogenous peroxidase, sections were incubated in fresh 3% H₂O₂ methanol liquid for 10 min at room temperature, and then antigen retrieval was performed by microwave treatment for 20 min in 0.01 M citrate buffer (pH 6.0). After washing thrice in PBS and blocking endogenous biotin activity with normal goat serum for 30 min, we incubated the sections overnight at 4°C with primary antibodies respectively.

The antibodies included mouse monoclonal anti-S-100 protein (S-100, clone 4C4.9, prediluted), mouse monoclonal anti-neuron-specific enolase (NSE, NSE-1G4, prediluted), mouse monoclonal anti-neurofilament (NF, DA2/FNP7/RMDO20.11, prediluted), mouse monoclonal anti-chromogranin A (CgA, LK2H10, prediluted) and rabbit polyclonal anti-synaptophysin (Syn, prediluted), all from Zymed Laboratory Inc., San Francisco, USA. The sections were then incubated with secondary antibody for 30 min at room temperature following washing thrice in PBS again. Staining was performed with a streptavidin-biotin peroxidase kit, and sites of bound antibody were visualized using liquid DAB + substrate + chromogen system. The sections were lightly counterstained with Mayer's hematoxylin, then dehydrated, hyalinized and

Table 1. Clinical data of six cases of ganglioneuroma

case #	sex	age (yrs)	location	size (cm)	duration	related nerve
1	male	6	left upper neck	4 × 3	4 yrs	hypoglossal
2	female	9	left parapharyngeal	3 × 3	2 weeks	non-specified*
3	female	62	left upper neck	8 × 4	1 yr	non-specified
4	female	57	right neck	8 × 7	13 yrs	non-specified
5	male	9	bilateral neck	4 × 2	2 yrs	C3, C4**
6	female	53	right upper neck	4 × 4	3 months	non-specified

*related nerves were too small for estimating their origins **3rd and 4th cervical vertebra

covered for observation.

Control slides included in each experiment consisted of tissue previously shown to express the protein of interest as positive controls; negative controls were prepared by substituting PBS for each primary antibody, and no detectable staining was evident. The immunoreactivity showed brown granules in cytoplasm of both ganglion cells and Schwann cells. Some antibodies such as anti-S100 protein also showed nuclear staining. For the immunohistochemical study, 5 normal sublingual ganglions were selected as normal control.

Results

Of the 6 patients with neck ganglioneuroma, 2 were males and 4 were females, with a male to female ratio of 1:2. The ages at presentation were from 6 years old to 62 years old. The patients noted the neck lesions had been present for many years or only a couple of weeks (Table 1). Clinical examination revealed the tumor in one patient located in the left parapharyngeal region, one in the bilateral cervical region, two in the left cervical region and two in the right cervical region. All patients presented to the clinic with complaints of swelling in the neck and had no history of other tumors such as neuroblastoma. No familial background

Fig. 1. Macroscopic appearance of a neck ganglioneuroma. The tumor was oval in shape and well encapsulated. Its cut surface was solid and brownish, scattering small hemorrhagic foci.

was found among the patients. Computed tomography and ultrasonographic examination disclosed the tumors as firm, well-encapsulated masses. Generally the tumors were lobulated, painless, immobile masses, most having a diameter

Fig. 2. Histopathology of ganglioneuroma. Hematoxylin and eosin (HE) stain. (a) × 50; (b) × 200; (c) × 250; (d) × 300; (e) × 80. There were a lot of ganglion cells (lower left) distributed among the tangled masses of neurites and edematous stroma (a). High-power view of the ganglion cells showed that they had great nuclei, prominent nucleoli, and some satellitic cells around themselves (b). Lipofuscin was noted in the cytoplasm of some ganglion cells in the ganglioneuromas (c). There were some binucleated ganglion cells with nuclear hyperchromatism (d). Cystic degeneration was seen in the ganglioneuroma.

between 3 and 8.8cm. The masses were not associated with any systemic symptoms and the overlying skin was normal. Neither biopsy nor aspiration cytology was performed before the operation. Complete resection of the lesions was performed after achievement of general anesthesia. After surgery, the patients presented without complication.

On macroscopy, the tumors were well encapsulated and circumscribed. The cut surface of 3 cases was solid, homogeneous and gray or yellow brown in color. Hemorrhage and cystic degeneration was found in 3 cases (Fig. 1). Under light microscope, the tumors was observed to consist of primarily Schwann cells, tangled masses of neurites in bundles, and variably distributed large ganglion cells, widely scattered or clustered (Fig. 2a). These cells were generally greater than 20 μ m and polygonal with prolonged axons or dendrites. The ganglion cells showed abundant eosinophilic granular cytoplasm and single nuclei, often eccentrically placed with prominent nucleoli (Fig. 2b). The ganglion cell bodies were surrounded by a layer of small flat satellite cells. Lipofuscin was often observed in the cytoplasm (Fig. 2c). Binucleate ganglion cells showing nuclear hyperchromasia were also noted in 2 cases (Fig. 2d). Surrounding the ganglion cells were Schwann cells and nerve fibers. The

Schwann cells were spindle-shaped and were arranged in intersecting bundles. The nuclei were rod-shaped and hyperchromatic, and the chromatin was exquisite. The tumors were rich in blood vessels and thickening vessel walls, and a thrombus could be observed occasionally. The tumor stroma was loose and edematous or myxoid. Cystic degeneration and focal necrosis could be observed in 3 cases (Fig. 2e). Small foci of calcification were noted in 2 cases. Cluster lymphocytes were common and sometimes a few mast cells were seen.

Table 2 gives an overview of the result of immunoreactivity. Immunohistochemical stains demonstrated variable intensity of antigen expression in ganglion cells. Interestingly, S-100 was not positive for the ganglion cells (Fig. 3a), while NF (Fig. 3b), NSE (Fig. 3c), Syn (Fig. 3d), and CgA (Fig. 3e) were positive for them. Schwann cells showed positive staining for all antibodies except for CgA (Fig. 3e), and Syn-positive cells were mostly located around the ganglion cells (Fig. 3d). Ganglion cells in normal control ganglions showed similar reactions for the antigens used in this study (Fig. 3f-j).

Follow-up time ranged from 30 months to 56 months, and the average follow-up time was 43 months. There was

Fig. 3. Immunohistochemical profiles of ganglioneuroma and control ganglion. Immunoperoxidase stains for S-100 protein (a, f), neuron-specific enolase (b, g), neurofilament (c, h), synaptophysin (d, i), and chromogranin A (e, j), hematoxylin counterstain. Ganglion cells in ganglioneuroma (a-e, bar = 25 μ m) showed positive staining for all the antigens (b-e) except for S-100 protein (a), which was almost the same as seen in normal ganglion (f-j, bar = 50 μ m).

Table 2. Immunohistochemical profiles between ganglioneuroma and normal ganglion

groups	number of cases	S-100 protein		neurofilament		neuron-specific enolase		synaptophysin		chromogranin A	
		SC*	GC**	SC	GC	SC	GC	SC	GC	SC	GC
ganglioneuroma	6	+	-	+	+	+	+	+	+	-	+
control ganglion	5	+	-	+	+	+	+	+	+	-	+

+positive staining; -negative staining; *Schwann cells; **ganglion cells

no recurrence and metastasis.

Discussion

Ganglioneuroma is a rare, benign neurogenic tumor arising from the sympathetic nervous system (4). It belongs to the family of neuroblastic tumors which includes also neuroblastoma and ganglioneuroblastoma. Ganglioneuroma may evolve from differentiating neuroblastomas (7-8). Infrequently, it has been reported with familial history (9). Neck ganglioneuroma is rare, and it may evolve from the cervical sympathetic nervous chain. Generally, the tumor is unique and unilateral, whereas multiple localizations associated with neurofibromatosis have been reported. In our study, no patients had a history of other tumors or familial background. Five cases were unilateral and one case was bilateral in the neck.

Histologically, the tumor was composed of intersecting bundles of spindle cells resembling neurofibroma or neurilemmoma, loose and myxoid stroma and the dysplasia of the ganglion cells which resembled normal ganglion cells both morphologically and immunohistochemically. Myxoid degeneration and edema were often evident in the tumor stroma. Immunohistochemistry can be helpful in ascertaining the origin of the tumor and in the differential diagnosis (10). In our study, the ganglion cells of ganglioneuroma showed positive staining for NF, NSE, Syn, and CgA, but negative for S-100. The results were consistent with the normal ganglion cells in the control group and elucidated the differentiation of the tumors. The Schwann cells showed positive staining for S-100 and negative staining for CgA, the opposite of the ganglion cells. The result is inconsistent with other reports (11-13) in which the ganglion cells in tumors showed positive immunohistochemical staining for S-100 and variable staining for Syn. The inconsistent findings by immunohistochemistry may be attributed to different tumor location, resource of primary antibody, treatment of the specimen, and method of the immunohistochemistry.

A specific diagnosis of ganglioneuroma is confirmed only by pathological study. Imageology examination is helpful for the preoperative diagnosis (14). The differential diagnosis includes paraganglioma, neurilemmoma and neurofibroma. Paraganglioma is often smaller than ganglioneuroma in diameter and when the tumor nest is surrounded by the fibrous stroma, there are no typical ganglion cells. Occasionally a few ganglion cells showing karyopyknosis degeneration can be noted in neurilemmoma or neurofibroma, but no binucleate or trinucleate ganglion cells can be observed in them, whereas ganglion cells, sometimes binucleate or trinucleate, can be found in ganglioneuroma under careful observation even though the ganglion cells may be quite few.

Neurogenic tumors are insensitive to the radiotherapy and chemotherapy, so the main treatment for the ganglioneuroma is surgical excision (5). In our study, the

tumors ranged from 3.0 to 8.8cm in diameter. Complete resection was performed for the well encapsulated tumors, and the prognosis is good. For the worse encapsulated tumors, preoperative preparation should be careful. For the tumor containing immature content, regular follow-up should be taken to prevent recurrence. In our study, all the 6 cases showed no recurrence or metastasis during a following-up time averaging 43 months.

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