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Neuroblastoma of Parotid Gland: Report of a Case and Immunohistochemical Characteristics

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A case of a parotid mass in a 2-year-old boy, postoperatively diagnosed as neuroblastoma, a rare tumour not previously reported in the parotid gland is presented. The neoplasm developed within the parotid gland as a painless mass without regional lymphadenopathy. Histopathologically, the tumour showed primitive nerve cells—neuroblasts—with round or oval dark basophilic nuclei and scanty cytoplasm. The cells were arranged in circular rosettes around an eosinophilic mass consisting of very fine filaments originating in the tumour cells or papillary configuration and sometimes scattered in the poorly developed stroma. Immunohistochemical evaluation of the tumour showed a positive immunoreactivity for vimentin, α and β subunits of S-100 protein, neurone-specific enolase (NSE), substance P, met-enkephalin and chromogranin but cytokeratins, desmin, actin, myosin, glial fibrillary acidic protein (GFAP) and calcitonin gene related peptide (CGRP) were negative. The histopathological and immunohistochemical findings conclude a diagnosis of neuroblastoma of the parotid gland.

Keywords: neuroblastoma, parotid gland, immunohistochemistry, neuron-specific enolase, vimentin, S-100 protein

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INTRODUCTION

NEUROBLASTOMA (NB) IS a highly malignant neoplasm of central nervous system, adrenal medulla and retroperitoneal tissues. In the head and neck region, the tumour occasionally arises as olfactory NB [1, 2]. A primitive neuroendocrine tumour, possibly a NB in the oral cavity has been reported, however, immunohistochemical characteristics were not available [3]. Benign and malignant neoplasms of neural tissue origin such as schwannoma, neurofibroma and malignant schwannoma in salivary gland have been classified as nonepithelial neoplasms of major glands [4]. Numerous experimental and immunohistochemical studies have suggested the stem cells of salivary neoplasms are neuroectodermal in origin, e.g. neural crest cell in origin [5, 6]. We describe a case of NB where the tumour was localised in the left parotid gland presenting as an asymptomatic solitary mass and a detailed immunohistochemical evaluation to conclude the diagnosis. The possible histogenesis of the present case is discussed as tumour tissue is further compared with normal ganglion cells and astrocytes for the immunohistochemical characteristics. To our knowledge, this is the first case report of NB arising in the parotid gland.

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CASE REPORT

A 2-year-old boy was seen at the Department of Oral and Maxillofacial Surgery of the 4th Military Medical University, Xi'an, People's Republic of China on 28 September 1984 for evaluation of an asymptomatic mass in the left parotid region of 3 months duration. An elastic, firm, non-tender, solitary, movable mass measuring approximately $3 \times 3{\cdot}5 \times 2$ cm was noted in the left parotid region. The intraoral findings were within normal limits and there was no palpable regional lymphadenopathy. The past medical history was noncontributory.

Fine needle aspiration cytology was performed in suspicion of a parotid tumour. Microscopically, the tumour showed a few small cells with round or oval nuclei but was not suggestive of a parenchymal neoplasm of the parotid gland. The absence of other neoplasia was ascertained from anamesis and radiographic examination of the skull, chest, stomach, pelvis and extremities.

The tumour was excised on 8 October 1984 by partial parotidectomy preserving the facial nerve. The mass on the superficial lobe of the parotid was found to extend anteriorly to the anterior border of the masseter muscle at the site of the

parotid duct. The cut surface of the tumour was greyish white without necrosis. Examination of frozen sections suggested a ductal carcinoma of the salivary gland. The postoperative recovery was uneventful. However, the patient was lost to follow-up but reported dead due to recurrence of the disease at the primary site 6 months after the surgery.

The sections of the tumour were later evaluated by three examiners, immunohistochemical examination was performed at the Department of Oral and Maxillofacial Surgery of Asahi University and the final diagnosis was made as NB of the parotid gland.

MATERIALS AND METHODS

The specimen obtained at surgery was fixed in 10% neutral buffered formalin and embedded in paraffin. Serial sections at 4 μm were stained with haematoxylin and eosin and for further histopathological examination by immunohistochemical evaluation. The immunohistochemical staining was performed by a three-stage avidin–biotin complex method described by Hse *et al.* [7]. The primary antibodies used were monoclonal antibody to proliferating cell nuclear antigen (PCNA), 1:20; cytokeratin KL1, 1:40 (Immunotech, France); cytokeratin K8.12, 1:40 (Bio-Yeda, Israel); desmin, 1:50 (Euro-diagnosics); vimentin, 1:50; neuron-specific enolase (NSE), 1:50; glial fibrillary acidic protein (GFAP), 1:100; S-100 protein α and β, 1:100 (Dakopatts, Denmark); calcitonin gene-related peptide (CGRP), 1:2,000 (Chemicon, U.S.A.) and polyclonal antibody to chromogranin, 1:300; met-enkephalin, 1:100 (Incstar, U.S.A.); actin, 1:20; myosin, 1:20; and substance P, 1:500 (Chemicon). The ABC immunohistochemistry kit was purchased from Dakopatts. For positive control and comparative study, ganglion cells from ganglioneuroblastoma and astrocytes from astrocytoma were evaluated. Negative control where the primary antibody was replaced by phosphate buffered saline (PBS) showed negative results in all instances.

RESULTS

Histopathological findings

Haematoxylin and eosin stained sections showed the tumour was composed of primitive nerve cells—neuroblasts. The tumour cells were round or oval with dark basophilic nuclei and scanty cytoplasm. The cytoplasmic boundaries were poorly defined and mitotic figures were infrequent. The cells were arranged in circular rosettes around an eosinophilic mass consisting of very fine filaments originating from the tumour cells (Fig. 1a). In some areas there were giant cells, irregular in shape with deeply basophilic nuclei, scattered in the neuroblastic tumour foci (Fig. 1b). In another area, tumour cells of similar morphology, as present in the rosettes, were arranged in papillary projections (Fig. 1c). There were numerous blood vessels in the tumour stroma which were often angiomatoid.

Immunohistochemical findings (Table 1)

The PCNA immunoreactivity was localised in the nuclei of tumour cells mainly arranged in rosette or papillary projections (Fig. 1d). No immunoreactivity of cytokeratins recognised by monoclonal antibody KL1 and K8.12 was observed, however, tumour cells had moderate to strong positive reactions for vimentin (Fig. 1e). Positive immunostaining with S-100α was found in almost all of the tumour cells (Fig. 1f), however, S-100β was strongly positive only in a limited number of tumour cells and the giant cells (Fig. 1g). NSE was abundant in all the tumour cells (Fig. 1h). A strong immunoreactivity of substance P and met-enkephalin (Metenk) was observed in most of the tumour cells while chromgranin was positive in all the tumour cells with weak to intermediate level of reaction (Fig. 1i–k). No reaction product of GFAP and CGRP was seen in the tumour cells. Desmin, actin and myosin were negative in the tumour cells.

Sections of ganglion cells and astrocytes were used as positive controls and were evaluated for the immunoreactivity of the markers used in the present study. Most of the ganglion cells showed positive immunoreactivity with vimentin whereas satellite cells were consistently negative for this marker. S-100 β was strongly positive in satellite cells but negative in ganglion cells while S-100α was present in both cell types (Fig. 2a–c). Reaction products of met-enkephalin, substance P, chromogranin (Fig. 2d–f) and NSE were conspicuous in the cytoplasm of ganglion cells (Fig. 2g) while CGRP was very slight or negative (Fig. 2h). No immunoreactivity of cytokeratin and GFAP was observed in ganglion cells.

Table 1. Immunoreactivity of tumour cells in parotid neuroblastoma compared with ganglion, satellite and astroglial cells in ganglioblastoma and astrocytoma

Antibody	Neuroblastoma cells	Ganglion cells	Satellite cells	Astroglial cells
Cytokeratin KL1 (55–57kD)	−	−	−	−
Cytokeratin K8.12 (No. 13, 16)	−	−	−	−
Vimentin	+	+	+	±
Actin	−	−	±	±
Myosin	−	−	±	±
Desmin	−	−	−	−
Glial fibrillary acidic protein (GFAP)	−	−	−	+ + +
S-100α	+ + +	+ +	+ + +	+ +
S-100β	− to + + +	−	+ + +	+ + +
Met-enkephalin	+ + +	+ + +	−	±
Substance P	+ + +	+ + +	−	+ + +
Chromogranin	+ to + +	+ + +	−	+ +
Neuron-specific enolase	+ + +	+ + +	−	±
Calcitonin gene related peptide (CGRP)	−	+ +	−	±

− Negative; ± trace; + weak; + + intermediate; + + + strong.

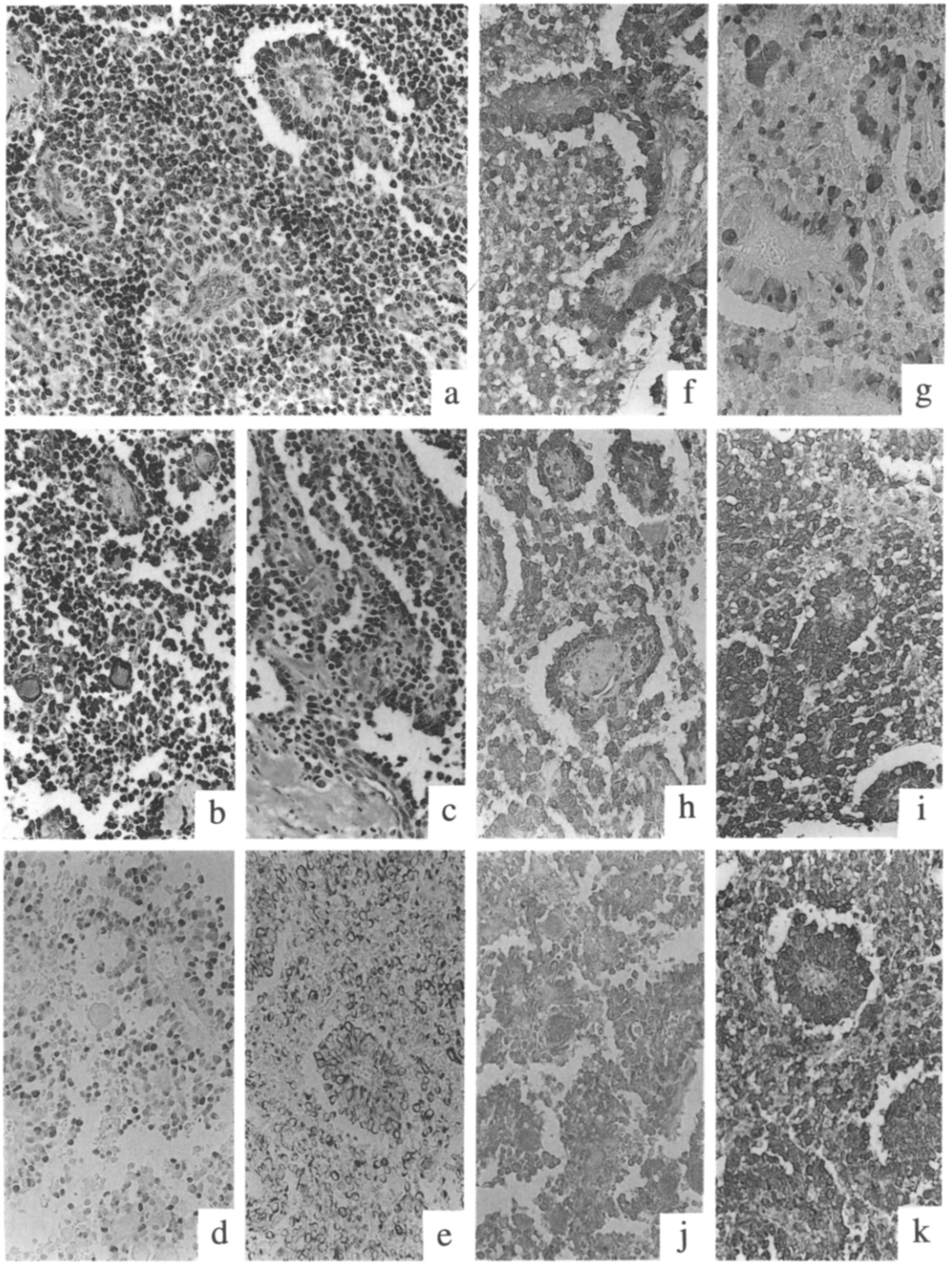

Fig. 1. (a) Neuroblastoma of parotid gland: histopathological appearance shows undifferentiated neuroblast like cells showing uniform small basophilic nuclei with scanty cytoplasm in a poorly developed stroma. Rosette formation by the tumour cells can be seen. × 100. (b) Giant cells of neural origin are seen in the tumour mass where the tumour cells are scattered without forming rosette. (c) Some tumour cells of neuroblastoma are arranged in papillary projections. (d) Proliferating cell nuclear antigen (PCNA) positive nuclei are mainly concentrated in the rosette forming tumour cells. (e) Vimentin immunostaining: all the tumour cells show a weak or moderate to strong immunoreactivity for vimentin. (f) S-100α immunostaining. Tumour cells in rosettes and papillary projections show a strong S-100α immunoreactivity. The nerve fibre is devoid of S-100α immunostaining. (g) S-100β immunostaining. Tumour cells located in papillary projections show a strong immunoreactivity of S-100β. Not all tumour cells are positive to S-100β. (h) Neuron-specific enolase. All the neoplastic cells show a diffuse positive reaction of monoclonal NSE antibody. (i) Substance P immunostaining. A strong immunoreactivity of substance P is seen in all the tumour cells. (j) Met-Enk immunostaining. Tumour cells show a strong immunoreactivity for Met-Enk. (k) Chromogranin immunostaining. A diffuse reaction product of chromogranin immunoreactivity is seen in the tumour cells and stroma.

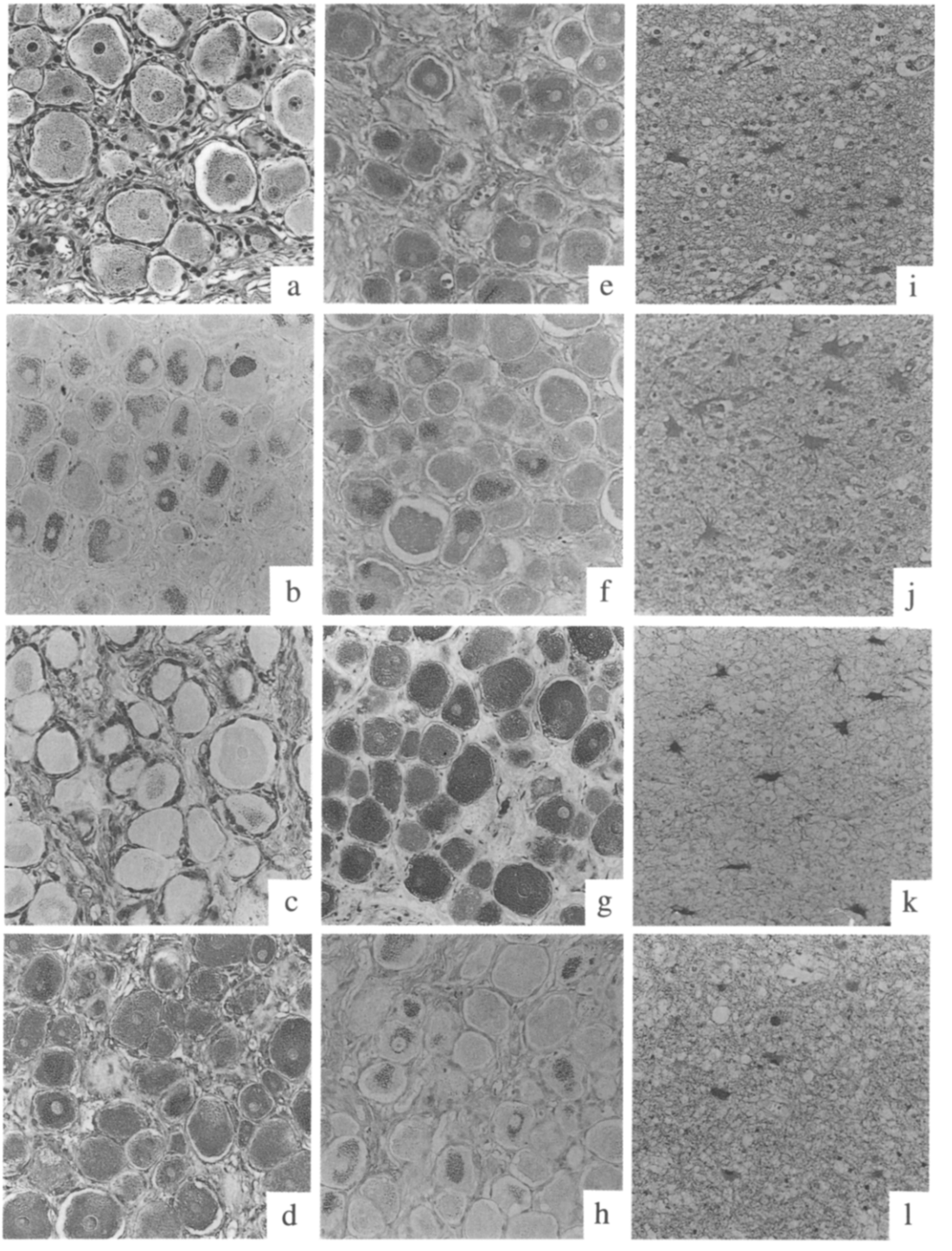

Fig. 2. (a–b) Control sections of ganglion cells and astrocytes. (a) Histology of ganglion cells showing central nuclei with abundant cytoplasm, satellite cells and interstitial connective tissue. × 100. (b) Vimentin immunostaining. Positive reaction product of vimentin is seen in the cytoplasm of ganglion cells. (c) S-100β immunostaining. Satellite cells show positive immunoreactivity of S-100β. (d) Met–enkephalin immunostaining. The ganglion cells show positive immunoreactivity. (e) Substance P immunostaining. Moderate to strong immunoreactivity is seen in ganglion cells. (f) Chromogranin immunostaining. Chromogranin immunoreactivity is particularly concentrated in the cytoplasm of ganglion cells. (g) NSE. An intense activity of NSE is seen in ganglion cells. (h) CGRP immunostaining. Few ganglion cells show positive CGRP immunoreactivity. (i) Substance P immunostaining. Astrocytes show a strong reaction for substance P. × 100. (j) Chromogranin immunostaining. Astrocytes show a weak immunoreaction of chromogranin. × 100. (k) GFAP immunostaining. Astroglial cells and their process show a strong immunoreactivity of GFAP. × 100. (l) S-100β immunostaining. Astrocytes show a positive reaction of S-100β subunit. × 100.

Astroglial cells were strongly positive for GFAP, S-100 β, substance P and moderately for chromogranin (Fig. 2i–l).

DISCUSSION

The tumour we investigated, histopathologically, showed a classical picture of NB as previously reported at other sites [8]. As the tumour was diagnosed retrospectively on the basis of histopathological and immunohistochemical features no supporting clinical evidence for NB such as increased urinary catecholamines and vanylmandelic acid (VMA) were available. Peripheral neuroectodermal tumours are uncommon, and may be confused with other small round cell tumours such as Ewing's sarcoma, alveolar rhabdomyosarcoma and Merkel cell tumour [9]. However, a rare tumour in an unexpected site adds a further diagnostic dilemma, and the present case was initially diagnosed as salivary duct carcinoma. The immunohistochemical studies ruled out the possibility of salivary duct carcinoma which was suggested in the frozen section diagnosis during primary surgery, as the tumour was negative to cytokeratins and EMA, but positive to vimentin, NSE, S-100 protein, substance P, met-enkephalin and chromogranin. Positive immunoreactivity of vimentin, NSE and S-100 protein have been consistently reported in NB [2, 10, 11]. The histopathological features, on the other hand, with round cells and in particular giant cells with peripheral 'wreath-like' nuclei which can be considered to be highly suggestive of alveolar rhabdomyosarcoma were evident in the specimen. Poorly differentiated round cell tumours may express NSE, vimentin and S-100 protein and, therefore, do not confirm NB or exclude rhabdomyosarcoma. However, the absence of myogenic differentiation suggested by no immunoreactivity for desmin, actin and myosin reasonably excluded the possibility of rhabdomyosarcoma, although, at rare instances small round cell tumour may have both neurogenic and myogenic elements [12]. Cell surface membrane antigen and cytoskeleton protein analysis is useful for the differential diagnosis of rhabdomyosarcoma and Ewing's sarcoma [13].

NB in adrenal medulla and retroperitoneal tissues mainly affects infants and children and is often accompanied by widespread metastasis. The age incidence of olfactory NB is bimodal, one peak occurring in adolescence and the other in the sixth decade, although it may be found at any age [1, 2].

The histopathological, ultrastructural and immunohistochemical features of NB have been well documented. Ultrastructurally, it has been found to have abundant cytoplasmic filament processes and dense core endocrine vesicles or neurosecretory granules [2]. Neuroendocrine cells in undifferentiated minor salivary gland carcinoma have been reported suggesting that neuronal differentiation does occur in salivary gland tumours [14]. Markers of neuroectodermal differentiation, somatostatin in Warthin's tumour [15] and vasoactive intestinal peptide (VIP) in parotid acinic cell carcinoma [16] have been reported. The majority of salivary gland tumours, however, rarely express somatostatin and VIP but usually express S-100 protein, GFAP and NSE. Recent studies, by immunohistochemistry and tissue culture, on the histogenesis of salivary gland tumours have proposed that the stem cells are of neural crest in origin [5, 6]. Primitive neuroendocrine tumours are, however, rare in salivary glands and to our knowledge this is the first case of NB in the parotid gland.

Squamous differentiation with cytokeratin expression has been reported in olfactory neuroblastoma [10]. In *in vitro* experiments, NB cell lines with differentiation into smooth muscle cells with alpha-smooth muscle actin and/or desmin have been demonstrated in epithelial-like NB cells lines [13]. Frierson *et al.* have observed characteristic immunohistochemical expression of NSE and S-100 protein in olfactory neuroblastoma where, in addition, a panel of antibodies to synaptophysin, keratin, GFAP, vimentin, chromogranin and EMA were employed and suggested S-100 protein and NSE as specific neural marker of olfactory neuroblastoma [11]. In the present case, we found no reaction of keratin proteins suggesting no component of adenocarcinoma or squamous carcinoma to be present.

It is well established that NSE and GFAP are markers of neuronal and glial cells, respectively [17] and S-100 protein has been used in the immunohistochemical identification of normal gial cells in the central nervous system, normal schwann cells of peripheral nervous system and their tumours [18]. Immunohistochemical studies have reported neuroendocrine markers in central neurocytoma [19], ganglioneuroblastoma [20] and childhood NB [21]. Central neurocytoma formed by the cells committed to neuronal phenotype and NB as in the present case as well as those reported by others shared some biochemical properties, nevertheless advanced neuronal differentiation may be absent. Central neurocytomas have been found to express neuroendocrine markers NSE, synaptophysin and rarely S-100 protein but not chromogranin A and GFAP [22]. NSE immunoreactivity is consistently present in the neuroblastoma [10, 11, 17, 20–24]. NSE immunostaining has been found to be a very sensitive marker of metastatic NB cells in the bone marrow [25, 26]. In the present study, NSE immunoreactivity was found to be a useful and sensitive marker for the identification of NB cells. S-100 protein α and β were strongly positive in varying number of neuroblastic tumour cells including neurogenic giant cells and S-100β in ganglioneuroblastoma.

Neurofilaments (NF: 68, 120 and 200kD proteins), the intermediate filament proteins are specific markers of neuronal differentiation. Intermediate filaments have been identified at ultrastructural level in NB, but at the immunohistochemical level the identification of NF do not correlate with the immunohistochemical detection of NF in paraffin embedded sections [2, 17]. However, presence of all NFs in fresh frozen section by immunohistochemical methods have been reported [23]. Neuroblasts at their early stages of differentiation are negative to NF but positive to vimentin and high molecular weight NF appears with loss of vimentin as they mature [10]. Therefore, either the NB cells due to their state of primitive differentiation do not possess NF, hence cannot be identified at immunohistochemical level or NF may be lost during tissue processing for paraffin embedding. In the present study we did not evaluate NF protein as in the paraffin-embedded sections the results are not consistent enough to conclude their identification.

Immunoreaction of met-enkephalin and substance P is not well known in NB or neurogenic tumours, however, immunoreactive substance P has been reported in anaplastic ganglioglioma of brain and met-enkephalin in some large ganglioid cells [27]. From the present results, both neuronal markers were expressed in the parotid NB. Chromogranin immunoreactivity has been documented in NB [28] where as small cell neuronal tumours of central neurocytoma and the majority of undifferentiated neuronal cells are negative [22]. GFAP is present in glial cells while NB cells are usually devoid of GFAP

reaction [10, 11] as seen in the present study so NB does not show glial differentiation. Calcitonin gene-related peptide is a recently characterised neuroactive substance expressed in a large proportion of small to medium diameter sensory ganglion neurons including those of trigeminal ganglion where it is often colocalised with substance P [29]. Although ganglion cells in the present study showed common immunohistochemical characteristics with NB cells, CGRP was negative in the latter. On the basis of markers used, the present case suggests an undifferentiated state of normal neural development leading to the neoplastic transformation. The other possible origin of the tumour may be from schwann cells, perineural fibroblasts or reserve stem cells of salivary gland showing primitive neuronal differentiation.

In conclusion, histopathological and immunohistochemical findings of positive immunoreactivity for vimentin, NSE, S-100 α and β, met-enkephalin, substance P and chromogranin but not for cytokeratins, desmin, actin and myosin allow us to reasonably conclude that the lesion is a primary NB of the parotid gland.

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