Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology

journal homepage: www.elsevier.com/locate/jomsmp

Original research

Immunohistochemical profiling is useful to distinguish oral neural benign neoplasms

Gabriela Nagata [a], Fabrício Passador-Santos [b], Patrícia Adachi [a], Suzana Cantanhede Machado de Sousa [a], Marília Trierveiler [a,*]

[a] Department of Oral and Maxillofacial Pathology, School of Dentistry, University of São Paulo, Avenida Professor Lineu Prestes, 2227, 05508-000, São Paulo, São Paulo, Brazil
[b] Department of Pathology, São Leopoldo Mandic Institute and Research Centre, Rua José Rocha Junqueira, 13, 13045-755, Campinas, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 20 March 2017
Received in revised form 14 June 2017
Accepted 28 June 2017
Available online xxx

Keywords:
Neurofibroma
Neurilemmoma
Traumatic neuroma
Immunohistochemistry
Benign neural neoplasms

ABSTRACT

Objective: The most common oral neurogenic lesions are traumatic neuroma, neurilemmoma, and neurofibroma. They share clinical and histological features, but have a different prognosis and treatment. Our aim was to use a small panel of immunohistochemistry antibodies to distinguish among them.
Methods: Eight cases of traumatic neuroma, 9 cases of neurilemmoma, and 11 cases of neurofibroma were selected and stained with primary antibodies against S-100 protein, laminin, and epithelial membrane antigen (EMA) using the streptavidin-biotin-peroxidase method of immunohistochemistry.
Results: The S-100 protein was positive in the endoneurium of traumatic neuroma, Schwann cells, and Verocay bodies of the neurilemmomas and revealed a variable number of positive cells amongst spindle-shaped cells that compose neurofibromas. Laminin was positive in the endoneurium and in the perineurium of traumatic neuromas. In neurilemmomas, all neoplasic cells and the capsule were positive for laminin. For neurofibromas, laminin positivity followed the patterns observed for S-100 protein. EMA was positive in the perineurium of traumatic neuroma and in the capsule of neurilemmomas. Most cases of neurofibroma were completely negative for EMA, but three cases showed rare, scattered positive cells.
Conclusion: The use of this small immunohistochemical panel to evaluate the presence and localization of S-100 protein, laminin, and EMA can help to distinguish among these three lesions and provide a more specific diagnosis. Consequently, this will provide a better prognosis for patients.

1. Introduction

Neurogenic neoplasms represent approximately 12% of all benign soft tissue neoplasms [1,2]. In the oral cavity, the most common lesions are traumatic neuroma, neurilemmoma (also known as benign schwannoma), and neurofibroma [1]. Traumatic neuroma is a non-neoplastic lesion developing from the proliferation of the proximal end of an injured nerve as a result of trauma or surgery. It presents as firm swelling with a smooth surface that more commonly arises from the mental foramen region and lower lip [3–5]. Histologically, traumatic neuromas consist of haphazardly arranged nerve fascicles comprised of axons with their investitures of myelin associated with Schwann cells. These fascicles are involved with perineurium, which is a sheath of connective tissue formed by flattened cells that are strongly adhered and also embedded within a densely collagen fibrous matrix [6].

In areas where nerves are small and the extracellular matrix has less collagen deposition, traumatic neuroma can be easily confused with neurofibromas [2]. Neurofibromas and neurilemmomas are defined as benign nerve sheath neoplasms, because both exhibit a high proliferation of cells that make up the nerve tissue [2].

Neurilemmomas are slow-growing, encapsulated lesions more commonly found during the second and third decades of life [7]. Approximately 25–45% of all neurilemmomas occur in the head and neck [8]. Of these, approximately 1–12% occur intraorally [9] with the tongue being the most common site [10,11].

The most important histologic features of neurilemmomas are the presence of a capsule and the identification of Antoni A and Antoni B patterns. Antoni A areas are more organized and composed of spindle cells arranged in fascicles that often form a palisade

☆ AsianAOMS: Asian Association of Oral and Maxillofacial Surgeons; ASOMP: Asian Society of Oral and Maxillofacial Pathology; JSOP: Japanese Society of Oral Pathology; JSOMS: Japanese Society of Oral and Maxillofacial Surgeons; JSOM: Japanese Society of Oral Medicine; JAMI: Japanese Academy of Maxillofacial Implants.

* Corresponding author at: Faculdade de Odontologia da USP, Disciplina de Patologia Oral e Maxilofacial, Av. Prof. Lineu Prestes, 2227, São Paulo, SP 05508-000, Brazil.
E-mail address: trierveiler@usp.br (M. Trierveiler).

http://dx.doi.org/10.1016/j.ajoms.2017.06.013
2212-5558/

arrangement around central acellular, eosinophilic areas known as Verocay bodies [2,11,12]. Antoni B areas are less organized, hypocellular, and contain more myxoid loosely arranged tissue [5]. When Antoni B pattern is predominant, the differential diagnosis between neurilemmoma and neurofibroma may be difficult to establish without the use of special stainings [13].

Malignant transformation is exceedingly rare and, in the oral cavity, neurilemmomas always appear as solitary lesions that do not present a relationship with other abnormalities [5,14].

Neurofibromas are the most frequent benign neoplasms originating from the peripheral nerve sheath. Variants of neurofibroma include localized cutaneous, diffuse cutaneous, localized intraneural, plexiform, and massive soft tissue forms [15].

Approximately 25% of all neurofibromas occur in the head and neck region, [16] and lesions in the oral cavity are not uncommon [4]. Most neurofibromas in the head and neck region tend to be solitary tumors. In the oral cavity, these tumors have been described most frequently in the tongue, gingivae, [13,17–19] buccal mucosa, lips [13,17,18] and palate [17,19]. Histologically, neurofibromas consist mostly of Schwann cells and fibroblasts, but they also contain other cell types, including mast cells, pericytes, endothelial cells, and cells with intermediate features between fibroblasts and perineurial-like cells [20,21].

Neurofibromas can also be associated with neurofibromatosis syndrome type 1, caused by a mutation that affects the 17q11.2 region, which is a locus responsible for the transcription of neurofibromin a protein considered to be a tumor suppressor. Other features of neurofibromatosis syndrome beyond multiple neurofibromas are *café au lait* patches, Lish nodules, and axillary freckling [14]. When patients have NF1 syndrome, neurofibromas can transform into a malignant peripheral nerve sheath tumor in 8–13% of cases [22]. In addition, the prognosis of the malignant peripheral nerve sheath tumor is poor, with less than half of patients being cured [23].

Surgical excision is the most common treatment for traumatic neuroma, neurilemmoma, and neurofibroma; however, as neurofibroma has a considerable risk for malignant transformation, close follow-up is necessary after surgical excision of the tumor [4].

Traumatic neuroma, neurilemmoma, and neurofibroma share several clinical and histological features, which leads to difficulty in determining the correct diagnosis. Because of the difference in prognosis, the concern in accurately diagnosing these lesions justifies the efforts of identifying methods that can help distinguish these neural lesions.

Immunohistochemistry has often been used in pathology as a diagnostic tool when there is any clinical or morphological doubt regarding a case. Many molecules have been studied in normal neural tissue and lesions. Among these molecules, the most cited is the S-100 protein, which is predominantly found in the nervous system and expressed by Schwann cells, but not by perineurial cells and endoneural fibroblasts [24]. Laminin is an adhesion molecule present in the extracellular matrix and produced by various cells, including Schwann cells. It has the potential to demonstrate the cell periphery by immunostaining the basal membrane around those cells [25,26]. The epithelial membrane antigen (EMA) is expressed by the cytoplasmatic membrane of perineurial cells as well as epithelial and plasma cells [27–29]. Table 1 summarizes the previously described immunoprofile of these nerve components.

We believe that a specific immunohistochemical profile based on the presence and localization of these proteins will help in distinguishing among the three described neural lesions. Therefore, the aim of this study was to use a small panel of immunohistochemistry antibodies to distinguish among these lesions, which share similar clinical and histological features but show a different prognosis.

Table 1
Immunoprofile of the normal nerve components.

Structure	S-100 protein	Laminin	EMA
Schwann cell	+	+	–
endoneurium	+	+	–
perineurium	–	+	+
epineurium	–	–	–

+ positive.
– negative.

2. Material and methods

This study was previously approved by the ethics committee of our institution. A total of 28 cases of oral neural lesions were retrieved from the files of the Oral Surgical Pathology Service, including 8 cases of traumatic neuroma, 9 cases of neurilemmoma, and 11 cases of neurofibroma. The selected cases were those that fulfilled the clinical and histopathological diagnostic criteria previously described [2,4]. The hematoxylin and eosin (H&E) stained sections were reviewed by all of the authors of this work.

For immunohistochemistry, 3 μm sections were obtained from formalin-fixed, paraffin-embedded specimens and submitted to antibodies against S-100 protein (polyclonal), laminin (polyclonal), and epithelial membrane antigen (EMA, clone E29). All of the antibodies were obtained from DAKO (DAKO, Carpinteria, CA, USA). A streptavidin-biotin method was chosen for this study using 50 mM Tris-buffered saline (TBS), pH 7.6.

After dewaxing and rehydration, the sections being stained for laminin and EMA received antigen retrieval treatment. For laminin, this treatment included incubation with a type XIV protease (Sigma-Aldrich, Saint Louis, MO, USA) diluted in TBS (2.8 units/ml) at 37 °C for 30 min. For EMA, the treatment was carried out in a 10 mM citrate solution (pH 6.0) at 95° C for 30 min in a water bath. The sections were then incubated for 30 min in 6% hydrogen peroxide/methanol (v/v) solution to quench endogenous peroxidase activity. Primary antibodies were diluted in Tris-bovine serum albumin (BSA) buffer (S-100 protein 1:1000; laminin 1:200; EMA 1:50) and the sections were incubated with the antibodies for 30 min. A secondary biotinilated antibody and streptavidin-biotin-peroxidase complex were incubated using the LSAB Dako kit (DAKO). Diaminobenzidine was used as the chromogen, followed by counterstaining with Mayer's hematoxylin. All steps from primary antibody incubation to counterstaining were performed on an Autostainer (Dako).

As a positive control, fragments of normal oral mucosa were used for the three antibodies. The negative control included buffer without the primary antibody. The localization, intensity, and extent of staining were evaluated using a light microscope.

3. Results

Figs. 1A–C, 2A and B, and 3A and B show morphological aspects of the lesions studied.

3.1. S-100 protein

In traumatic neuroma samples, the S-100 protein was positive in the nuclei and cytoplasm of spindle-shaped cells inside proliferating nerve fascicles. However, the perineurium, which is the structure that surrounds fascicles, was negative for this marker. The epineurium was also negative, although it was not visible in all cases (Fig. 1D).

Neurilemmoma, which is mainly formed by Schwann cells, showed intense positive staining for S-100 protein in both the cytoplasm and nuclei. Nevertheless, the peripheral region of the lesions,

Please cite this article in press as: Nagata G, et al. Immunohistochemical profiling is useful to distinguish oral neural benign neoplasms. J Oral Maxillofac Surg Med Pathol (2017), http://dx.doi.org/10.1016/j.ajoms.2017.06.013