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Clinicopathologic and immunohistochemical features of oral neurofibroma

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Abstract

Objective. The purposes of this study were to assess clinical, histopathological and immunohistochemical features of 22 oral neurofibromas (NFs) and discuss with previously described literature, addressing the main aspects regarding the differential diagnosis. ***Materials and methods.*** Immunohistochemical reactions included S-100, CD34, GLUT-1, EMA, Ki-67, p53 and Collagen IV and histochemical reactions for Alcian blue. ***Results.*** Clinically, the preferential location was the maxillary bones, tongue and buccal mucosa. Microscopically, widely spread spindle-shaped cells with scant cytoplasm and elongated nuclei were observed. Immunostaining revealed that the tumor cells weakly expressed GLUT-1, Collagen IV, Ki-67 and p53. They were variably positive for CD34, S-100 protein and membrane epithelial antigen (EMA). ***Conclusions.*** The different types of nerve sheath cells observed in the present series reinforce the presence of heterogeneous population in NFs. The strong positivity for S-100 suggests that the lesions were more composed by S-100-positive Schwann cells than other cells. Besides, the high number of CD34-positive cells suggests that this marker can be useful for the differential diagnosis of NFs against PEN, traumatic neuromas and Schwannomas. Finally, the low immunostaining for p53 and Ki-67 may indicate that NFs massively composed by S-100-positive Schwann cells present low potential of aggressiveness and malignant transformation.

Key Words: *neurofibroma, oral cavity, immunohistochemistry, clinicopathological aspects, differential diagnosis*

Introduction

Neurofibromas (NFs) are benign heterogeneous tumors originating from the peripheral nerve sheath, mainly characterized by proliferation of a mixture of Schwann cells, perineurial cells and endoneurial fibroblasts [1,2]. They can assume localized, diffuse or plexiform growth patterns [2] and may occur as solitary (isolated) or multiple lesions associated or not with neurofibromatosis type 1 (NF-1) [3]. Clinically, NFs are usually asymptomatic, affecting predominantly adults, showing no gender preference or prevalence in females, and may be found at any site on the body but most often in the head and neck [3,4]. Additionally, NFs present slow growth, which can cause a delayed diagnosis [2–4].

Benign oral peripheral nerve tumors include schwannoma, neurofibroma, nerve sheath myxoma, palisade encapsulated neuroma, mucosa neuroma associated with multiple endocrine neoplasia III, traumatic neuroma and granular cell tumor [1,3,5]. Although these tumors share a neural origin, they exhibit notable heterogeneity in terms of microscopic and pathogenetic aspects [1]. Studies focus on ultrastructural and immunohistochemical analysis have been performed in order to clarify the histogenesis and pathogenesis of these lesions, as well as to help their, sometimes, difficult diagnosis [1].

On this basis, the purpose of the present study was to assess clinicopathological and immunohistochemical features of 22 neurofibromas (NFs) of the oral cavity and discuss the literature regarding NF and other important neural lesions in order to provide insights for differential diagnosis.

Materials and methods

All experiments were undertaken with the understanding and written consent of each subject and in accordance with ethical principles (World Medical

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Association Declaration of Helsinki). The study was independently reviewed and approved by an institutional ethical board (IRB).

Clinical data

Clinical data (anatomic distribution, gender, age, ethnic group, multiple or isolated lesion, association with NF-1 and size) were recorded and expressed in percentages.

Tissue samples and histopathological assessment

A total of 22 cases, previously diagnosed as isolated neurofibroma, were retrieved from the Surgical Pathology Service archives of the University of São Paulo. All specimens were fixed in formalin and embedded in paraffin. Hematoxylin-eosin-stained 5 μm sections from all specimens were reviewed. Clinical and histopathological features were accessed by previous recorded data, reviewed and reclassified when necessary showing that the 22 lesions were distributed in 16 isolated sites and six multiples.

Immunohistochemistry and histochemistry reactions

All specimens were submitted to Alcian blue staining and for immunohistochemical reactions, 3 μm sections were obtained from formalin-fixed, paraffin-embedded tissue, following the streptavidin-biotin method (System-HRP K0690, DakoCytomation, Carpinteria (city) CA). The antibodies (DakoCytomation Carpinteria), dilutions and antigen retrieval included CD-34 (1:50, 10 mM citrate solution, pH 6.0), GLUT-1 (1:200, 10 mM citrate solution, pH 6.0), EMA (1:75, 10 mM citrate solution, pH 6.0), Ki-67 (1:75, EDTA, pH 9.0), p53 (1:75, EDTA, pH 9.0), S-100 (1:1000, water bath) and Colagen IV (1:50, 1% pepsin). Positive and negative controls were included in all reactions.

Immunohistochemical analysis

Immunoreactivity was graded with a semi-quantitative method as described previously [6]. The number of positively stained cells was evaluated using the following scale: (−), no positive cells in the tumor; (+), less than 5% of tumor cells positive; (++), between 5–50% of tumor cells positive; and (+++), more than 50% of tumor cells positive.

Results

Clinical data

Clinical features are shown in Table I. Samples were obtained from 22 patients: 16 female (72.7%) and six male (27.3%). The mean age was 38.5 years, ranging from 7–71 years for females and from 14–66 years for males. The neurofibromas presented as soft, slightly elevated mass and rarely exceeding 2 cm. The most prevalent ethnic group was leucoderma (68.2%), with melanoderma representing 18.2%. Non-specified ethnic group was reported in three cases. The preferential location was the maxillary bones (nine cases in the upper maxilla and eight cases in the mandible) followed by tongue and buccal mucosa. Among the 22 cases, 18 cases showed peripheral presentation involving only soft tissue (81.8%), while four cases, all located in the lower maxilla, were intraosseous (18.2%). None of the clinical diagnoses included neurofibroma as a hypothesis. Sixteen cases were represented by solitary neurofibromas (72.7%), while six cases present as multiple neurofibromas (27.3%). Among the five cases that presented as multiple neurofibromas, only one had a previous diagnosis of NF-1.

Table I. Distribution of the clinical and histopathological features of the 22 cases of NFs in the oral cavity.

Clinical and histopathological features		Number of cases	Percentage (%)
Age	≤ 50 years	11	50
	> 50 years	11	50
Gender	Male	6	27.3
	Female	16	72.7
Ethnic group	Leucoderma	15	68.2
	Melanoderma	4	18.2
	Non-specified/other	3	13.6
Site	Upper maxilla	9	40.9
	Lower maxilla	8	36.4
	Tongue	2	9
	Jugal mucosa	1	4.5
	Non-specified	2	9
Distribution	Isolated	16	72.7
	Multiple	6	27.3
Association with NF-1	Yes	1	4.5
	No/Non-specified	21	95.5
Capsule-like	Yes	8	36.4
	No	14	63.6
Inflammation	Yes	11	50
	No	11	50
Mast cells	Yes	14	63.6
	No	8	36.4
Tactile-like bodies	Yes	7	31.8
	No	15	68.2

Histopathological features

The following data are seen in Tables I and II. Although most of the cases were relatively circumscribed, the neurofibromas were not encapsulated. In

Table II. Clinical, histopathological and immunohistochemical features of the 22 new cases of oral neurofibroma.

Case	Lesion	Variant	S-100	EMA	GLUT1	CD34	COL IV	p53	Ki67
01	Multiple	Localized	++	++	–	+	–	–	+
02	Isolated	Diffuse	+++	+++	–	+	–	–	+
03	Isolated	Diffuse	+	–	–	+	–	–	–
04	Isolated	Diffuse	+++	++	–	+	–	–	–
05	Isolated	Diffuse	+	+	–	+	+	+	+
06	Multiple***	Plexiform	+++	–	+++	+++	+	–	–
07	Multiple	Localized	+++	+++	–	++	–	–	–
08	Isolated	Diffuse	++	–	–	–	–	+	–
09	Isolated	Diffuse	+++	++	–	+++	–	–	–
10	Isolated	Diffuse	++	+	–	+++	+	–	–
11	Isolated	Diffuse	+++	–	–	+	–	–	+
12	Isolated	Localized	+++	+	–	–	–	+	–
13	Isolated	Localized	+++	+	–	–	+	+	+
14	Multiple	Diffuse	+++	+	–	+	+	+	+
15	Isolated	Localized	+++	+	–	+++	+++	+	–
16	Isolated	Plexiform	++	++	–	+	–	–	–
17	Isolated	Localized	+++	–	–	++	+	–	–
18	Isolated	Localized	+++	–	–	+++	+++	–	–
19	Isolated	Localized	+	–	–	–	–	–	–
20	Isolated	Diffuse	+	+	+	+	+	–	–
21	Isolated	Diffuse	+++	+	–	+++	–	–	–
22	Multiple	Diffuse	–	–	–	–	–	–	–

Case 06***: NF1 associated.

detail, eight cases (36.4%) were circumscribed by a capsule-like structure and 14 (63.6%) were non-circumscribed (or not encapsulated). The diffuse variant was more frequent (54.6%) than the localized one (36.4%). Only two cases (9%) presented plexiform type (PT), one of them presenting as an isolated NF and the other reporting NF-1.

Histologically, neurofibromas were composed of uniform and spaced spindle-shaped cells with elongated, ovoid-to-thin nuclei and scant cytoplasm surrounded by a collagenous matrix. Tumor cells were situated in a delicate and often myxoid stroma (Figure 1A). Peripheral nerve fibers were observed in the stroma (50%), as well as mild mononuclear inflammatory infiltrate (90%), mast cells (63.6%) and blood vessels (86.4%). Tactile-like bodies resembling Meissner and Pacinian corpuscles were observed in seven cases (31.8%), one of them of the plexiform type.

The two plexiform neurofibromas presented a classical histopathological pattern exhibiting a tortuous mass of expanded nerve branches with an abundant mucoid matrix and bundles of wavy, spindle cells. One of them occurred in the buccal mucosa (reporting NF-1) and the other in the mandible (solitary neurofibroma).

Immunohistochemical and histochemical results

These data can be seen in detail in Table II and in Figure 1B–F. Immunostaining revealed that the tumor cells were negative to GLUT-1 in 20 cases of neurofibromas analyzed. The tumor cells weakly expressed Collagen IV, Ki-67 and p53. However, a strongly positive stain to S-100 with only five cases revealing less than 5% of positive cells could be noted. Variably positive stain for CD-34 and membrane epithelial antigen (EMA) was detected. All cases showed a positive histochemical reaction for Alcian blue.

Discussion

Due to occasional histologic overlap, diagnosis of benign neural tumors can be challenging. In view of this, the purpose of this study was to evaluate clinical, histopathological and imunohistochemical features of 22 neurofibromas, in order to bring knowledge to correctly diagnose them. Our study focuses on: (1) presenting the clinical, histopathological and immunohistochemical aspects of NF; and (2) comparing the results to the literature regarding NF and other neural lesions to determine useful information for its differential diagnosis.

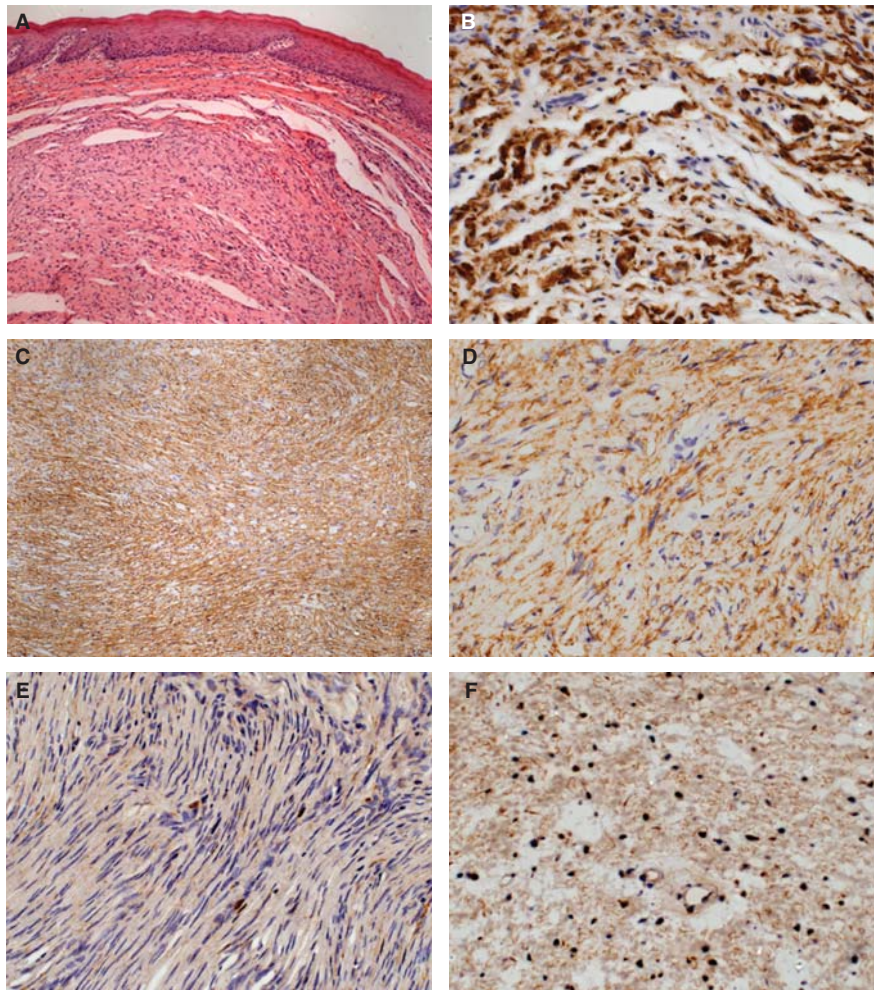

Figure 1. Histopathological and immunohistochemical aspects of NFs. (A) Light microscopy of neurofibroma. Hematoxylin-eosin (10×); (B) Immunohistochemical aspects of S-100 staining. Streptavidin-biotin. Very strong and widespread immunoreactivity for S-100 in neurofibroma (40×); (C and D) Aspects of CD-34 and EMA immunostaining. Streptavidin-biotin. Very strong and widespread imunnoreactivity for CD-34 (10×) and EMA (40×); (E) Immunohistochemical aspects of Ki-67. Streptavidin-biotin. Very faint and widespread staining (40×); (F) Immunoexpression of p-53 protein. Streptavidin-biotin. Mild nuclear and widespread staining (40×).

The literature reports that the frequency of solitary neurofibromas in the oral cavity remains ~ 6.5%, especially in lesions not associated with neurofibromatosis type 1 (NF-1) [3]. In the oral cavity, the preferential site is tongue, followed by buccal mucosa and lips [1], but it can also affect the gingiva, palate, major salivary glands and maxillary bones [3]. In the present study (Table I), the most prevalent site was the maxillary bone (upper and lower) and the least common site was buccal mucosa. In contrast to the literature, only two cases affected the tongue. On the other hand, our data are in agreement with the literature regarding the gender, age and ethnic group prevalence, showing a preference for white females older than 40 years [3]. All lesions studied here presented a benign clinical course characterized by the absence of symptoms and slow growth pattern that could be responsible for delay in the diagnosis, contributing to a late search for treatment, as frequently described in the literature [4,5].

Among the cases analyzed in the present study, 16 were isolated NF and only six were multiple. Since the clinical appearance of NF is similar to many other lesions of the oral cavity, its diagnosis can be challenging and, thus, biopsy is mandatory for the final diagnosis. Moreover, complementary immunohistochemical reactions are helpful in many times. In the present series, except for one case that had a previous diagnosis of NF-1, no clinical hypothesis included NF as a possible diagnosis, which points out the importance of a careful anamnesis. The examination should consider medical and familiar histories, as well as the use of complementary analysis as adjuvant helpers to the establishment of a correct diagnosis.

Histochemical reaction represented by Alcian blue was able to identify the collagen component, which was corroborated by S-100 staining (Figure 1B). In agreement with the literature, our NFs showed a heterogeneous mixture of S-100 negative and positive cells. While endoneurial cells, perineurial cells

and nerve sheath fibroblasts were S-100-negative, Schwann cells showed a positive staining for S-100 [5]. Supporting the neural lineage this study showed a positive staining for S-100 protein and epithelial membrane antigen (EMA) [7], as well as for CD-34 (Figure 1B–D). Additionally, the erythrocyte glucose transporter protein 1 (GLUT1), a marker for perineurial cells [8], showed weak staining in only two cases of the NF studied. The variable immunostaining pattern seen in the samples analyzed in the present study supports that the number of Schwann cells, perineurial cells and endoneurial fibroblasts may vary among the NF lesions and also within various areas of the same tumor, as reported previously [1]. Besides, the markers used here showed consistent results according to the current literature. Although the role or function of the CD34-positive cells in neural lesions remains unknown, this marker is considered useful in the analysis of NF1-related oral soft tissue tumors [9] and to stain endoneurial fibroblasts [10]. Moreover, the neural-associated CD34-positive cells are believed to represent a separate population of endoneurial cells that may serve as precursors or as supportive cells to other neural cells [1,8,11]. Taken together, these data can suggest the existence of an important supportive niche represented by this separate population of endoneurial cells (CD34-positive cells) within the cells of NF.

Malignant transformation from neurofibroma to malignant peripheral nerve sheath tumors (MPNSTs) has been reported, especially in patients with NF-1 [12]. Besides, in cases of MPNSTs arising from benign peripheral nerve sheath tumors, the early diagnosis has a direct impact towards a better prognosis [13]. For this reason, many immunohistochemical and molecular studies have recently suggested the Ki-67 and p53 as promising markers to improve the success in the early diagnosis of MPNSTs [13,14]. On this basis, the present investigation performed reactions, including p53 and Ki-67 markers, to exclude malignant transformation. Fortunately, in the present series, both p53 and Ki-67 were weakly positive in only six cases (among diffuse and localized types), reinforcing the benign pattern of the lesions analyzed here (Figure 1E and F). Although it is widely accepted that plexiform neurofibromas associated with the NF-1 increase the malignant transformation potential, the only case (case 6, Table II) that presented this association showed an absence of immunoexpression for p53 or Ki-67, diminishing a possible risk of malignant transformation. Among the variable pattern, in our series, the stronger immunostaining was presented by S-100, suggesting that the 22 neurofibromas included in this study were mostly composed by S-100-positive Schwann cells over other cells. Taken together to the low immunostaining for p53 and Ki-67 this result can also indicate that NFs massively composed of S-100-positive Schwann cells present low potential of aggressiveness and malignant transformation.

In general the diagnosis of NF should be based on specific pathologic features associated or not with complementary immunohistochemical reactions. Differential diagnosis of NF should include a range of lesions such as schwannoma, nerve sheath myxoma, palisaded encapsulated neuroma (PEN), traumatic neuroma, perineurioma and granular cell tumor (GCT). In order to guide this process, the present study reviewed, briefly, the literature to highlight important histopathological features and immunohistochemical markers comparing NFs to other benign neural lesions. This can be seen in detail in Tables III and IV [1,2,15].

According to the literature, oral benign neural tumors demonstrate variable immunoreactivity for S-100, which can be exemplified by the high positivity found in schwanommas and PEN in contrast with NF [1,4,16]. In the present study, although the NFs were not compared to other benign lesions, S-100 was the only marker that presented a diffuse and strong stain in almost all cases. Although the analyzed NFs presented a variable staining for CD-34, the positive immunoreaction for this marker may help in the differential diagnosis of NF against cases of PENs, schwannomas and traumatic neuromas, since these lesions showed only few scattered CD34-positive cells and/or staining restricted to capsules or Antony B tissue when these structures are present [1]. According to the present results, in the case of positive immunostaining, the CD-34-positive cells were diffusely distributed and were more numerous at the periphery of the lesions, in fibrous areas and in multivacuolated cells floating in myxoid areas.

In conclusion, the different types of nerve sheath cells observed in the present series reinforce the presence of a heterogeneous population in NFs. Although variable staining for S-100, EMA and CD34 was found in the present NFs, these data, associated with negativity for GLUT1, should be helpful for differential diagnosis. The low immunostaining for p53 and Ki-67 may indicate low potential of aggressiveness and malignant transformation of this lesion.

Table III. Immunohistochemical staining of oral benign neural tumors (data from the literature).

Tumor	S-100	EMA	CD34
Neufibroma	+	+	+
Schwannoma	+++	++*	++
Granular cell tumor	++	–	–
Traumatic neuroma	+	–	+
PEN	++	–	+
Perineurioma	–	+	–

PEN, Palisaded encapsulated neuroma; * Capsular staining.

Table IV. Differential diagnosis of benign neural tumors (review of the literature).

Tumor	Type	Histopathological contents
Neurofibroma	Non-encapsulated	Mixture of Schwann cells, perineurial cells and endoneurial fibroblasts
Schwannoma	Encapsulated	Schwann cells: cellular palisaded pattern (Antoni type A) and a loose paucicellular pattern (Antoni type B)
Granular cell tumor	—	Round or polygonal cells with small nuclei and abundant granular cytoplasm
Traumatic neuromas and PEN	Encapsulated	Proliferation of both Schwann cells and axons
Perineurioma	Non-encapsulated	Elongated spindle cells presenting bipolar cytoplasmic processes, fusiform nuclei and eosinophilic cytoplasm disposed embedded in a mixed collagenous and myxoid stroma

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