Original Article

Clinicopathological and immunohistochemical analysis of oral melanocytic nevi and review of the literature

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ABSTRACT

Introduction: Oral melanocytic nevi (OMNs) are uncommon benign melanocytic tumors, histologically similar to their cutaneous counterparts. The aim of this study was twofold: to contribute to the epidemiology with a literature review with the first Spanish series of OMNs, and to report on clinicopathological, immunohistochemical and demographic findings.

Materials and methods: A retrospective analysis of cases attended over the period 1999–2010 was carried out using data drawn from the pathology unit files at two public hospitals in the Spanish region of Andalusia, serving between them a population of 823,614 inhabitants (11%).

Results: Ten cases of OMNs were retrieved, accounting for 0.18% of the total 5499 oral biopsies performed over the period. The female-to-male ratio was 1.5:1; mean patient age was 30. The palate was the most common location (70%). Relative frequencies of histologic types were as follows: subepithelial (40%), compound (30%), junctional (20%) and junctional (10%). Immunohistochemical examination showed strong S-100 protein expression, variable reactivity to HMB-45 and high c-Kit expression by junctional melanocytes. Ki-67 was ≤3 in all cases.

Conclusions: Although this first clinicopathologic analysis of OMNs reported in Spain was based on a small patient series, the results are in line with those reported in larger series and additionally provide new demographic data. Since OMNs and early melanomas are usually detected at routine dental examination, detailed oral exploration should always be performed, and in case of doubt a biopsy should be taken to ensure an accurate diagnosis.

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1. Introduction

Oral mucosal melanocytic nevi (OMNs) are uncommon benign melanocyte tumors histologically resembling those found in the skin.

Although characterized until a few years ago as hamartomas, melanocytic nevi are now regarded as true neoplasms, due to their common expression of oncogenic mutations in BRAF and less frequently in NRAS, HRAS and GNAQ genes [1].

OMNs account for between 0.067% and 1.5% of accessed oral biopsies [2] with an incidence of 4.35 cases per 10 million persons per year [3].

Most OMNs are acquired neoplasms [2,3], although infrequent- ly they may be congenital or a manifestation of a rare genetic disorder [4]. A recent review of the English-language literature revealed only five well-documented cases of oral congenital melanocytic nevi [5].

Two-thirds of reported OMNs are found in females, and mean age at diagnosis is 35 [6,7]. Nevi are usually small—on average 0.5 cm [7]—well-circumscribed, round-to-oval lesions; the surface may either be flat (macula) or raised (papules); more rarely, prominent lesions such as nodules [2,3] or polypoid masses have been described [8]. Histologically, the most common type is the subepithelial nevus followed by the common blue nevus; compound nevus and junctional nevus are less frequent [2,3,7,9]. OMNs are asymptomatic lesions discovered by chance on routine dental examination [2,3]. Since an accurate clinical diagnosis is not usually made at that point, excision is required to rule out other pigmented solitary lesions or even early melanoma [2,3].

This study sought to augment the epidemiological knowledge with the first Spanish series of OMNs, based on cases drawn from the pathology unit files at two public hospitals in the Andalusia region, and to report on demographic, epidemiological, clinicopathological and immunohistochemical findings.
Table 1
Epidemiological, clinical, surgical, pathological and immunohistochemical findings of OMNs.

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex/age</th>
<th>Site shape†</th>
<th>Size (mm)</th>
<th>Clinical diag.</th>
<th>Surg. proc.</th>
<th>Histologic type</th>
<th>Immunohistochem (%)</th>
<th>Request pathol report</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F/18</td>
<td>Palate F</td>
<td>2</td>
<td>Pigmented lesion</td>
<td>Excision</td>
<td>Junctional</td>
<td>&gt;90</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>F/18</td>
<td>Gingiva R</td>
<td>6</td>
<td>Fibroma</td>
<td>Excision</td>
<td>Compound</td>
<td>&gt;90</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>M/39</td>
<td>Palate F</td>
<td>4</td>
<td>Tumor</td>
<td>Incision</td>
<td>Compound</td>
<td>&gt;90</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>M/33</td>
<td>Oral mucosa R</td>
<td>3</td>
<td>Nevus</td>
<td>Excision</td>
<td>Subepithelial</td>
<td>&gt;90</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>F/21</td>
<td>Palate R</td>
<td>2</td>
<td>Nevus</td>
<td>Excision</td>
<td>Subepithelial</td>
<td>&gt;90</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>F/18</td>
<td>Palate R</td>
<td>10</td>
<td>Soft tumor</td>
<td>Excision</td>
<td>Subepithelial</td>
<td>&gt;90</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>F/25</td>
<td>Palate R</td>
<td>3</td>
<td>Nevus</td>
<td>Excision</td>
<td>Subepithelial</td>
<td>&gt;90</td>
<td>70</td>
</tr>
<tr>
<td>8</td>
<td>F/47</td>
<td>Palate F</td>
<td>4</td>
<td>Melanotic macule</td>
<td>Excision</td>
<td>Common blue</td>
<td>&lt;90</td>
<td>80</td>
</tr>
<tr>
<td>9</td>
<td>M/50</td>
<td>Palate R</td>
<td>3</td>
<td>Nevus</td>
<td>Excision</td>
<td>Common blue</td>
<td>&gt;90</td>
<td>80</td>
</tr>
<tr>
<td>10</td>
<td>M/31</td>
<td>Oral mucosa R</td>
<td>7</td>
<td>Melanotic macule</td>
<td>Excision</td>
<td>Common blue</td>
<td>&gt;90</td>
<td>80</td>
</tr>
</tbody>
</table>

† Shape: F; flat; R: raised.
†† Odontology.
‡ Maxillofacial surgery.
§ Dermatology.

Estrogen receptor was also tested and no labeling was seen in any case.

2. Material and methods

A retrospective systematized evaluation was performed of all oral melanocytic pigmented lesions documented between January 1999 and December 2010 in the Pathology files of two public hospitals in the Spanish region of Andalusia: Torrecárdenas Hospital (Almería) and Virgen Macarena Teaching Hospital (Seville). The two hospitals serve a total population of 823,614 inhabitants, i.e. 10.5% of the region’s inhabitants (n = 7,849,799 in 2005).

Oral biopsies (total 5499) accounted for 1.67% of all specimens. Biopsy data were retrieved from Pathology Unit files using the following keywords: mucosa, mouth, oral cavity, cheek, alveolar mucosa, gingiva, palate, tongue, lip mucosa and floor of the mouth. Later, a new database search was carried out focussing on oral biopsies alone, including the terms nevi, nevus, nevocellular, melanocytic, melanotic, intramucosal, Spitz and combined. Nevus located on skin side of the lip, oral nevi for which data were incomplete, lesions diagnosed as melanocytic hyperplasia and non-melanocytic nevi were excluded from the study. Thus, a total of 46 cases of melanotic lesions (33 melanotic macula, 3 melanoma and 10 OMNs) were described. A total of 10 OMNs were the total number of cases encountered of these lesions and were prepared for evaluation. Histology slides were re-assessed and classified in accordance with well-established criteria [3,6] into the following categories: conventional melanocytic nevi (junctional, compound and subepithelial, rather than intramucosal) and common blue nevi. The junctional nevus is formed by a nest of compact melanocytes, known as thèques, occurring at the epithelial–subepithelial junction; the subepithelial nevus (usually termed intramucosal nevus) contains melanocytes located solely in subepithelial connective tissue; the compound nevus comprises thèques of melanocytes at the epithelial–connective tissue interface, together with nests of melanocytes in the subepithelial connective compartment; the common blue nevus is characterized by a subepithelial proliferation of pigmented, elongated and dendritic melanocytes, spreading in short fascicles, usually aligned parallel to the overlying epithelium.

All specimens were fixed in 10% buffered formalin, embedded in paraffin, serially sectioned and routinely stained with hematoxylin and eosin. Immunohistochemical study was performed with the Dako EnVision peroxidase/DAB complex system (Dako®, Glostrup, Denmark), using the following primary antibodies: S-100 protein, HMB-45, Estrogen receptor and Ki-67 (MIB1), (Dako®, Glostrup, Denmark), and c-Kit (Novocastra®, Newcastle, UK). The immunoreaction was evaluated as percentage of labeled cells in 10–15 randomly-counted high power fields (400×).

Clinical information was retrieved from the submitted biopsy request form. Follow-up data and some additional details were collected from the hospital files and also from the contributors. No dermoscopic exploration was made in any of the cases. All except one case (case 3) were excisional biopsies. Follow-up ranged from 3 to 12 years (mean 7 years).

3. Results

The 10 cases of OMN accounted for 0.003% of all biopsies and 0.18% of the 5499 oral biopsies performed over the study period. By contrast, melanotic macula accounted for 0.60% (33 cases) and primary oral melanoma for 0.054% (3 cases) of all accessed oral biopsies in the same period. Clinical, histopathological and immunohistochemical data, together with the name of the clinical unit requesting pathology reports, are summarized in Table 1.

The female-to-male ratio was 1.5:1, and mean patient age at excision was 30 (range 18–50); mean age was 24.2 (range: 18–33) for patients with subepithelial nevus and 43 (range: 31–50 years) for those with common blue nevus.

The palate was the predominant location (7 cases) followed by the oral mucosa (2 cases) (Fig. 1) and gingiva (1 case). Seven lesions were described as raised and 3 as flat. Mean size was 4.4 mm (range: 2–10 mm). Clinical diagnoses included nevus (4; 40%) and melanotic macula (2; 20%). No cases were diagnosed as melanoma.

![Fig. 1](image-url) Slightly raised grayish lesion, near commissure. Histologically identified as common blue nevus (case 10).
Fig. 2. (A) Proliferative melanocytic cells entirely located in the subepithelial connective tissue compartment. Subepithelial melanocytic nevus. H&E 200× (case 7). (B) 1. Common blue nevus showing elongated and spindle-shaped melanocytic cells heavily pigmented at the subepithelial tissue. Cells are parallel to the surface epithelium. H&E 200× (case 10). (B) 2. Detail showing dendritic cells with cytoplasmic pigment. H&E 400× (case 10). (C) Stromal mucinous change (black arrow) in a subepithelial melanocytic nevus. H&E 100× (case 4). (D) Isolated pleomorphic nuclei (black arrow) and numerous ectasic vessels. Subepithelial melanocytic nevus. H&E 100× (case 7).
Histologically, the 10 OMNs were classified as subepithelial [4] (Fig. 2A), common blue [3] (Fig. 2B), compound nevus [2] and junctional nevus [1]. Melanin pigment was present in all cases. Two cases of subepithelial nevi presented special features: one displayed a mucinous stromal change (Fig. 2C) and the other contained isolated pleomorphic nuclei and prominent ectasic vessels (Fig. 2D). No cases of either combined nevus or Spitz nevus were recorded.

Immunohistochemistry (Table 1) revealed diffuse nuclear and cytoplasmic staining to S-100 (Fig. 3A) and irregular cytoplasmic staining, mainly in upper melanocytes, to HMB45, in conventional melanocytic nevi (Fig. 3B). All blue nevi cases showed diffuse immunostaining to S100 and HMB45. C-Kit showed high transmembrane immunoreactivity, especially in junctional melanocytes (Fig. 3C). The Ki-67 (MIB1) index was less than 3% in all cases (mean: 1.6%). Alpha estrogen receptor immunostaining was negative in all cases.

Follow-up varied from 3 to 12 years (mean 7 years). All except one case (case 3) underwent excisional biopsies; although nevi were incompletely removed in two cases, no recurrences were noted.

Finally, the following units requested pathology reports: maxillofacial surgery, 5 cases (50%); odontology, 4 cases (40%); and dermatology, 1 case (10%).

4. Discussion

Cutaneous melanocytic nevi are very common; a study in the Scottish population found that by the third decade of life the mean mole count in both males and females had reached 27.5 [10]. By contrast, OMNs are very rare: a review of the literature up to 2002 [2] found fewer than 300 reported cases of OMN, mostly either in isolation or in small series. An additional Medline database search from 2003 to 2015 disclosed a further 382 documented cases of OMNs, grouped into 40 papers, three of which [2,3] accounted for 81% of all cases. Thus, the English-language literature up to 2015 contains 682 documented cases of OMN.

Very few studies have addressed the incidence and relative frequency of OMN. In three wide-ranging reviews, OMNs accounted for between 0.067% and 0.15% of all oral biopsies [2]. Here, the rate was higher (0.18%).

The most representative OMN series [2,3] are shown in Table 2. These studies, which comprise a total of 440 cases, i.e. 64.5% of all reported oral nevi, confirm the preference of OMNs for the palate, and the predominance of the subepithelial type followed by the common blue nevus type. Similar findings were recorded here for the most common histologic types, with only small differences in proportions. The table includes the present series, in which two cases of subepithelial nevi with uncommon features are highlighted: one with mucinous stroma and other with isolated pleomorphic melanocytes intermixed with ectasic vessels. The latter changes have been reported in senescent melanocytic nevi of the skin, but not hitherto in mucosal nevi [11].

All cutaneous subtypes have also been observed in the oral mucosa, with the exception of the halo nevus, the pigmented spindle-cell nevus and the deep penetrating nevus. The clinical appearance of OMNs is not diagnostic, so a biopsy is usually required to exclude other pigmented lesions such as melanocytic maculas, amalgam tattoos or other pigmented or vascular lesions and, more important, early malignant melanoma [2,3]. Treatment should be a complete excisional biopsy, with a view to preventing recurrence. Though the risk of malignancy is negligible, some authors [2,3,6] recommend excision of OMNs, especially in all unexplained pigmented oral conditions.

Fig. 3. Immunostain. (A) Diffuse cytoplasmic and nuclear staining to S-100 in a subepithelial melanocytic nevus. 200× (case 6). (B) Cytoplasmic staining to HMB-45. Note progressive loss of expression in the deepest cells. Subepithelial melanocytic nevus. 200× (case 5). (C) Strong positivity for c-Kit in a junctional melanocytic nevus. 400× (case 1).
Immunohistochemical examination of OMNs—as for their cutaneous counterparts—tends to reveal a characteristic profile [12], marked by strong S-100 expression in normal and neoplastic (benign and malignant) melanocytes; S-100 is thus regarded as a highly sensitive marker [12]. Staining for HM45 tends to be more irregular and patchy, since deep mature nevus cells display no staining. It is important to bear in mind that diffuse HM45 immunostaining is suggestive of possible malignancy. MIB1 (anti-Ki67) may be useful for the differential diagnosis of malignancy in junctional and subepidermal melanocytic masses when the proliferation index is ≥10%, although some malignant melanomas may have indices ≤5% [12]. C-Kit does not differentiate between benign and malignant melanocytic lesions, but may be useful as a therapeutic target in mucosal melanomas [13]. Since immunohistochemical findings here pointed to a benign immunophenotype pattern in all cases, only four antibodies were tested.

In the last few years various new antibodies, mainly cycle regulatory proteins such as p21 and cyclin D1, protein Skp2, as well as the cell proliferation markers minichromosome maintenance and geminin [14,15], have been tested in order to clarify the pathogenesis of oral malignant melanoma and to investigate differences between OMN and oral malignant melanomas. Studies report strong labeling in malignant melanomas whereas melanocytic nevi tend to be negative or, more rarely, weakly positive [14,15].

Although OMNs and oral malignant melanomas share certain features, there is not sufficient evidence to regard OMNs as obligatory precursor lesion [2,3]. In fact, only one case of malignant melanoma has been reported [16], in association with a blue nevus, located in the mucosa of the upper lip; however the literature contains a number of published cases of oral malignant melanomas associated with various types of melanosis [17].

The findings for this limited OMN series are in line with those of larger series, and also provide demographic data on this melanocytic lesion in Spain.

**Disclosure of interest**

The authors declare that they have no competing interest.

**References**


