Case Report
Melanotic oncocytic metaplasia of the nasopharynx: a case report with a focus on immunohistochemical analyses and literature review

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Received December 3, 2014; Accepted January 28, 2015; Epub February 1, 2015; Published February 15, 2015

Abstract: Melanotic oncocytic metaplasia (MOM) of the nasopharynx is an extremely rare lesion, with only 21 cases reported in English literature to date. MOM typically occurs near the Eustachian tube opening in Asian men in their 60s to 70s. Here, we present a case of MOM in a 57-year-old Japanese man who is a heavy smoker. The patient did not have complaints; MOM was diagnosed incidentally as 4 flat elevated lesions with brown to black discoloration, ranging from 2 to 3 mm in maximal diameter, were found in the right torus tubarius. Upon immunohistochemical examination, melanocytes displayed reactivity for 3 out of 4 melanocytic markers; immunopositivity for S-100 protein, Melan-A, and MITF and immunonegativity for HMB-45 was observed. Normal melanocytes in the nearby surface respiratory epithelium displayed the same pattern of immunoreactivity. Immunopositivity for S-100 protein and immunonegativity for HMB-45 have been previously reported in MOM. Reduction of stimulation of melanocytes in a longstanding lesion like MOM may explain the immunonegativity for HMB-45. S-100 protein, in conjunction with more specific marker for melanocytes, Melan-A or MITF, could prove the definite presence of melanocytes in this case of MOM. As it has been shown by previous reports that MOM pursues a benign course, it will be sufficient to follow up the patients regularly for the remaining 3 lesions.

Keywords: Melanotic oncocytic metaplasia, nasopharynx, immunohistochemistry

Introduction
Melanotic oncocytic metaplasia (MOM) of the nasopharynx is an extremely rare lesion, first described by Shek et al. in 1995 [1]. There have been only 21 cases reported in English literature to date [1-12]. MOM has typically been diagnosed near the Eustachian tube opening in Asian men in their 60s to 70s. MOM lesions are usually a few millimeters in size with a slight elevation and show brown to black discoloration. Multiple lesions and bilateral involvement have sometimes been encountered in patients [5]. The associated representative symptoms such as otitis media and tinnitus may be caused by compression of the Eustachian tube [11].

Although MOM may be macroscopically misidentified as early nasopharyngeal carcinoma, malignant melanoma, or nevus, it is microscopically distinct from these three diseases; MOM is characterized by an admixture of both melanin pigmentation and oncocytic metaplasia in the same gland [9]. The source of melanin was attributable to the numerous melanocytes found to coexist in MOM. Upon immunohistochemical examination, the melanocytes were found to be positive for S-100 protein and negative for HMB-45, both of which are melanocytic markers; only one study on MOM tested for immunonegativity for HMB-45. S-100 protein, in conjunction with more specific marker for melanocytes, Melan-A or MITF, could prove the definite presence of melanocytes in this case of MOM. As it has been shown by previous reports that MOM pursues a benign course, it will be sufficient to follow up the patients regularly for the remaining 3 lesions.

In this study, we present an additional case of MOM, where the melanocytes stained positively for S-100 protein and negatively for HMB-45, as found by IHC analysis, as was expected. We further analyzed the immunoreactivity of the melanocytes for two additional melanocytic
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markers, Melan-A and MITF, and found that the cell showed positive staining for both the markers. This pattern of expression signature of melanocytes in MOM is discussed in this report and extensive review of the literature was conducted to extract characteristics of MOM.

Clinical findings

A 57-year-old Japanese man, who was regularly followed up and treated at a local clinic for hypertension and hyperlipidemia, was referred because of elevated carcinoembryonic antigen (CEA; 8.9 ng/ml; normal range: < 5 ng/ml) levels and a positive result for fecal occult blood test. He is a heavy smoker with a history of smoking 30 cigarettes per day for the past 40 years (Brinkman index: 1200). Upper and lower endoscopic examination revealed no abnormality. By computed tomography (CT), several nodules with central cavities in the bilateral lung were identified. Metastatic or inflammatory lesions were suspected, but the patient refused a TBLB. The head and neck region was examined to search for the origin of a possible metastatic tumor. No palpable mass was found in this region. The patient underwent laryngoscopy, and 4 flat, elevated lesions with brown to black discoloration, ranging from 2 to 3 mm in the maximal diameter, were found in the right torus tubarius (Figure 1A, 1B). Melanoma was suspected, and a biopsy was collected from the largest lesion; examination revealed MOM. Examination of the nasal cavity, pharynx, and larynx revealed no additional lesions. Subsequently, fluorodeoxyglucose positron emission tomography was performed. No abnormal accumulation was found in the lung or any other organs. Six months later, the patient was reexamined by CT. The lesions that had been initially detected had disappeared and were therefore considered inflammatory lesions. However, the patient’s CEA level was still elevated (6.2 ng/ml); the increased CEA level was suspected to be a result of heavy smoking. As the lesions of the right torus tubarius were not enlarged, he was referred back to the local clinic.

Pathological findings

The biopsied tissue was fixed in 10% buffered formalin. The specimen was then embedded in paraffin. Sections were cut for hematoxylin and eosin (H&E) staining (2.5 μm thick sections), Fontana-Masson staining (2.5 μm thick sections), and immunohistochemical analysis (IHC; 4 μm thick sections). IHC analysis was performed using an automated slide stainer (Bench-Mark GX; Ventana Medical Systems, Tucson, AZ, USA). Giemsa staining was used as a counterstain for the specimen used for IHC analysis.

Figure 1. Laryngoscopical examination revealed lesions in the right torus tubarius. (A) One of the 4 flat elevated lesions found in the right torus tubarius. It displayed brown to black discoloration and ranged from 2 to 3 mm in maximal diameter. (B) (A) in higher magnification.
Figure 2. Histopathological findings. A. The surface of the lesion was covered by respiratory epithelium with goblet cells. It was composed of pre-existing seromucous glands with diffuse oncocytic metaplasia (× 20). B. Oncocytes presented abundant eosinophilic granular cytoplasm. Brown pigments were also observed in the cytoplasm of many
Microscopically, the lesion was well demarcated, but was not encapsulated. The surface was covered by respiratory epithelium with goblet cells. The lesion was composed of pre-existing seromucous glands with diffuse oncocytic metaplasia (Figure 2A). Oncocytes presented abundant eosinophilic granular cytoplasm. Brown pigments were also observed in the cytoplasm of many oncocytes (Figure 2B). Upon closer inspection, melanocytes were found to be hardly discernible. Mitotic figures or atypia of oncocytes were not observed (Figure 2C). The brown pigments were positive for Fontana-Masson staining (inset), indicating melanin or lipofuscin.

IHC analysis revealed melanocytes in the basal layer and intervening glandular cells. The melanocytes were positive for S-100 protein (polyclonal, 1:1000; Dako, Glostrup, Denmark) (Figure 3A), HMB-45 did not show immunoreactivity (HMB-45, 1:100; Dako) (Figure 3B). The melanocytes were found to be immunoreactive to Melan-A (A103, 1:50; Dako) (Figure 3C) and MITF (D5, 1:100; Dako) (Figure 3D). Melanocytes also existed in the surface respiratory epithelium near the lesion, which showed the
same staining pattern on IHC for the 4 antibodies (Figure 4A-D). As a counterstain of IHC, Giemsa staining was performed and displayed metachromasia, where the color of pigments changed from brown to green. This phenomenon was observed in all IHC specimens. This observation, together with the positive Fontana-Masson staining, demonstrated that the pigments were indeed melanin.

The diagnosis of MOM was rendered, supported by the result of H&E staining, special staining, and IHC analysis, along with the results of macroscopic observations.

Discussion

This case of the extremely rare lesion, MOM, is the 22nd reported case (Table 1) [1-12]. MOM comprises both oncocytic metaplasia and melanin pigmentation of the epithelium in the same gland. Oncocytic metaplasia of the glandular epithelium alone is sometimes encountered, which is considered an age-related phenomenon in the nasopharynx [13] and is common in the salivary gland [14]. Because oncocytic metaplasia is more frequently encountered than melanin pigmentation in the nasopharynx, it is speculated that melanin pigmentation occurred after oncocytic metaplasia. It has also been suggested that smoking may be a predisposing factor for melanin pigmentation. This speculation is supported by the observed relationship between oral melanin pigmentation and smoking in some Asian populations [15]. Male predominance can be explained by the fact that there are more male than female smokers among Asians [5].
### Table 1. Cases of melanotic oncocytic metaplasia of the nasopharynx

<table>
<thead>
<tr>
<th>Case</th>
<th>Author</th>
<th>Year</th>
<th>Gender/Age</th>
<th>Number of lesions</th>
<th>Site</th>
<th>Symptom</th>
<th>Smoking history</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Shek et al. [1]</td>
<td>1995</td>
<td>M/67</td>
<td>Single</td>
<td>Eustachian opening</td>
<td>Otitis media</td>
<td>Unknown</td>
</tr>
<tr>
<td>2</td>
<td>Shek et al. [1]</td>
<td>1995</td>
<td>M/63</td>
<td>Single</td>
<td>Eustachian opening</td>
<td>Tinnitus</td>
<td>Unknown</td>
</tr>
<tr>
<td>3</td>
<td>Kurihara et al. [2]</td>
<td>1997</td>
<td>M/69</td>
<td>Multiple</td>
<td>Left nasopharynx</td>
<td>None</td>
<td>Unknown</td>
</tr>
<tr>
<td>4</td>
<td>Hirakawa et al. [3]</td>
<td>1999</td>
<td>M/64</td>
<td>Multiple</td>
<td>Bilateral Eustachian opening, left pharynx, and nasal cavity</td>
<td>Discomfort in the throat</td>
<td>Unknown</td>
</tr>
<tr>
<td>5</td>
<td>Xue et al. [4]</td>
<td>1999</td>
<td>M/70</td>
<td>Multiple</td>
<td>Bilateral Eustachian opening</td>
<td>Tinnitus</td>
<td>Unknown</td>
</tr>
<tr>
<td>6</td>
<td>Sakaki et al. [5]</td>
<td>2004</td>
<td>M/80</td>
<td>Multiple</td>
<td>Right nasal cavity and pharynx</td>
<td>Hoarseness</td>
<td>10/day × 50 yr</td>
</tr>
<tr>
<td>7</td>
<td>Sakaki et al. [5]</td>
<td>2004</td>
<td>M/69</td>
<td>Single</td>
<td>Left Eustachian opening</td>
<td>Hoarseness</td>
<td>40/day × 60 yr</td>
</tr>
<tr>
<td>8</td>
<td>Sakaki et al. [5]</td>
<td>2004</td>
<td>M/74</td>
<td>Single</td>
<td>Left nasopharynx</td>
<td>Rhinorrhea</td>
<td>Unknown</td>
</tr>
<tr>
<td>9</td>
<td>Sakaki et al. [5]</td>
<td>2004</td>
<td>F/74</td>
<td>Multiple</td>
<td>Right Eustachian opening</td>
<td>Discomfort in the throat</td>
<td>Unknown</td>
</tr>
<tr>
<td>10</td>
<td>Sakaki et al. [5]</td>
<td>2004</td>
<td>M/68</td>
<td>Single</td>
<td>Nasopharynx</td>
<td>None</td>
<td>Unknown</td>
</tr>
<tr>
<td>13</td>
<td>Takano et al. [6]</td>
<td>2004</td>
<td>M/62</td>
<td>Multiple</td>
<td>Left Eustachian opening, and bilateral torus tubarius</td>
<td>Discomfort in the left ear</td>
<td>Unknown</td>
</tr>
<tr>
<td>15</td>
<td>Liao et al. [8]</td>
<td>2005</td>
<td>M/-</td>
<td>Multiple</td>
<td>Bilateral nasopharynx</td>
<td>None</td>
<td>Unknown</td>
</tr>
<tr>
<td>16</td>
<td>Kondo et al. [9]</td>
<td>2010</td>
<td>M/73</td>
<td>Multiple</td>
<td>Bilateral torus tubarius</td>
<td>Nasal congestion</td>
<td>Non-smoker</td>
</tr>
<tr>
<td>17</td>
<td>Li et al. [10]</td>
<td>2010</td>
<td>M/58</td>
<td>Multiple</td>
<td>Bilateral nasopharynx</td>
<td>Epistaxis</td>
<td>20/day × 40 yr</td>
</tr>
<tr>
<td>18</td>
<td>Na et al. [11]</td>
<td>2012</td>
<td>M/72</td>
<td>Multiple</td>
<td>Bilateral torus tubarius</td>
<td>Headache and impaired hearing</td>
<td>40/day × 50 yr</td>
</tr>
<tr>
<td>19</td>
<td>Na et al. [11]</td>
<td>2012</td>
<td>M/71</td>
<td>Multiple</td>
<td>Left torus tubarius and soft palate</td>
<td>Hoarseness</td>
<td>20/day × 40 yr</td>
</tr>
<tr>
<td>20</td>
<td>Na et al. [11]</td>
<td>2012</td>
<td>M/51</td>
<td>Multiple</td>
<td>Right torus tubarius</td>
<td>Pain of the tongue</td>
<td>Unknown</td>
</tr>
<tr>
<td>21</td>
<td>Chang et al. [12]</td>
<td>2014</td>
<td>M/63</td>
<td>Multiple</td>
<td>Nasopharynx</td>
<td>Epistaxis</td>
<td>40/day × 40 yr</td>
</tr>
<tr>
<td>22</td>
<td>Present case</td>
<td>2014</td>
<td>M/57</td>
<td>Multiple</td>
<td>Right torus tubarius</td>
<td>None</td>
<td>30/day × 40 yr</td>
</tr>
</tbody>
</table>

M, male; F, female; yr, year.
Melanocytes are consistently present in MOM as a source for melanin pigmentation in oncocyes [1-12]. Melanocytes have been reported to exist in the epithelium and stroma of the nasal cavity [16] and are postulated to colonize in MOM. Ultrastructurally, oncocyes in MOM lack premature melanosomes, in which melanin is produced [3]. Hence, the melanin pigment in MOM is believed to be passed on from the adjacent melanocytes via their dendrites [3].

Upon IHC examination of previous reports, there were some cells in MOM consistently found to be positive for S-100 protein and negative for HMB-45, both of which are melanocytic markers. Only one previous study confirmed the presence of melanocytes in MOM using a third melanocytic marker, Melan-A [12]. As S-100 protein is the most sensitive marker for melanocytes [17], positive staining for S-100 was expected. However, positive staining for S-100 protein does not conclusively identify cells as melanocytes due to its lack of specificity [17]. Although HMB-45 is a more specific marker for melanocytes than S-100 protein [17], it stains premelanosome glycoprotein abundant in adult melanocytes during stimulation and normal adult skin melanocytes are not stained [18]. This is a likely explanation for why HMB-45 staining was not observed in previous studies. It may also be presumed that melanocytes in MOM are not in a highly stimulated state at the time of biopsy, indicating a previously developed and longstanding nature of MOM. In contrast, we speculate that melanocytes were stimulated during MOM development, judging by the abundant melanin pigmentation. Another melanocytic marker, Melan-A, which is the same as MART-1, a transmembrane protein of melanocytes [19], is more sensitive than HMB-45 [20]. Melan-A/MART-1 stains normal skin melanocytes [19], explaining the positive staining of melanocytes in MOM for Melan-A. MITF is an integral transcriptional regulator in melanocytes and expression of this gene is required [21]. This fact may explain the finding that melanocytes in MOM are positive for MITF.

All the reported cases of MOM followed a benign clinical course; there have not been any cases of recurrence or progression of MOM [11]. Simple excision, including excisional biopsy, is a suitable treatment of choice for MOM [6]. In conclusion, this is the 22nd reported case of MOM occurring in an Asian man who is a heavy smoker. Asian ethnicity and the history of heavy smoking are likely related to the development of MOM. In addition to 2 frequently used melanocytic markers, S-100 protein and HMB-45, 2 additional melanocytic markers with high specificity, Melan-A and MITF, were used in this case. The use of these additional markers increases the accuracy of the diagnosis, as S-100 is less specific as a marker for melanocytes and HMB-45, a more specific marker, hardly recognizes melanocytes not being stimulated. Some cells of MOM in this case were shown to stain positively for Melan-A and MITF, which indicated the definite presence of melanocytes. Using S-100 protein and HMB-45 alone would not be sufficient for evaluating benign and dormant melanocytic lesions. Since MOM pursues a benign course, it will be sufficient to follow up regularly with the patient to evaluate progression of the remaining 3 lesions that were not biopsied.

Disclosure of conflict of interest
None.

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References
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