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“Oral Melanoma: Histopathological and Molecular Alterations”

Autor:

Fátima Mariana Martins Soares

Aluna do 5º Ano do Mestrado Integrado em Medicina Dentária

Contacto: fatimamarianasoares@gmail.com

Orientador:

Prof. Doutor Pedro Sousa Gomes

Professor Auxiliar

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“Para ser grande sê inteiro: nada

Teu exagera ou exclui.

Sê todo em cada coisa. Põe quanto és

No mínimo que fazes. (...)”

Fernando Pessoa

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ABSTRACT

Introduction: Oral Melanoma is a rare entity accounting for 0.8 to 8% of all melanomas and 0.5% of all oral neoplasms. The melanocytic lesions that appear on the oral cavity are based on genetic mechanisms with no association to ultraviolet (UV) light exposure, but the etiologic factors for the former are not established yet. Oral melanoma usually develops in people over 50 years old and has a male predisposition. The palate and maxillary gingiva are the most affected sites. In the majority of cases oral melanoma is an asymptomatic disease, without pain in early stages, which contributes to the delay in the diagnosis.

Objective: This work aims to review the existing literature related with oral melanoma, detailing its molecular and histopathological alterations.

Materials and Methods: A comprehensive literature review was performed using the PubMed, Medline, EBSCO and SCOPUS electronic databases, in order to identify publications about Oral Mucosal Melanoma, in the English language, between 2006 and 2016, and with full text available.

Discussion: The American Joint Committee on Cancer (AJCC) considered necessary the creation of a separate classification system from the one used for all other neoplasms of the head and neck region, which reflected the high recurrence rate and low overall survival of oral mucosal melanoma. The 5-year survival rate reported for oral melanoma ranges from 12% to 55%.

The most common mutation harbored in oral melanoma is found in the tyrosine-protein kinase (KIT). Mutations on v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*) oncogene are uncommon in oral melanoma and are more frequent in cutaneous melanoma. Alterations in *p53* gene allow oral melanoma to metastasize and have a more aggressive pattern.

Oral melanoma is an aggressive disease with high tendency to local recurrence and metastization. The best chance of survival is associated with the complete surgical removal of the tumor with negative margins. Radiotherapy, chemotherapy and immunotherapy can be used as adjuvants after resective surgery.

Conclusion: Oral mucosal melanoma and cutaneous melanoma differ greatly with respect to biological behavior and genetic alterations. Consequently, further prospective

studies and randomized clinical trials are required in order to better understand oral melanoma and to validate the optimal treatment protocols. Dental professionals play a critical role in the detection of mucosal melanoma, as throughout the clinical examination conducted in routine medical appointments, they should be alert to any alterations in the oral cavity.

Key Words: Melanoma, Oral Melanoma, Mucosal Melanoma, Oral Malignant Melanoma, Histopathological alterations, Molecular alterations.

RESUMO

Introdução: O melanoma oral é uma entidade rara que representa 0,8 a 8% de todos os melanomas e 0,5% de todas as neoplasias orais. As lesões melanocíticas que aparecem na cavidade oral têm origem em mecanismos genéticos que não estão associados com a exposição à luz ultravioleta, mas os seus fatores etiológicos ainda não estão completamente estabelecidos. O melanoma oral desenvolve-se em pessoas com mais de 50 anos de idade e mais frequentemente em homens. Os locais mais afetados são o palato e a gengiva maxilar. Na maioria dos casos o melanoma oral é uma doença assintomática, sem dor nas fases iniciais, o que contribui para o atraso no seu diagnóstico.

Objetivo: Este trabalho visa a realização de uma revisão da literatura existente acerca do melanoma oral, detalhando as suas alterações moleculares e histopatológicas.

Materiais e Métodos: Recorreu-se às bases de dados eletrónicas PubMed, Medline, EBSCO e SCOPUS, de forma a identificar publicações sobre o melanoma da mucosa oral, em Inglês, entre 2006 e 2016, e com texto integral disponível.

Discussão: O *American Joint Committee on Cancer* (AJCC) considerou necessária a criação de um sistema de classificação separado do usado nas restantes neoplasias da cabeça e pescoço, que refletisse a alta taxa de recorrência e a baixa sobrevida global do melanoma da mucosa oral. A taxa de sobrevivência a 5 anos, para o melanoma oral, varia entre 12% a 55%.

A mutação mais comumente encontrada no melanoma oral é na proteína tirosina-quinase KIT (KIT). Mutações no Homólogo B1 do oncogene viral de sarcoma murino v- raf (*BRAF*) são muito raras no melanoma oral e mais frequentes em pacientes com melanoma cutâneo. Alterações no gene *p53* conferem ao melanoma oral a capacidade de metastizar e a aquisição de um padrão mais agressivo.

O melanoma oral é uma doença agressiva, com alta tendência para a recorrência local e metastização. A melhor hipótese de sobrevivência está associada à remoção cirúrgica completa do tumor com margens negativas. A radioterapia, quimioterapia e imunoterapia podem ser utilizadas como adjuvantes, após a cirurgia ressectiva.

Conclusão: O melanoma da mucosa oral e o melanoma cutâneo são muito diferentes relativamente ao comportamento biológico e às alterações genéticas. Assim, são necessários mais estudos prospetivos e ensaios clínicos randomizados de forma a

compreender melhor esta condição patológica e a validar protocolos de tratamento adequados. O Médico Dentista desempenha um papel fundamental na detecção do melanoma oral, pois durante o exame clínico que realiza em todas as consultas deve estar alerta a quaisquer alterações na cavidade oral.

Palavras-Chave: Melanoma, Melanoma Oral, Melanoma da Mucosa, Melanoma Maligno Oral, Alterações Histopatológicas, Alterações Moleculares.

INTRODUCTION

Melanocytes are the pigment producing cells of the body which derive from the neural crest and migrate to the skin and mucosa, during embryogenesis. (1-3) Apart from the skin, melanocytes can also be found in mucosal surfaces of the body that derive from the ectoderm, such as sinuses, nasal passages, oral cavity, vagina, anus and conjunctiva. (4-6) Skin melanocytes have a protective function against the harmful effects of sun exposure; however, the function of these cells in the mucosa is still largely unknown. (7) When melanin production or melanocyte proliferation occurs, a wide range of occurrences can develop, ranging from physiologic pigmentations to malignant neoplasms. (7) If melanocytes suffer malignant transformation, melanoma develops. (7, 8)

Oral Melanoma is a rare entity accounting for 0.8 to 8% of all melanomas and 0.5% of all oral neoplasms. (2, 4, 9) Head and Neck Mucosal Melanomas (HNMM) account for less than 10% of the melanomas that develop on the head and neck region (4, 7), half of which appear on the oral cavity. (7, 10) The World Health Organization defined mucosal melanoma as a malignant neoplasm of melanocytes or of melanocytes' precursors. (10) According to the Melanoma Research Foundation, mucosal melanoma is a rare form of melanoma that occurs in the mucous membranes, such as the nasal passages, throat, vagina, anus or mouth, making up only about 1% of melanoma cases. (6)

Oral melanoma was first described by Weber in 1859 and recognized as a clinical entity by Lucke in 1869. (11) The incidence of HNMM is approximately 4 per 10 million individuals, per year, in the USA, and for oral melanoma specifically, is around 1.2 cases, per 10 million individuals, per year. (7, 12) Mucosal melanomas are more frequent in some areas of Africa, Japan or Uganda than in Western countries. (4, 13, 14)

Despite the majority of malignant melanocytic lesions appear on the skin, about 90%, they can develop anywhere in the body where melanocytes are found. (9) The melanocytic lesions that appear on the oral cavity are based on genetic mechanisms with no association to UV light exposure, as it happens for the lesions on the skin. However, the etiologic factors for the formers are not established yet. In fact, no obvious risk factors or even family history was associated with oral melanoma development. (4, 15, 16)

Regardless, some authors believe that tobacco use and chronic irritation can influence the development of mucosal melanoma. (4, 17)

Oral melanoma appears initially as a highly pigmented macule, colored black or brown, with ill-defined and irregular borders (10, 18), which can later originate a nodule or an ulcer. (19) In the upper aerodigestive tract, oral and sinus cavities are the most affected areas. (4) Usually, oral melanoma develops in people over 50 years old, with an average age of 61 years (4, 9, 20), more frequently on males. (9, 17, 20) The most common sites in the oral cavity are the palate and maxillary gingiva (80% of the cases), but it can also be found on the lips, jugal mucosa and floor of the mouth. (4, 17, 21) Oral melanoma lesions can be pigmented or amelanotic, which represents a greater challenge in diagnosis. (6, 22)

In the majority of cases oral melanoma is an asymptomatic disease, without pain in early stages, which contributes to the delay in the diagnosis. (13) For that reason, mucosal melanoma usually presents itself as a large lesion in an advanced stage, at diagnosis. (23)

Some authors believe that oral melanoma lesions should not be biopsied, but completely excised, because incisional biopsy can cause the risk of dissemination of malignant melanocytes and lead to metastization. (4, 19, 24) However, despite the absence on the identification of any precursor lesion, one third of the patients had preexistent mucosal melanosis. (1, 4, 13) Therefore, other authors believe that it is mandatory to perform an incisional biopsy of these pigmented lesions, as well as other suspicious lesions, to rule out the diagnosis of oral melanoma. (1, 13, 17) In fact, the majority of mucosal melanomas arise *de novo* from an otherwise normal mucosa, but about 30% are preceded by oral pigmentation for several months or years. (4, 14, 17)

To confirm the diagnosis, a panel of immunohistochemical markers is usually performed using the S-100 protein, Melan-A, HMB-45, tyrosinase (T311), microphthalmia transcription factor (Mitf), Vimentin and also by the demonstration of intracellular expression of melanin. (1, 13, 17)

Mucosal melanoma is a very aggressive disease, rated among the most malignant epithelial tumors of the human body, with tendency to local recurrence and distant metastasis. (4, 14, 16) Resecting surgery is the primary modality of treatment advocated by the majority of the authors (4) but it can be associated with radiotherapy, chemotherapy and immunotherapy, even though the effectiveness of these therapies, either as primary

treatment or in combination with surgery, is largely unknown. (13, 16, 17) The leading cause of death is systemic recurrence of the disease (4) and therefore, the majority of patients with HNMM will not survive beyond 5 years. (2)

Due to its rarity and the difficulty of developing prospective studies, oral melanoma is poorly described and little studied. As a result, there is a lack of data regarding its epidemiology, etiology and pathogenesis, and the establishment of standardized therapeutic guidelines is a great challenge. Hence, there is no consensus on the optimal systemic therapy. (2, 17, 25) The best chance of survival is associated with the complete surgical removal of the tumor with negative margins. However, wide free margins are difficult to obtain in the head and neck region without high morbidity and profound changes in the quality of life of the patients. (1, 3, 4) Early diagnosis and a multimodal aggressive treatment are the only means available to the medical community to offer the best results to patients with mucosal melanoma. (3) Patient prognosis can also be enhanced if the cancer is detected early and managed properly. (26)

In accordance, this work aims to review the existing literature on oral melanoma, detailing the molecular and histopathological alterations. Since oral melanoma is such a rare and aggressive disease for which etiological factors and optimal treatment strategy have not yet been defined, it is crucial to understand the molecular level alterations, verified in this condition. The study of the most common mutations affecting *KIT*, *p53*, *p16* and many other molecular pathways, enables the understanding of the mechanisms behind oral melanoma development and their trigger factors, in ways that promote the adequacy of existing therapeutic approaches and the establishment of new therapies.

MATERIALS AND METHODS

A comprehensive literature review was performed using the PubMed, Medline, EBSCO and SCOPUS electronic databases, in order to identify publications about Oral Mucosal Melanoma, in the English language, between 2006 and 2016, and with full text available. The following search terms were used: “oral melanoma”, “oral melanoma AND histopathological alterations”, “oral melanoma AND molecular alterations”, “oral malignant Melanoma AND histopathological alterations”, “mucosal melanoma AND molecular alterations”, “mucosal melanoma AND histopathological alterations”. The articles used for this paper were selected based on the review of the abstract and the relevance to the topic.

In addition to the articles resulting from the research in the mentioned databases, other articles, found to be relevant to the topic were also included, as well as information collected from reference books in the field of Oral Pathology, as indicated in the References section.

DISCUSSION

ETIOLOGY

Oral melanoma is a rare clinical entity with an undefined etiology and broadly unknown risk factors. In this way, it is not possible to prevent the establishment or development of this neoplasm, or even to identify patients at higher risk for development of oral melanoma. Regardless, some authors have suggested the possible role of tobacco use, formaldehyde exposure, chronic irritation (e.g. ill-fitting dentures) and exposure to inhaled and ingested environmental carcinogens at high body temperatures, in the transformation of melanocytes. (14, 17, 27) Regardless all the above mentioned, a study by Furney, et al., demonstrated that the genetic mutations found in oral melanoma cells were not the same as the ones caused by tobacco use in other neoplasms. This lead to the idea that tobacco use may not be involved in oral melanoma development. (28)

In the majority of cases oral melanoma is asymptomatic, but symptoms such as swelling, bleeding, ulceration, pain, ill-fitting dentures, tooth mobility, pigmented lesions, delayed healing of extraction sites or bone erosion may be present in a later stage of the disease. (29-31) Therefore, the diagnosis happens in a delayed fashion and mostly by accident, and not because the patient suspected of any changes in the oral cavity. (14, 29) All of these factors contribute to a very poor prognosis.

CLINICAL DIAGNOSIS

Clinically, oral melanoma diagnosis cannot be made using the ABCDE rule, as for cutaneous melanoma. Given the fact that signals such as asymmetry, irregular borders, irregular color, dimensions larger than 6 mm in diameter and elevation of the lesion appear late in the course of the disease and in situations in which there is already significant vertical invasion of tumor cells into the underlying tissue, clinicians should not apply the former rule. (17) Therefore, mucosal melanoma can be clinically staged as: Stage I - lesion limited to the primary site (Tany N0M0); Stage II - lesion associated with lymph node metastasis (Tany N1M0) and Stage III - lesion associated with distant metastasis (Tany N1M1). (16, 17)

HISTOLOGICAL DIAGNOSIS

Given the fact that the majority of HNMM (71 to 91%) are localized and identified in Stage I, and the classification system mentioned above gives great importance to metastization, Prasad et al., developed a microstaging system with 3 levels for stage I. Level I - *in situ* melanoma with no evidence of invasion or microinvasion (microinvasion is defined as the presence of individual or agglomerated invasive melanocytes with fewer than 10 atypical melanocytes near the subepithelial junction); Level II - melanoma cells limited to the lamina propria; and Level III - invasion of the deep connective tissue, including skeletal muscle, bone, or cartilage. Level I is defined above the epithelial basement membrane, Level II below the epithelial basement membrane but above the perimysium, or periosteum, or perichondrium and Level III represents invasion through the perimysium, periosteum, or perichondrium. According to these authors, histological level constitutes one of the most important histopathological predictors of poor survival. Hence, the breakage of each of the barriers represents a worse prognosis. (16)

Furthermore, oral melanomas can be classified as: *in situ* melanoma, when the lesion is confined to the epithelium and the epithelial-connective tissue interface; invasive melanoma, if it is extended into the connective tissue; and lesions with a combined pattern of invasive melanoma with *in situ* component. (7, 10, 17) The majority of mucosal melanoma lesions are identified during vertical growth phase and with an invasive or combined pattern. (7, 10) Despite, histological staging of these tumors is not yet completely defined and due to anatomical differences between the mucosa and the skin, the histological microstaging methods of Clark and Breslow's thickness, used on cutaneous melanoma, may not be suitable for the mucosal melanoma staging. (16, 17)

Oral melanoma cells exhibit a broad range of cellular morphological features on which spindle shaped, plasmacytoid, epithelioid and “clear” cells can be identified. (17) In the majority of cases the mucosal melanoma cell population is polymorphic. (32) Despite, spindle and epithelioid cells are the most commonly found, often in combination. (32, 33) The microscopic patterns in which mucosal melanoma cells can be arranged include sheet-like, organoid, alveolar, solid, neurotropic or desmoplastic. (8, 10, 34)

According to the literature, patients with non-epithelioid cell oral melanoma seem to have a better prognosis, with expected increased survival. In contrast, patients with epithelioid cell mucosal melanoma exhibit a higher risk for the development of distant

metastasis and a worse prognosis, suggesting the association with a more aggressive variant of the tumor. (6)

Neoplastic melanocytes exhibit hyperchromatic, angular and large nuclei with prominent nucleolus and infrequent mitotic figures. (7, 10, 17) Furthermore, these cells have lost contact inhibition that characterizes melanocytes, thus being able to form cell clusters. (7, 17)

Given the wide spectrum of forms which melanoma cells can adopt, the lesion may mimic other malignancies such as sarcoma, plasmacytoma, carcinoma, neuroendocrine lesions or papilloma (e.g. spindle cell oral melanoma can mimic sarcoma; epithelioid mucosal melanoma may suggest salivary carcinoma; plasmacytoid oral melanoma may resemble olfactory neuroblastoma or plasmacytoma) (29)

DIFFERENTIAL DIAGNOSIS

Differential diagnosis of all lesions which may mimic this neoplasm is a crucial step in the diagnosis of oral melanoma. According to all the literature available on this subject, differential diagnosis may include benign pigmented lesions such as melanotic macule, melanosis associated with tobacco use, drug induced pigmentation, melanoacanthoma, melanotic nevus, racial pigmentation, vascular lesions, pigmentation associated with systemic disease (e.g. Addison disease or Peutz-Jeghers syndrom) amalgam tattoo and post-inflammatory pigmentation; malignant pigmented lesions of which Kaposi sarcoma is an example. Amelanotic melanomas must be differentiated from a variety of non-pigmented neoplasms, such as sarcomas, poorly differentiated carcinoma, lymphoma and small cell carcinoma. (3, 8, 32)

IMMUNOHISTOCHEMICAL DIAGNOSIS

The immunohistochemical diagnosis in oral melanoma is performed using melanocyte differentiation markers such as Melan-A, HMB-45, tyrosinase and Mitf. Mucosal melanoma cells also express Vimentin and S-100 protein. (1, 9, 13) Another way to confirm the diagnosis is through the demonstration of intracellular expression of

melanin. (1, 9, 13) Oral melanomas do not express cytokeratins and epithelial membrane antigens. (10, 13) The clinical diagnosis of amelanotic melanomas presents a great challenge, hence immunohistochemical diagnosis is essential.

Mitf is a melanocytic nuclear protein which plays a crucial role in melanocytes embryonic development, postnatal viability and pigmentation. (35) T311 is a critical enzyme during melanin biosynthesis, being a sensitive and specific marker for melanoma. (36) Comparing the immunological markers used nowadays in oral melanoma diagnosis, Mitf is more specific than S-100 protein and is as specific as HMB-45. (35) S-100 protein and T311 seem to have a higher sensitivity when compared with HMB-45, but the latter seems to be more specific. (3, 32) Despite that, there is not a unique melanocyte marker with 100% sensitivity. (32)

HISTOPATHOLOGICAL AND MOLECULAR ALTERATIONS

As occurs in most human cancers, the malignant transformation of melanocytes is dependent upon the sequential accumulation of molecular and genetic alterations that seem to be independent of UV light exposure. (8, 27) Therefore, the biologic behavior of oral melanoma must be different from its cutaneous counterpart. However, the genetic and environmental risk factors implicated in oral melanomagenesis have not been described. (28) Considering that the results of cutaneous melanoma studies do not necessarily apply to mucosal melanomas, there is a need to further investigate this entity and develop a proper diagnosis and therapeutic approach. (37-39)

The most common mutation harbored in oral melanoma is found in the tyrosine-protein kinase KIT (KIT) (15-39% of the cases). (3, 28, 38) This protein has a critical function in the normal development and activity of melanocytes in the embryonic and postnatal stages. (8) Therefore, genetic aberrations affecting *KIT* oncogene, such as amplifications or activating mutations, play an important role in tumor growth and therapeutic approaches. In fact, there are case reports which demonstrate the clinical activity of KIT inhibitors, such as Imatinib, in melanoma treatment. (3, 40)

p53 mutations are one of the most prevalent events in human cancers, including mucosal melanoma, on which is detected in around 2/3 of the cases. (7, 17) *p53* is a tumor suppressor gene and when DNA is excessively damaged, *p53* protein promotes G1 arrest,

increases the function of DNA repair genes and induces apoptosis. (41) If p53 protein is mutated, it fails to regulate cell cycle and, therefore, to induce apoptosis, imparting the cell resistance to chemotherapy and radiotherapy. (31) This abnormality is associated with the ability of the neoplasm to metastasize, and associated with a more aggressive pattern of the disease. (7) In cutaneous melanoma, mutation of *p53* is uncommon. (41) According to Tanaka, et al., in oral melanoma, genetic aberrations affecting *p53* are correlated with high stage development of the neoplasm. (39) In summary, alterations in *p53* can function as a predictor of the malignant potential of oral melanoma and be used in diagnosis, prognosis and therapeutic approach. (39)

Cyclin-dependent kinase inhibitor 2A (*p16*) inhibits melanocytic proliferation leading to cell arrest, through the activation of p16cdkn2a pathway, which prevents the cell cycle to progress from G1 to S phase. (41) Therefore, alterations in this pathway leads to uncontrolled cell growth. The p16cdkn2a pathway is involved in the development of melanoma in sites with less sun exposure and the loss of *p16* expression was identified in about 50% of oral melanomas. (8, 42)

Mutations in *p53* and inactivation of *p16* are early events in tumorigenesis of mucosal melanoma and are associated with worse prognosis. (41, 43) These abnormalities suggest that the loss of programmed cell death and G1/S arrest play a role in the development of oral melanoma. (41, 42)

Oral melanocytic nevi are one of the differential diagnosis for oral melanoma. These lesions are more prevalent in the palate, which is the most common site for mucosal melanoma development in the oral cavity. (7, 44) This has led some authors to support that oral melanocytic nevi represents a precursor lesion of oral melanoma. (30, 44) However, in a study by Andrade et al., *p16* expression in oral melanocytic nevi was not altered, leading to the conclusion that the former benign lesion may not be a precursor of oral melanoma. (42) Regardless, oral melanocytic nevi prophylactic excision is recommended since their nature is unknown. (7)

B-cell lymphoma 2 (*Bcl-2*) encodes an anti-apoptotic protein that may also be mutated in oral melanoma. Its function is to prolong cell survival. (41) Expression of *Bcl-2* is also an early event in tumorigenesis but, contrary to *p53* and *p16*, it predicts better prognosis and longer survival of the patients. (41)

Mutations on v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*) oncogene are uncommon in oral melanoma and can only be found in 10% of the cases. (3, 40)

Furthermore, the abnormalities found on *BRAF* are highly associated to neoplasms arising in sites with intermittent sun exposure. (45, 46)

Lower incidence of *BRAF* oncogene mutations associated with a higher incidence of *KIT* oncogene mutations suggest a divergent genetic etiology and renders to oral melanoma uniqueness, when compared to the cutaneous counterpart. (13, 25)

Cyclin D1 (*CCDN1*) is a positive regulator of the G1/S checkpoint of the cell cycle. (42) In a study by Andrade et al., an increased expression of this molecule in oral melanomas was verified, as it happens in cutaneous and uveal melanomas, suggesting that cyclin D1 has a role in the pathogenesis of oral melanoma. (42) Cyclin-dependent kinase 4 (*CDK4*), a member of the cyclin dependent kinase (cdk) family, encodes a protein that binds to *CCND1*, allowing cell cycle progression. (46) In a study of Curtin, et al., amplifications in *CDK4* protein were more frequent in acral and mucosal melanomas. (46) *p16*, which functions as a negative regulator for *CDK4-CCDN1* complex, also showed losses in its *locus*, more commonly in mucosal and acral melanomas, losing the ability to control G1/S checkpoint. (46) If *CDK4* is amplified, it excessively binds to *CCDN1* leading to an uncontrolled progression of the cell cycle, increasing oral melanoma growth. (46)

Retinoblastoma (*Rb*) gene and the pRb2/p130 protein are growth suppressor agents, which are dysfunctional in several neoplasms. (39) According to Tanaka, et al., patients whose tumors were positive for pRb2/p130, had a higher 5-year cumulative survival rate when compared with pRb2/p130 negative tumors. (39) Thus, pRb2/p130 protein expression was associated with low stage of development of oral melanoma and a better clinical outcome. (39)

p21, a cyclin-dependent kinase inhibitor, functions as a tumor suppressor and its downregulation is associated to tumor invasiveness and metastasis formation. In melanoma, its expression is lower in metastatic, rather than in primary lesions, indicating a more aggressive phenotype. (42)

p27, a cyclin-dependent kinase inhibitor, is a suppressor of the G1/S cell cycle transition. (42) *p27* protein is highly expressed in benign lesions and it is lost as melanoma develops. (42) It has been demonstrated that the loss of heterozygosity (LOH) in the gene *locus* 12p13 leads to the loss of *p27* protein expression, allowing melanoma progression and the formation of metastasis. (17, 42, 47)

There are many other molecular pathways altered in melanoma that may assist on the explanation on how this disease develops. *Mitf*, which is regulated by *KIT*, regulates important genes for melanoma proliferation and it is amplified in approximately 15-20% of melanomas. (8) Phosphatase and Tensin Homolog (*PTEN*), a part of the phosphatidylinositol 3' kinase (PI3K) pathway, is a tumor suppressor gene responsible for regulating cell division, migration, spreading and apoptosis, which is also depleted in melanoma, contributing to tumor growth. (8, 46) The dysregulation of the Mitogen-activated protein kinase (MAPK) pathway, also known as Ras/Raf/MEK/ERK pathway, plays a crucial role in the development of various neoplasms, including melanoma, since it regulates cell survival, invasion and growth. (8, 33) The alterations in this pathway are associated with the mutations of several genes, such as *Ras*, *B-Raf*, *PI3K*, *PTEN* and *Akt*. (33) Since this pathway is constitutively activated in the majority of human melanomas, cells seem to be able to resist to programmed cell death, allowing tumor cells to growth and proliferate. (33, 48)

Melanocyte-specific gene 1 (*MSG-1*) may be useful for the understanding of oral melanoma pathogenesis given the fact that it is thought to be involved in embryogenesis, melanogenesis and malignant transformation of melanocytes. (17, 37) However, in a retrospective study by Sedghizadeh et al., *MSG-1* was expressed in only one mucosal melanoma case. Thus, this gene may be more associated with tumor development induced by UV light. (37)

STAGING

A specific staging system for oral melanoma has been established in 2010. (49) Prior to this date, patients were staged according to the tumor node metastasis (TNM) classification system of the AJCC, as for the cutaneous melanoma. This was a very simple staging system and did not reflect the true prognostic factors of oral melanoma. (2, 3)

According to the AJCC, a neoplasm can be classified has: Stage I - generally denotes cancers that are smaller or less deeply invasive with negative nodes; Stage II and III - defines cases with increasing tumor or nodal extent; and Stage IV - identifies those who present with distant metastases (M1), at diagnosis. (49)

Due to the aggressive nature of mucosal melanoma represented by the high recurrence rate and low overall survival, the AJCC considered that it was necessary to create a separate classification system from the one used for all other neoplasms of the head and neck region, which reflected these characteristics. Thus, the staging system for HNMM starts with Stage III, for primary cancers limited to the mucosa. (Tables 1 to 4). (13, 49) Lesions in an advanced stage are classified as T4a and T4b and stages I and II are not recognized for mucosal melanoma. Moreover, *in situ* mucosal melanoma was considered extremely rare by the authors and also excluded from the staging. (49)

Table I: AJCC Staging system for HNMM - Primary Tumor. Adapted from EDGE, et al., 2010. (49)

Primary Tumor

T3	Mucosal disease
T4a	Moderately advanced disease: Tumor involving deep soft tissue, cartilage, bone, or overlying skin
T4b	Very advanced disease: Tumor involving brain, dura, skull base, lower cranial nerves (IX, X, XI, XII), masticator space, carotid artery, prevertebral space, or mediastinal structures

Table II: AJCC Staging system for HNMM - Regional Lymph Nodes. Adapted from EDGE, et al., 2010. (49)

Regional Lymph Nodes

NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis present

Table III: AJCC Staging system for HNMM - Distant Metastasis. Adapted from EDGE, et al., 2010. (49)

Distant Metastasis

M0	No distant metastasis
M1	Distant metastasis present

Table IV: Anatomic stage and prognostic groups for HNMM. Adapted from EDGE, et al., 2010. (49)

ANATOMIC STAGE/PROGNOSTIC GROUPS

Stage III	T3	N0	M0
Stage IVA	T4a	N0	M0
	T3–T4a	N1	M0
Stage IVB	T4b	Any N	M0
Stage IVC	Any T	Any N	M1

TREATMENT

As it was mentioned before, the overall survival rate for oral melanoma is low, in fact the 5-year survival rate ranges from 12% to 55%. (9, 50, 51) Furthermore, this is a very aggressive disease with tendency to local recurrence, which ranges from 64 to 92% (1, 52), and distant metastasis. (4, 14, 16) In contrast with cutaneous melanoma, the optimal treatment protocol for oral melanoma remains undefined. (53)

Oral mucosa is composed of an epithelial layer, lamina propria and the submucosa. The lamina propria is thin and the absence of a reticular layer, as opposing to the skin, enables oral melanoma cells to spread more rapidly than those in the cutaneous melanoma. Therefore, mucosal melanomas infiltrate and metastasize quicker and have a worse prognosis than their cutaneous counterpart. (4, 21) The leading cause of death is systemic recurrence of the disease and the majority of patients with HNMM will not survive beyond 5 years. (2, 4) In fact, mucosal melanoma metastatic cells can infiltrate any organ in the body but they have a clear predisposition for the lungs, liver, brain, lymph nodes and intestine. For that reason, the majority of patients die of disseminated disease. (4, 9, 16) This demonstrates the need for the development of systemic therapy for oral melanoma to prevent metastization, and that local control does not equal to patients long-term survival. However, until the present, no systemic therapy has been recognized as effective in the treatment of oral melanoma. (3, 50, 54)

The mainstay of treatment is wide resective surgery with clear margins (ideally, at least 1.5 cm of normal tissue), since it offers the best chance of survival and local control for Stages III and IVA lesions. (9, 13, 55) Surgery can be associated with radiotherapy, chemotherapy and immunotherapy, even though the effectiveness of these

therapies, either as primary treatment or in combination with surgery, is largely unknown. (9, 13, 17)

For Stages IVB and IVC surgery is not recommended, so radiotherapy is used as primary treatment, and chemotherapy and immunotherapy as adjuvants. (13)

The majority of the studies suggest that postoperative radiotherapy increase local control but without increasing survival rate, due to the high incidence of distant metastasis. (55-57) Its use is also critical in cases of positive margins after surgery, and also for inoperable cases. (53, 55, 56)

Chemotherapy is mostly used as an adjuvant or palliative treatment, as well as immunotherapy (e.g. IFN α , Ipilimumab), but both with poor outcomes and no improvement on prognosis. (4, 53, 56) Mucosal melanomas express Cancer Testis Antigens (CTA) which can be used as a target for immunotherapeutic treatment, using monoclonal antibodies to CTAs. (27)

Biochemotherapy has considerable activity against this neoplasm and has already demonstrated correlation with improved survival in patients with advanced and metastatic disease. (50, 58)

In cases of *Kit* mutations, Imatinib, a *Kit* inhibitor, can be used with therapeutic benefits. (38, 40) If the tumor is *Bcl-2* positive, therapies to down-regulate this anti-apoptotic oncogene can be considered for treatment purposes. (41)

The use of sentinel lymph node is not completely defined in oral melanoma. (17, 53, 58) However, the high tendency to regional relapses associated with oral melanoma highlights the need for treatment of positive cervical lymph nodes. Hence, if lymph node metastasis are clinically detected, elective neck dissection should be performed (Level I to III). The most recent studies demonstrate the necessity to perform elective neck dissection for oral melanomas since there is a higher risk of cervical lymph node metastasis, when compared with other HNMM. (13, 54, 56)

The benefits of the use of adjuvant therapy in terms of survival for oral melanoma patients remains to be proven with randomized prospective clinical trials. (57, 58) Until then, clinicians will continue to extrapolate the results from cutaneous melanoma studies.

CONCLUSION

Nowadays the medical community continues to adequate the knowledge obtained with cutaneous melanoma studies and its treatment strategies to oral melanoma cases. This constitutes the wrong approach given the fact that, as we could conclude from this work, these neoplasms differ greatly with respect to both the biological behavior, as well as the genetic alterations known to trigger disease establishment and development. As there are no prospective studies on this subject, treatment protocols are based in the clinical knowledge of the medical oncologist or surgeon, which is at the basis of the scientific evidence pyramid. Hence, further prospective studies and randomized clinical trials are required in order to better understand oral melanoma and to validate the optimal treatment protocols, which offers patients an improvement in outcome and a longer survival rate. Due to the fact that oral melanoma is extremely rare, these studies have to be multicentric.

Dental professionals play a critical role in the detection of mucosal melanoma, as throughout the clinical examination conducted in routine medical appointments, they should be alert to any alterations in the oral cavity. Particular attention must be given to any changes in color of the mucosa of the palate and maxillary gingiva.

Despite being a controversial subject, the majority of the reference treatment centers, as well as the AJCC, advocate the incisional biopsy for oral melanoma diagnosis. Therefore, the best approach for prevention is to conduct a biopsy whenever there are doubts on clinical diagnosis, as the consequences which come from a delayed diagnosis are ominous to the patients' lives.

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ATTACHMENT 1

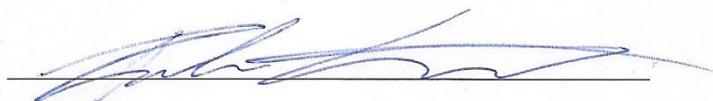


PARECER

Informo que o Trabalho de Monografia desenvolvido pela estudante Fátima Mariana Martins Soares com o título *Oral Melanoma: Histopathological and Molecular Alterations*” está de acordo com as regras estipuladas na FMDUP, foi por mim conferido e encontra-se em condições de ser apresentado em provas públicas.

Porto, 27 de maio de 2016

O orientador,



(Pedro Sousa Gomes)

Professor Auxiliar

ATTACHMENT 2



DECLARAÇÃO

Monografia de Investigação/Relatório de Atividade Clínica

Declaro que o presente trabalho, no âmbito da Monografia de Investigação/Relatório de Atividade Clínica, integrado no MIMD, da FMDUP, é da minha autoria e todas as fontes foram devidamente referenciadas.

Porto, 27 de maio de 2016



(Fátima Mariana Martins Soares)