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CASE REPORT

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Diagnosis of HPV-associated oropharyngeal squamous cell carcinoma in a middle-income country: report of two new cases

Abstract:

Human papillomavirus (HPV)-associated oropharyngeal squamous cell carcinoma (OPSCC) is a distinct subset characterized by better treatment responses and higher survival rates. Clinical and morphological aspects are crucial for diagnosis. Here we report two additional cases of HPV-associated OPSCC. Case 1: A 46-year-old male smoker and alcoholic presented with a 6 cm asymptomatic destructive ulcer involving multiple areas of the oropharynx. Microscopic analysis revealed non-keratinizing squamous cell carcinoma (SCC) with strong and diffuse cytoplasmatic and nuclear positivity for p16 in the tumor cells on IHC and ISH for HPV16/18 revealed the presence of viral DNA in a dot-like pattern. Case 2: A 53-year-old male smoker and alcoholic complained of dysphagia and exhibited erythematous areas in the uvula. Microscopically, a basaloid SCC was observed. Assessment of p16 expression showed diffuse moderate nuclear and cytoplasmatic positivity and ISH for DNA HPV16/18 also demonstrated dot-like signals. The present cases highlight the clinical and microscopical aspects of HPV-associated OPSCC. Pathologists and clinicians must remain vigilant in identifying HPV-associated OPSCC, even in cases where there is a history of alcohol and tobacco consumption. Assessment of p16 immunohistochemical patterns and ISH analysis are crucial to better understand the scenario of HPV-associated OPSCC in middle-income countries.

Keywords: HPV-associated squamous cell carcinoma; Oropharynx; Uvula; Immunohistochemistry; In situ hybridization.

INTRODUCTION

High-risk (HR) human papillomavirus (HPV) infection has been consistently associated with oropharyngeal squamous cell carcinomas (OPSCCs)[1,2] and has been considered a reliable prognostic biomarker[3]. The clinicopathologic and demographic profile of these patients is different from HPV-unrelated OPSCC and often affects younger men presenting higher levels of education, in addition to multiple oral sex partners, non-smoking, and nondrinking habits[4].

Statement of Clinical Significance
HPV-associated oropharyngeal squamous cell carcinoma (OPSCC) generally shows better survival outcomes. However, clinical evaluation is crucial especially for patients with tobacco use, which can negatively impact these outcomes. p16 immunohistochemistry and in situ hybridization for HPV DNA in diagnosing OPSCC are necessary. Careful diagnosis is essential, considering that treatment de-intensification may impact survival in smokers.

Microscopically, these tumors are generally non-keratinizing SCC, composed of full-thickness dysplastic papillary epithelium. On IHC, neoplastic cells show diffuse and strong positivity for surrogate marker p16 in at least 70% of the malignant cells. Nuclear pleomorphism, increased mitotic activity, and absence of keratin formation[5] are also noted. Individuals with HPV-associated OPSCC frequently demonstrate better responses in treatment and increased survival rates compared to HPV-unrelated counterparts[6]. The scenario of SCC etiology has changed in high-income countries with

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the decrease in smoking. However, in Brazil, the main risk factors for SCC remain in tobacco and alcohol abuse[7]. Interestingly, the improved survival associated with HPV-positive status in OPSCC patients may be suppressed by excessive smoking[8]. In the context of Brazil, a middle-income country, this study aimed to report two additional cases of HPV-associated OPSCC in heavy smokers and alcohol consumers.

CASE REPORT

A 46-year-old man (Case 1), a smoker and an alcohol consumer was referred for diagnosis of a destructive ulcer involving the soft palate, tonsil, posterior wall of the oropharynx, and base of the left tongue, with 3 months of evolution. The lesion was asymptomatic and measured about 6 cm (Figures 1A and 1B).

An intraoral incisional biopsy was realized, and the surgical specimen was sent for anatomopathological analysis.

A 53-year-old man (case 2), also a user of tobacco and alcohol, presented with a chief complaint of dysphagia with 1 month of evolution. Clinical examination revealed an erythroleukoplastic area in the uvula (Figure 2A).

A biopsy was performed, and the specimen was also sent for anatomopathological analysis.

Microscopic analysis of both cases revealed the presence of non-keratinizing SCC. Large nests and lobules of tumor cells with increased mitotic activity and central necrosis, surrounded by a lymphoid stroma were observed. No dysplasia was noted in the superficial epithelium of case 1 (Figures 1C and 1D). The neoplastic cells frequently exhibited hyperchromatic nuclei and ovoid morphology with indistinct cellular borders. In case 2, invasive areas were observed, with a basaloid morphology and discrete dyskeratosis.

Both cases were evaluated for HPV status using IHC to evaluate p16 expression, followed by Chromogenic in situ hybridization (CISH) for HPV subtypes 16 and 18. IHC analysis of p16 CDKN2A/p16INK4a expression (EPR20418, Abcam, Rabbit Recombinant Monoclonal, 1:500) was performed. Tissue sections of 3 µm were obtained and were deparaffinized and hydrated before antigen retrieval using EDTA/Tris solution (pH 9.0) during 15 minutes in an electric pressure cooker. Blocking of endogenous peroxidase activity was performed with 6% H_2O_2 20 vol. for 15 minutes. After the pretreatment, the tissue sections were incubated with the primary antibody for 2 hours at room temperature. The detection system used was EnVision-Dual Link System-HRP (Dako, Carpinteria, CA), following the manufacturer protocol and staining with diaminobenzidine tetrahydrochloride (Dako, Carpinteria, CA) for 5 minutes. Counterstaining was performed with Carazzi's hematoxylin. To detect and confirm the presence of high-risk HPV, CISH using DNA probes for HPV subtypes 16/18 was performed. Similarly, 3 µm tissue sections were deparaffinized and hydrated, followed by epitope retrieval in citrate buffer (pH 6.0), and hybridized with an HPV subtypes 16/18 biotinylated DNA probe (Dako, Carpinteria, CA) and labeled using the Tyramide signal amplification system for Biotinylated probes (Dako, Carpinteria, CA), according to the manufacturer protocol.

IHC analysis of p16 showed strong and diffuse nuclear and cytoplasmic staining in almost 100% of the

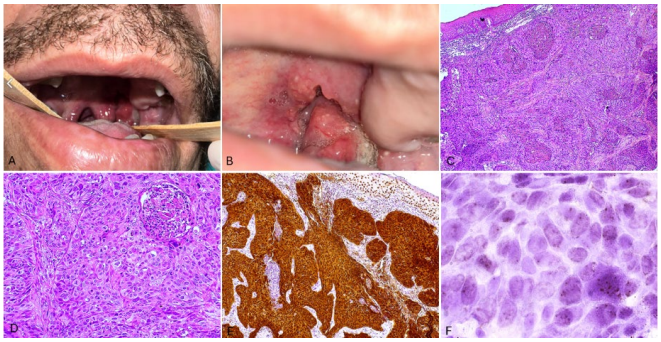

Figure 1. Case 1 - Clinical and microscopical aspects of HPV-related oropharyngeal squamous cell carcinoma. A, B) Extensive and destructive ulcer involving the oropharynx region. C, D) Photomicrographs revealed a malignant infiltration of non-keratinizing epithelial cells (H&E, original magnification x50 and x100). E) p16 IHC staining, demonstrating overexpression in the nuclei and cytoplasm of all tumor cells (original magnification x100). F) In situ hybridization for DNA HPV16/18 in a dot-like pattern (original magnification x1000).

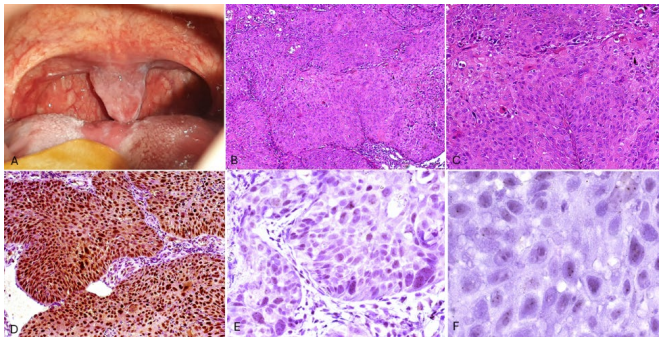

Figure 2. Case 2 - Clinical and microscopical aspects of HPV-related oropharyngeal squamous cell carcinoma. A) Erythroleukoplastic lesion in the uvula. B, C) Islands and nests of non-keratinizing basaloid epithelial malignant cells (H&E, original magnification x100 and x200). D) Diffuse moderate nuclear and cytoplasmic staining for p16 in the neoplastic cells (original magnification x200). E, F) In situ hybridization for DNA HPV16/18 in dot-like pattern (original magnification x400 and x1000).

tumor cells in case 1 (Figure 1E), and diffuse moderate nuclear and cytoplasmic positivity in more than 70% of the tumor in case 2 (Figure 2D). CISH for HPV16/18 confirmed the viral integration in the nuclei of malignant cells in a dot-like pattern for both cases (Figure 1F and Figure 2E, 2F). Based on this, the final diagnosis for the two cases was HPV-associated OPSCC, and the patients were referred to a cancer center for treatment.

DISCUSSION

Recent guidelines have indicated that HPV tumor status be investigated for all OPSCC cases. Basaloid non-keratinizing OPSCC associated with nuclear and cytoplasmic immunoreactivity for p16 indicates the diagnosis of HPV-associated OPSCC. Positivity for p16 is confirmed by nuclear and cytoplasmic staining with moderate to strong intensity in at least 70% of the malignant cells. In cases of p16 overexpression or questionable morphologies, HPV-specific testing (ISH or polymerase chain reaction) is also recommended[4,9,10]. Overexpression of p16 was noted in both nuclear and cytoplasmic compartments in case 1, and moderate cytoplasmic expression was noted in case 2. The presence of high-risk HPV was confirmed by ISH analysis in a dot-like pattern.

We present one case of an asymptomatic extensive ulcer and another involving the uvula with dysphagia. Primary squamous cell carcinoma (SCC) of the uvula is an uncommon disease[11]. HPV-associated OPSCC is generally diagnosed through the identification of a neck mass indicative of secondary nodal involvement[12]. Nodal metastasis was not detected in any of the presented cases.

The current cases were diagnosed in two males aged 46 and 53 years. A male predominance of HPV-associated OPSCC is observed worldwide. Several hypotheses for this male predominance have been suggested:

a) men have more sexual partners;
b) when oral sex is performed on mucosal surfaces (female genitals), the transmission of infection is more efficient when compared to oral sex on the keratinized epithelium (such as the penis); and
c) some level of systemic immunity may be protected against oral HPV infection in women who have cervical HPV infection[13-15].

According to age, HPV-unassociated OPSCC has a mean of 60 to 70 years, and patients with HPV-associated OPSCC have a mean age of 50 to 56 years at the time of the diagnosis[4].

Both cases involved smokers and alcoholic patients, reflecting the current Brazilian reality of SCC. Ongoing clinical trials related to the de-intensification of HPV-related OPSCC therapies due to improved treatment responses have been conducted[16]. These strategies have concentrated on modifying nonsurgical approaches[17,18]. However, even for HPV-positive OPSCC, tobacco use may eliminate the benefit of improved survival[8]. In 2010, the interaction between HPV status and tobacco exposure in OPSCC patients was identified, and the principal distinction between "low-risk" and "intermediate-risk" tumors was reduced survival in patients who were smokers[19]. Recent studies showed that Brazilian patients with HPV-associated OPSCC and with a history of tobacco did not have better survival rates compared to HPV-negative patients[7,20]. A study suggested that until the effect of tobacco use can be quantified with appropriate datasets, de-escalation of treatment for HPV-associated OPSCC smokers should be approached with caution[8].

Unlike oral cavity cancer, the incidence of OPSCC has increased significantly in several developed countries from Europe, North America, and Oceania in the past 3 decades, and this was attributed to an increase of HPV infection[21]. However, no significant increases in OPSCC incidence have been observed in Latin America and other low- and middle-income regions like South and Southeast Asia. Thus, in these countries, the role of HPV infection in the carcinogenesis of OPSCC remains relatively low[22]. In addition, a recent meta-analysis showed that 60 to 70% of OPSCC cases in high-income countries are associated with HPV infection against only 11.1% in Brazil[23]. It has been suggested that changes in sexual behaviors among recent birth cohorts, mainly in developed countries, have led to increased oral HPV infection and, as a consequence, increasing the incidence of HPV-associated OPSCC. On the other hand, tobacco and alcohol consumption are still the most frequent risk factors for OPSCC in Latin American countries and parts of Asia[22].

Brazil has the highest OPSCC incidence rates among Latin American countries, and according to the National Cancer Institute (INCA, its acronym in Portuguese), states from the Southeast region such as São Paulo, Minas Gerais, and Rio de Janeiro are responsible for the majority of diagnostic cases[24]. Recent studies from Brazil have estimated a significant increase in the incidence for HPV-associated OPSCC in young patients in the last decades[25,26]. The prevalence rates range from

6.1 to 59.1% in Brazil, with the highest rates found in São Paulo State, which demonstrates the HPV heterogeneity in the country[20]. A possible reason for the worldwide increased incidence of HPV-associated OPSCC is the changing sexual behaviors, especially oral sex practices[21]. Although the incidence of HPV-associated OPSCC has gradually increased, there is an atypically low prevalence in Brazil[27].

The INCA estimates approximately 15,100 new cases of oral and oropharyngeal cancer in Brazil in 2024[28]. Louredo et al. highlighted that public university oral pathology laboratories (PUOPL) have played an essential role in the diagnosis of these cases throughout the country. They reported that approximately 10% of all patients with oral and oropharyngeal cancer treated in Brazil last decade were diagnosed free of charge by only sixteen PUOPL from 13 States[29]. Although the country has experienced an expansion of the population's access to oral health care over recent decades, the diagnosis and treatment of cancer remain challenging. Consequently, the oral and oropharyngeal cancer diagnosis is usually delayed, allowing for local extension and regional metastasis. Therefore, most patients are still diagnosed with advanced-stage disease, and this delay impacts directly the survival rates[30,31]. According to Costa et al. 2023, reasons for late diagnosis and delayed time to treatment initiation in Brazil are often multifactorial and usually include lack of access to initial appropriate health care for correct diagnosis, lack of awareness of cancer signs and symptoms, and patient delay in seeking healthcare[32].

CONCLUSION

Pathologists and clinicians should be attentive to the clinical and morphological aspects of HPV-associated OPSCC. Additionally, pathologists must be prepared to identify the immunohistochemical pattern of p16 and assess ISH for high-risk HPV in suspected cases. Moreover, for low to middle-income countries, it is important to highlight that despite the de-intensification of treatment for HPV-associated OPSCC, recent studies have indicated negative effects on survival for tobacco users, even in cases of HPV detection.

AUTHORS' CONTRIBUTIONS

SNA: data curation, formal analysis, writing – original draft. HKFB: data curation, visualization, writing – review & editing. BVRL: data curation, visualization, writing – & editing. MAL: writing – review & editing. ARSS: writing – review & editing. PAV: conceptualization, supervision, writing – review & editing.

CONFLICT OF INTEREST STATEMENT

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