

Verrucous carcinoma with foci of invasive squamous cell carcinoma: Report of a case and discussion of current concepts

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Abstract:

Verrucous carcinoma (VC) is an uncommon type of low-grade, well differentiated and non-invasive squamous cell carcinoma (SCC). The development of invasive SCC within a VC is rare and distinction from the classical oral SCC is a frequent dilemma for pathologists. We report a case of a 78 years old male former smoker, alcoholic, presenting a painless leukoplastic verrucomatous lesion in mandibular alveolar ridge measuring approximately 4cm diameter, with 2 months evolution. Histological examination of the incisional biopsy revealed proliferation of well-differentiated squamous epithelium forming high exophytic hyperkeratinized papillary projections and deep blunt buds. In focal areas, invasive tumor epithelial nests with more apparent cytologic atypia were observed. Immunohistochemical analysis showed intense expression of p16 but weak positivity for ki-67. The diagnosis was VC with microinvasive SCC areas. The patient underwent surgery with a safety margin and is under follow-up. "Hybrid" lesions composed of typical VC and invasive SCC accounts for about 20% of oral VC. A discussion on the criteria of differential diagnosis and biological behavior of these lesions is also provided.

Keywords: Carcinoma, Verrucous. Carcinoma, Squamous Cell. Ki-67 Antigen.

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INTRODUCTION

Verrucous carcinoma (VC) is a well-recognized variant of the squamous cell carcinoma (SCC) of the oral cavity with unique morphology, characterized by exophytic appearance, presenting locally destructive growth but no metastasis tendency. It was first identified as a clinical and histologic entity by Ackermann in 1948 in a study of 31 patients. This rare tumor is more common in females in the seventh and eighth decades of life and the most frequent localization is buccal mucosa, followed by lip-commissure, gingiva, tongue and hard palate¹.

The histological presentation of VC is well defined, and it is characterized by epithelial exophytic projections forming high sharp ridges with keratin-filled invaginations, and downgrowth as blunt papillas that seem to compress the underlying connective tissue, with minimal or no cytological atypia^{1,2}. The non-invasive growth pattern and minimal or no cytological atypia of tumor cells are topical features to separate these tumors from classical invasive oral squamous cell carcinoma².

However, “hybrid” lesions comprised of typical VC associated with foci of invasive SCC have been previously reported³. The hybrid VC associated with SCC can represent up to 20% of these tumors in the oral cavity, and the differential diagnosis is considered an important diagnostic dilemma, since some studies report that these lesions behaves like SCC rather than VC regarding their metastatic tendency⁴, whereas others suggest that the clinical behavior of these tumors more closely matches pure VC³.

As there are only some few reports of VC in the literature, herein the authors describe a case of this hybrid lesion in a 78 years old male patient and provide a discussion of the parameters of differential diagnosis with other verrucous lesions of the oral cavity, well as the clinical significance of the invasive areas.

CASE REPORT

A 78-year-old white male was referred to a private dentistry service with a chief complaint of a white tumor underneath the tongue. Intraoral examinations revealed an extensive verrucous leukoplactic lesion, with 4.0 cm in the largest diameter, asymptomatic, located in the mouth floor (Fig. 1), with 2 months evolution. Anamnesis revealed that the patient worked in farming, was former smoker, and ex-alcoholic. Medical history was non-contributory. The presumptive diagnoses were verrucous carcinoma × benign verrucous hyperplasia × squamous cell carcinoma. Incisional biopsy was performed and the tissue sample was sent to histological examinations.



Figure 1. Clinical presentation of the lesion as an extensive painless verrucous leukoplactic lesion, with 40 mm in the largest diameter, located in the mouth floor.

Microscopically, the tumor showed high exophytic papillomatous proliferation of squamous epithelium forming high with blunt rete processes towards the subepithelial area. The hyperkeratinized epithelial surface formed “keratin plugs” between the epithelial projections. Intense lymphocytes infiltrate was seen in the connective tissue (Fig. 2A / 2B). The overall analysis was consistent with VC. However, in focal areas of nests and islands of atypical squamous cells invading the surrounding connective tissue, with increased mitotic activity, invading the surrounding connective tissue were observed (Fig. 2C / 2D).

The extent of invasion, measured as the distance from the deepest point of invasion to the nearest focus of pure VC, was less than 2 mm. An immunohistochemical study of p16 (Ab-7, 1:100, Neomarkers, Fremont, CA, USA) and Ki-67 (MIB-1, 1:50, Dako, Glostrup, Denmark) antigens was performed. Tumor cells exhibited intense diffuse positivity for p16 (Fig 3A / 3B), whereas Ki-67 immunoexpression was weak, with labeling index of 13.4%, but increasing to 23.7% when only histological fields adjacent to the invasive areas were considered (Fig. 3C / 3D). These findings were consistent classic VC with focal transformation into invasive SCC.

The patient was subjected to wide local excision of the lesion, with proper mucosal and soft-tissue margins. Histological analysis of the surgical specimen showed the very same histological findings and confirmed the presence of multiple areas of tumor cells microinvasion. However, the surgical margins of the resected specimen were negative for tumor cells. The patient is now free of tumors, and followed up 18 months after the operation.

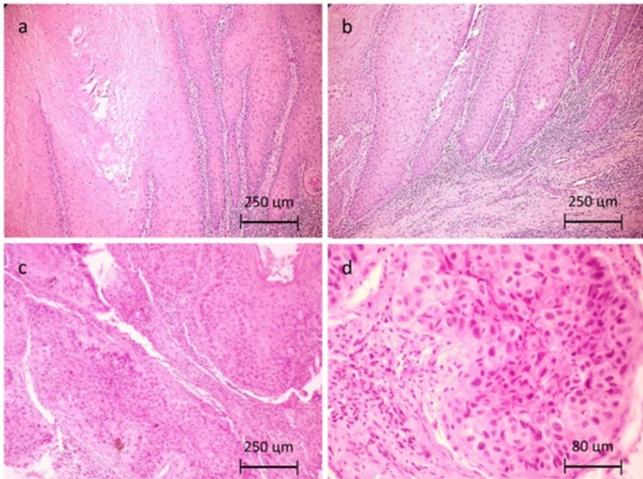


Figure 2. Photomicrographs of the histological sections of the biopsied tissue (stained in HE). (a) Papillomatous proliferation of the hyperkeratinized well-differentiated squamous epithelium forming high exofitic sharp ridges with spaces in between filled with keratin ("keratin plugs") (100 ×). (b) Proliferative squamous epithelium showing blunt rate processes towards the subepithelial area and intense lymphocytic infiltrate in the connective tissue (100 ×). (c) Focal formation of nests and islands of squamous cells invading the surrounding connective tissue (100 ×). (d) Clear cytological atypia, such as cellular/ nuclear pleomorphism and hyperchromatism in the invasive area (400 ×).

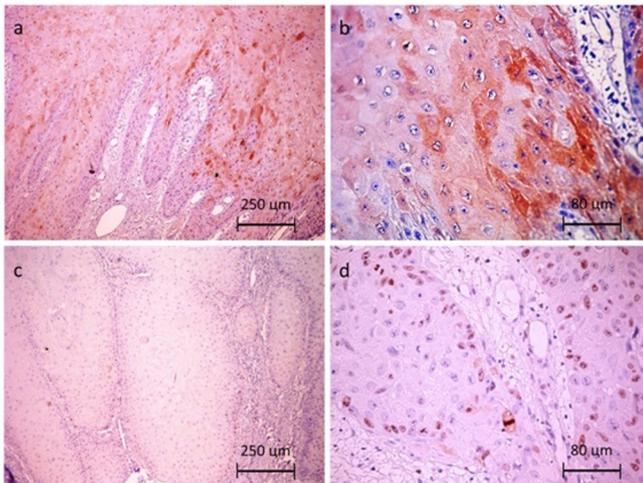


Figure 3. Photomicrographs of the histological sections of the biopsied tissue (LSAB). (a, 100 ×) and (b, 400 ×) Tumor cells exhibiting intense and diffuse nuclear and cytoplasmic positivity for p16INK4A in the stratum spinosum, but (c, 100 ×) and (d, 400 ×) weak immunoeexpression of Ki-67.

DISCUSSION

The differential diagnosis of the verrucous leukoplastic lesions of the oral cavity is challenging as they include a spectrum of benign, potentially malignant, and frankly malignant lesions, with distinct biological behaviors. One of the main diagnostic dilemma is the overlapping of histological features observed in VC and benign verrucous hyperplasia (BVH), a premalignant

condition with capacity to transform into squamous cell carcinoma⁵.

The histopathological criteria purposed by Kallarakkal et al.⁶ for distinguishing BVH from VC are: (i) long and narrow heavily keratinized verrucous processes or broad and flat verrucous processes that are less keratinized; (ii) absence of invasion of the hyperplastic epithelium into the lamina propria as compared with the adjacent normal mucosal epithelium; and (iii) (c) presence of cytologic/architectural features of dysplasia.

Based on these features, the histology of the current case fulfills the criteria of VC rather than BVH, since cell atypias limited to some few focal areas and shows downward growth pattern and "plug-forming" intense hyperkeratosis typically observed in VC.

To date, VC has been considered to be a variant of well-differentiated squamous cell carcinoma. However, once invasive changes and metastasis are not expected, it has been suggested that this tumor should be recognized as a unique subtype of *in situ* carcinomas, with exophytic growth⁷. Furthermore, evidence of cell atypia and focal invasion should be regarded histological signs of transformation into SCC².

Likewise, in the current case, cell atypia were limited to areas of invasive SCC whereas VC areas were composed of typically well-differentiated squamous keratinocytes, which was suggestive of SCC arising within VC. However, Patel et al.⁸ have stated that the depth and extent of invasion (SCC areas) within VC are critical for the establishment of tumor prognosis. The authors have demonstrated that modal metastasis and local recurrence were significantly more common in tumors presenting invasive areas over the cut off value of 2 mm than in pure VC or VC showing either epithelial dysplasia or minimal areas of conventional SCC. As in the current case the extent of SCC invasive areas were less than 2 mm, we assumed that it more closely matched conventional VC rather than SCC.

Ki67 immunostaining was seen mainly in basal cells, with proliferative index of 13.4%, surprisingly much lower than the mean of $79.2 \pm 25.5\%$ reported by Mohtasham et al.⁹. Since Ki67 expression has been regarded as indication of the fraction of actively replicating cells, it is possible to suppose that the proliferative potential of the tumor was very low. However, if separately analyzed, the invasive areas showed higher proliferative index.

Although this finding seems to point at a possible change in the proliferative pattern of tumor cells in such invasive areas in comparison with non-invasive conventional verrucous carcinoma, the reliability of this

index as a marker of tumor biological behavior remains questionable. In fact, although Ki67 expression in oral cancer has been previously associated with more aggressive tumors¹⁰, other studies failed to show significant differences in the expression of this marker between oral verrucous and invasive squamous cell carcinomas^{5,9}.

Despite all this controversy, the increased number of Ki67 positive cells in addition to more intense cytological atypia in isolated islands and nests of epithelial cells were interpreted as a morphological sign of SCC arising within VC.

p16 is a tumor suppressor gene that encodes an inhibitor of cyclin D1-CDK4/6 complexes responsible for Rb protein phosphorylation and consequent transition from G1 to S phase of the cell cycle. Overexpression of p16 in oral cancer, such as SCC and VC, has been considered as a possible marker of high-risk HPV infection.

Although p16 strong positivity observed in the current case might pose a possible link between this tumor and HPV infection, a study performed in 36 VC, including tumors associated with SCC and p16-positive tumors, showed lack of transcriptionally active high-risk HPV³. These data seem to suggest that both VC and its related SCC are not HPV-driven tumors and that p16 positivity should not be regarded as a definitive marker of HPV infection.

CONCLUSION

We report a rare case of VC with focal SCC of the oral cavity. The differential diagnosis of VC remains a challenge and requires careful examination of these tumors, and clinical, histopathological and immunohistochemical data confrontation. However, as the biological behavior of VC with areas of invasive SCC is not well-established, other case reports with detailed clinicopathological descriptions and long-term follow-up are necessary to provide data to enable a better understanding of these tumors.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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