

D240 as a Potential Marker that Differentiate Verrucous Carcinoma from Squamous Cell Papilloma

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Abstract

- Background** Verrucous carcinoma is a distinct variant of oral squamous cell carcinoma characterized by slow growth and rare metastases. It may present diagnostic difficulties as it may be inaccurately diagnosed as squamous cell papilloma.
- Objective** The study performed a comparative immunohistochemical staining for both entities to obtain a possible method of differentiation.
- Methods** The study involved 13 samples of oral verrucous carcinomas and 10 samples of oral squamous cell papillomas which were stained immunohistochemically with antibodies to the lymphatic endothelial marker D240.
- Results** In all samples, the entire epithelium of verrucous carcinomas was positively stained with D240 whereas only the basal cell layer of squamous cell papillomas was positive.
- Conclusion** D240 could be used as a differentiating marker between oral verrucous carcinomas and squamous cell papillomas.
- Keywords** Verrucous carcinoma, Squamous cell papillomas, D240.

Introduction

Verrucous carcinoma (VC) is a rare variant of oral squamous cell carcinoma which was first described by Ackerman in 1948⁽¹⁾. It is distinct in its slow growth and ability to become locally aggressive if not treated properly. However, even with local tumor progression, regional or distant metastasis is rare⁽²⁾. Its occurrence originally was related to the use of chewing tobacco or snuff, although this was never substantiated by controlled epidemiologic investigations. Moreover, HPV appears to be of etiologic significance⁽³⁾, although not supported universally. VC predominantly occurs in older people, the majority of cases being observed in individuals in

their sixth decade or later, and has a higher incidence in males⁽⁴⁾.

On clinical examination, VC appears as a relatively well-circumscribed, elevated, nodular mass with a surface that may be pebbled, papillary, verrucous, or smooth. Depending on the degree of surface keratinization, it varies in color from white to red to admixtures of both⁽⁵⁾.

VC is broadly based and invasive, with plump papillary invaginations of thickened and infolding epithelium that lack the usual cytologic criteria of malignancy⁽⁴⁾. The exophytic surface is covered by abundant orthokeratin and/or parakeratin that also fill the crevices of deep surface invaginations. Because the lesions are well differentiated,

superficial biopsies often do not yield a diagnosis. Clinical correlation can be of great value because lesions tend to be more impressive visually than microscopically⁽⁵⁾.

The clinical and histologic differential diagnosis of VC includes benign and malignant squamous proliferations, including reactive inflammatory epithelial hyperplasia, squamous papilloma, conventional squamous cell carcinoma (SCC), and papillary squamous cell carcinoma (PSCC)⁽⁶⁾. Lack of cellular atypia serves to rule out conventional SCC and PSCC whereas distinguishing VC from squamous papilloma and reactive inflammatory epithelial hyperplasia may be more problematic^(4, 7).

D240 is a monoclonal antibody to podoplanin, it was primarily employed as a specific marker for lymphatic vessels, however; it was shown to be of use as a differentiation marker in a number of tumors^(8,9) and as a marker for malignant cells in other studies⁽¹⁰⁾.

This study compared the immunohistochemical staining pattern between VC and squamous cell papilloma using a lymphatic vessel marker (D240).

Methods

The archives of the department of oral pathology at the college of dentistry / Baghdad University were retrospectively reviewed for formalin fixed – paraffin embedded biopsy samples for verrucous carcinomas and squamous cell papillomas. The samples were checked for size adequacy and fitness to the histological diagnostic criteria, three samples were excluded due to these reasons. Five µm thickness tissue sections were cut from each tissue sample and mounted on positively charged slides (Fisher scientific, USA).

Slides were baked in hot air oven at 65 °C overnight. Sections were sequentially dewaxed through a series of xylene, graded alcohol and water immersion steps. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide followed by blocking the nonspecific antibody binding with normal goat serum this was followed

by the application of the primary antibodies for D240 (Dako Cytomation - USA) with a dilution of (1:100). Lymphatic vessels were considered as appositve control to the marker. The slides were incubated for 1 h at 37 °C and then kept at 4°C in a humid chamber overnight. Next day, after washing with PBS, biotinylated antimouse IgG were applied to the sections, incubated and rinsed with a stream of PBS. Conjugated antibodies were visualized with DAB chromogen. Sections were counterstained with Mayer’s hematoxylin for 1-2 min, dehydrated and mounted. Then a microscopical examination of the slides was performed.

Results

The archival review resulted in tissue samples from twenty three patients, thirteen of verrucous carcinomas and ten of squamous cell papillomas (Table 1 and figures 1, 2, 7, 8).

Diffuse positive immunohistochemical staining was shown in all verrucous carcinoma tissue samples extending from epithelial top to bottom in addition to strong positivge staining of the small sized lymph vessels in the underlying connective tissue stroma (Figures 3-6). Whereas only the basal cell layer of the squamous cell papilloma tissue samples showed positivity for D240 monoclonal antibody with strong positivity in large lymph vessels at the intervening papillae and underlying connective tissue stroma (Figures 9-12).

Table 1. Study samples’ age and sex

Tissue sample	No.	Age (years)	Sex	
			Female	Male
VC	13	56.07±16.32	5	8
SCP	10	26±17.66	4	6

VC = Verrucous carcinoma, SCP = Squamous cell papilloma

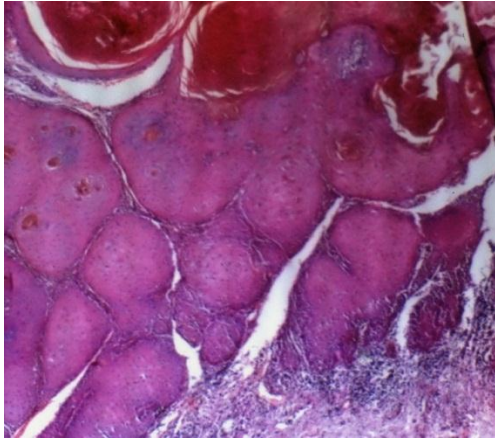


Figure 1. Verrucous carcinoma with surface keratin (H&E) X40

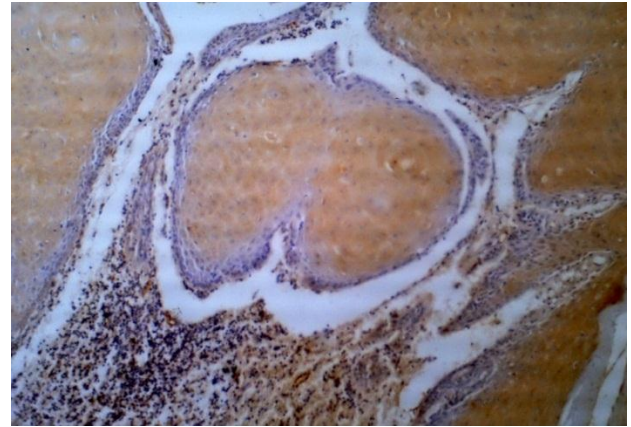


Figure 4: Verrucous carcinoma demonstrating diffuse staining pattern throughout the epithelial layers (Immunohistochemistry with D2-40) X100

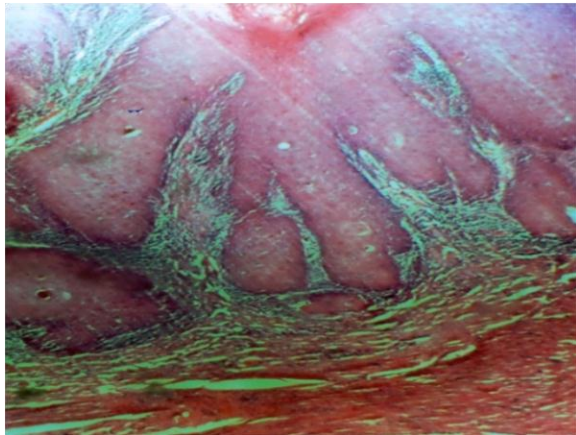


Figure 2. Verrucous carcinoma showing bulbous rete ridges and subepithelial infiltration of inflammatory cells (H&E) X40

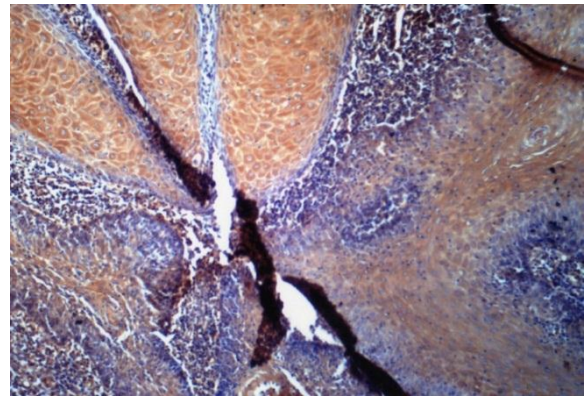


Figure 5. Diffuse tumor cell positivity of verrucous carcinoma and positive small lymphatic vessels peritumorally (Immunohistochemistry with D2-40) X200

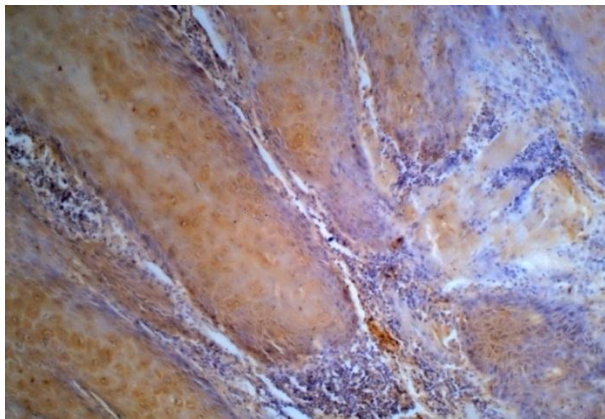


Figure 3. Verrucous carcinoma demonstrating diffuse staining pattern throughout the epithelial layers (Immunohistochemistry with D2-40) X100

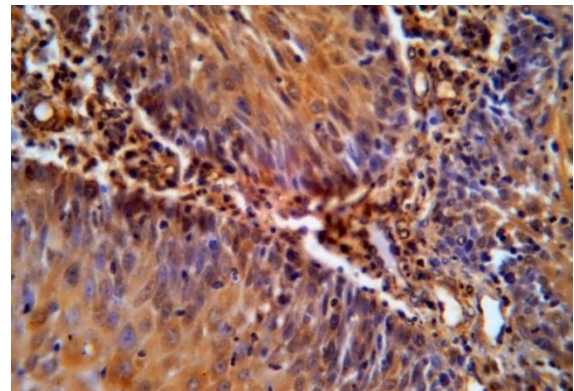


Figure 6. Advanced front of verrucous carcinoma with diffuse positivity for D2-40 antibodies with immunopositive small lymphatic vessels in-between (Immunohistochemistry with D2-40) X400

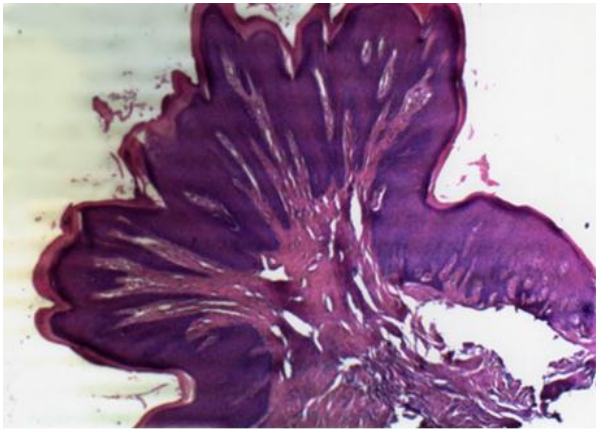


Figure 7. Squamous cell papilloma (H&E) X40

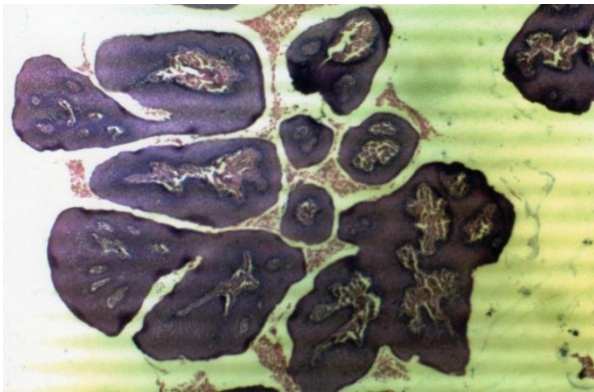


Figure 8. Squamous cell papilloma cut in cross section (H&E) X40

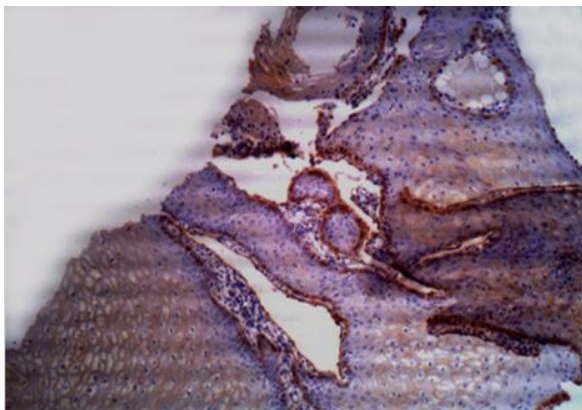


Figure 9. Strong basal cell positivity in squamous cell papilloma tissue sample (Immunohistochemistry with D2-40) X100

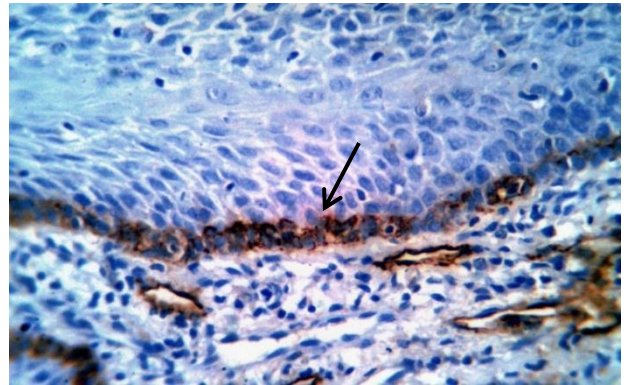


Figure 10. Squamous cell papilloma tissue sample demonstrating strong basal cell positivity with numerous positive subepithelial lymph vessels (Immunohistochemistry with D2-40) X400

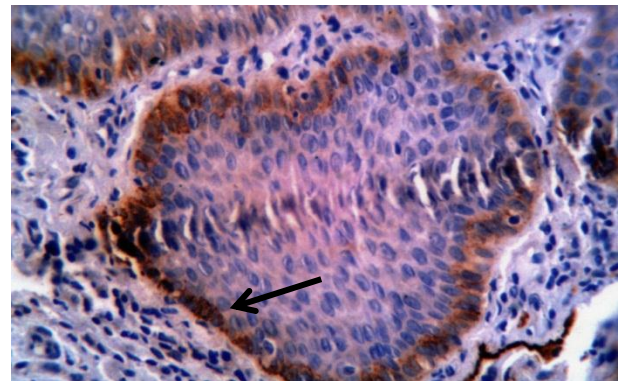


Figure 11. D2-40 immunopositivity confined to the basal cell layer in a squamous cell papilloma tissue sample (Immunohistochemistry with D2-40) X400

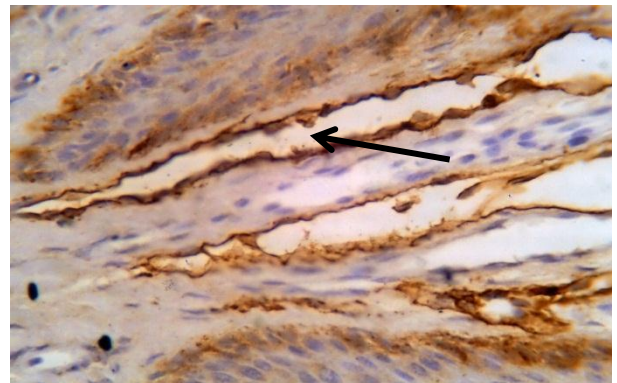


Figure 12. D2-40 positive large lymphatic vessels between positive basal cell layer in a squamous cell papilloma tissue section (Immunohistochemistry with D2-40) X400

Discussion

Verrucous carcinoma has been the subject of a debate concerning its diagnostic features and mode of treatment since its discovery^(1,4). It is a differentiated variant of squamous cell carcinoma and may present diagnostic difficulties as it may be erroneously diagnosed as squamous papilloma⁽¹¹⁾. Determining the DNA content by nuclear cytometry on Feulgen-stained histologic sections has been reported to be diagnostically useful in detecting cells with abnormal DNA content in VC; this finding may be helpful in differentiating VC from benign lesions⁽¹²⁾. Recording nuclear size with image analysis has been suggested to be helpful in differentiating VC from squamous papilloma, as the cells in VC are, in general, larger (>300 µm) than those in papillomas (<250 µm)⁽¹¹⁾. D240 is a monoclonal antibody directed against podoplanin, which is a 38 kDa type-1 transmembrane glycoprotein⁽¹³⁾ which has been reported to be expressed occasionally in normal epidermal basal cells⁽¹⁴⁾. It is widely used as a specific marker for lymphatic endothelial cells and lymphangiogenesis in many species, as podoplanin is expressed on lymphatic but not on blood vessel endothelium⁽¹⁵⁾ which was demonstrated in the lymphatic and vascular endothelial cells in this study, moreover; it has been reported to be upregulated in human squamous cell carcinomas⁽¹⁴⁾. This study is the first of its kind describing IHC of D240 monoclonal antibodies verrucous carcinoma in comparison to squamous cell papilloma. Previous studies were either based on cytomorphological features or counting mitotic figures^(11,12), immunohistochemical staining with D240 showed positivity confined to the basal cell layer in all the samples of squamous cell papillomas whereas the positive staining in verrucous carcinoma samples involved all the layers of epithelium, reflecting the disparity of the epithelia amid the lesions and facilitating their differentiation. The resulting staining pattern could be attributed to normal physiologic regeneration within basal cell layer in normal

epithelium and, in this study; squamous cell papilloma epithelium.

Consequently, and with no statistical analysis required, this method of comparative staining could be used to differentiate verrucous carcinomas from squamous cell papillomas.

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