Evaluation of Epithelial Dysplasia and Invasion through the Basement Membrane in Oral Verrucous Carcinoma: A study on 45 cases

*Akhtaruzzaman M,[1] Dey BP,[2] Abedin N,[3] Banu SG[4]

Abstract

Background: Oral Verrucous carcinoma (OVC) is a rare variant of well differentiated squamous cell carcinoma (SCC). Epithelial dysplasia and/or foci of invasion in OVC are indicators of worse prognosis. The aim of this study was to evaluate the epithelial dysplasia and invasion through the basement membrane in OVC.

Method: This was a cross-sectional observational study conducted at the Department of Pathology, BSMMU during 2021-2023 that included 45 cases of histologically diagnosed OVC. Demographic variables, presence or absence of epithelial dysplasia in routine H&E stain and presence or absence of invasion through the basement membrane by PAS stain were assessed. Thirty three cases were followed up and reports of the subsequent biopsies were collected. Research data were placed in a data sheet. Results of all cases were tabulated. Statistical analysis was performed on the tabulated data by Fisher's Exact Test.

Result: In this study, 33.3% cases revealed epithelial dysplasia in OVC and 11.5% cases showed invasive foci through the BM. We followed up the study cases and 15.6% cases were diagnosed as invasive SCC in subsequent biopsy. Statistical analysis revealed presence of invasion through the BM significantly higher in OVC cases having epithelial dysplasia. Similarly, in subsequent biopsy ISCC was found significantly higher in previously diagnosed cases of OVC having features of epithelial dysplasia.

Conclusion: Epithelial dysplasia in OVC signifies the increased possibility of its progression to conventional invasive SCC and is an important prognostic factor. So, earlier detection of epithelial dysplasia and/or foci of invasion in OVC will help the clinician in properly managing the patient.

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1. **Dr. Md. Akhtaruzzaman, Clinical Pathologist, Department of Laboratory Medicine, Dhaka Medical College Hospital, Shahbagh, Dhaka-1000, Bangladesh; akter22nd@gmail.com*
2. *Dr. Bishnu Pada Dey, Associate Professor, Department of Pathology, Bangladesh Medical University, Shahbagh, Dhaka-1000, Bangladesh; drbishnu@bsmmu.edu.bd*
3. *Dr. Nafisa Abedin, Senior Clinical Pathologist, Department of Laboratory Medicine, Dhaka. Medical College Hospital, Dhaka, Bangladesh; Email: nafisa.abedin@gmail.com*
4. *Professor Dr. Sultana Gulshana Banu, Professor, Department of Pathology, Bangladesh Medical University, Shahbagh, Dhaka-1000, Bangladesh; sgb.bsmmu@gmail.com*

**For correspondence*

Introduction

Oral cancer is the sixth most frequent cancer worldwide.[1] In the oral cavity, about 90% of reported malignancies are oral invasive squamous cell carcinomas. Despite advancements in diagnosis and treatment, oral malignancies, which have a five-year survival rate of about 45%, are still challenging for physicians to diagnose and treat.[2] Oral verrucous carcinoma is a low grade and uncommon variant of well differentiated squamous cell carcinoma.[3] Fridell and Rosenthal originally reported it in 1941 and Lauren V. Ackerman characterized it in 1948. Oral cavity and larynx are the commonest sites for verrucous carcinoma but it also occurs on the skin of genitalia, sole of the foot, oesophagus, nasal cavity and paranasal sinuses.[4] In oral cavity verrucous carcinoma most frequently arises in the buccal mucosa followed by lip commissure, gingiva, and hard palate. Human papilloma virus, alcohol, tobacco, betel nuts, and poor oral hygiene have all been observed as significant risk factors for oral verrucous carcinoma.[5] Male over fifth to sixth decade are more commonly affected.[4] The reported rate of verrucous carcinoma among all primary cancers of oral cavity is 3% worldwide.[6] In Bangladesh the prevalence of oral verrucous carcinoma is 2.5%.[1] OVC clinically presents as slowly growing, painless, grey white, fungating and exophytic growth on oral mucosa. Histologically, OVC is characterized by hyperkeratosis, parakeratosis and acanthosis in its superficial portions resembling a verruca. The tumour extends downwards with broad strands that often contain keratin filled cysts in their center. There are large, bulbous, downward proliferations that compress the collagen bundles of stroma and push them aside. Thus, OVC extends downwards in bulldozing or pushing manner but does not invade through the basement membrane into the underlying connective tissue stroma. OVC usually lacks epithelial dysplasia. Even in the deeper portions of the tumour nuclear atypia, individual cell keratinization and horn pearls are absent. Verrucous carcinoma is usually of indolent clinical behavior with excellent prognosis and remains for long time without distant metastasis. In course of time verrucous carcinoma may show epithelial cell dysplasia indicating progression to conventional squamous cell carcinoma which can invade through the basement membrane and metastasize subsequently if not treated properly.[7] Periodic acid-Schiff (PAS) stain is a special stain that delineates type IV collagen which is the main component of epithelial basement membrane. It can be used to see the breach in continuity of the basement membrane and invasion of tumour cells through the basement membrane into the underlying connective tissue stroma.[8] OVC is treated by surgical resection of the primary tumour with adequate margin alone whereas oral verrucous carcinoma with foci of invasion is treated as ISCC. These patients get surgical resection of the tumour with neck dissection and adjuvant radiation therapy if indicated by overall pathologic stage.[9] Oral verrucous carcinoma has a better prognosis than oral verrucous carcinoma with epithelial dysplasia and/or foci of invasive SCC.

The aim of this study was to evaluate epithelial dysplasia and invasion through the basement membrane in oral verrucous carcinoma.

Methods

This was a cross sectional observational study. It was conducted among 45 patients of oral verrucous carcinoma in the Department of Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU) during 2021-2023. Paraffin blocks and Hematoxylin and Eosin-stained (H&E) slides of histologically diagnosed cases of oral verrucous carcinoma were collected from Pathology department, BMU. H&E stained sections of each case

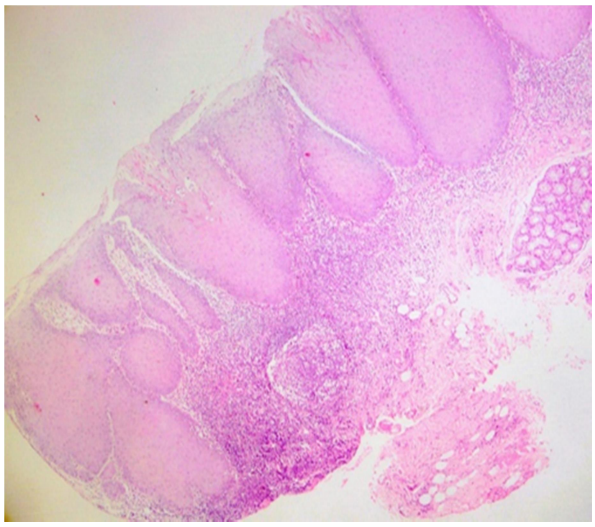
were re-evaluated microscopically to assess the histological diagnosis of oral verrucous carcinoma. Presence or absence of epithelial dysplasia was evaluated. One or more of the following characteristics were considered indicative of epithelial dysplasia: expansion of the basal layer by enlarged or hyperchromatic cells, decreased maturation with enlarged or hyperchromatic nuclei above the basal layers, cytologic atypia or pleomorphism, cells with high nuclear to cytoplasmic ratio above the suprabasal layer and multiple or atypical mitoses above the basal or suprabasal layers.[9] New sections were cut from the paraffin blocks and stained with PAS stain. The PAS stained sections were evaluated to see any discontinuity in the PAS positive basement membrane and presence or absence of invasion of tumor cells through the BM into the underlying connective tissue stroma. Study cases were followed up after six months to one year. History was taken from the patients or their relatives about the patients' health status, recurrence and subsequent biopsy. Histopathology reports of the subsequent biopsies were collected.

The statistical analysis was carried out using the Statistical Package for Social Sciences version 25.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics (frequencies and percentages) were used to summarize the patients' demographic characteristics and were presented in tables, figures and charts. The frequencies of different entities were expressed as percentage. Fisher's Exact test was used to analyze different categorical variables. A *p* value <0.05 was considered statistically significant.

Results

This study was a cross sectional observational study. It was conducted in the Department of Pathology, BSMMU. The study population were the patients who were diagnosed as verrucous carcinoma of oral cavity in the Department of pathology, BSMMU during 2021-2023. Patients of both sexes of all ages were included in the study. Demographic and histopathological variables i.e. age, sex, site of the lesion, predisposing conditions, epithelial cell dysplasia and invasion of tumour cells through the basement membrane were assessed. Study cases were followed up after six months to one year during this study period.

The mean age of the study population was 60.20±9.7 year (Range: 35-85 years). Majority patients were in age group 51-60 years (48.9%). Females were found affected slightly more than male and female to male ratio was 1: 0.96. Number of female patients was 23 (51.1%) and of male patients was 22 (48.9%). Different sites of oral cavity were involved by the verrucous carcinoma. In majority of patients, the affected site was left buccal mucosa (44.4%) and the second most common site was right buccal mucosa (31.1%). The study subjects presented clinically with exophytic, ulcerative, whitish elevated area or ulceroproliferative lesions. Majority of the patients presented with an exophytic lesion (51%) and the second most common clinical presentation was an ulcerative lesion (35%). In this study the most frequent predisposing condition was betel nut chewing (44.4%) and the second most frequent predisposing condition was tobacco smoking (40%). It was observed that 15 cases (33.3%) showed the presence of one or more features of epithelial dysplasia and 30 cases (66.7%) showed no epithelial dysplasia.



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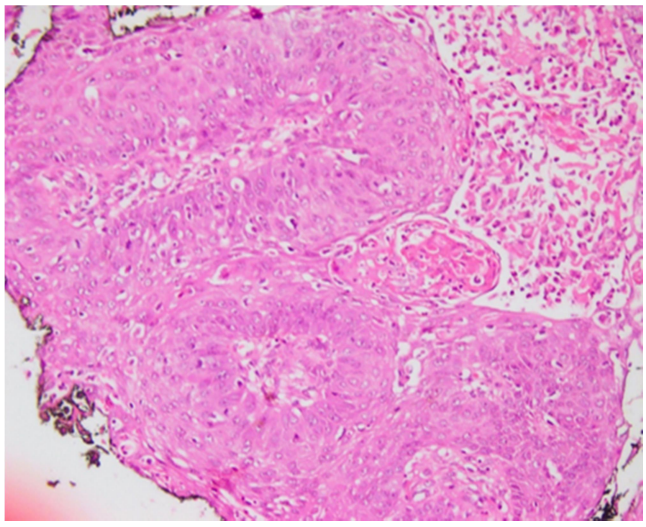

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Figure 1. Photomicrograph of histopathological slides of OVC (A) OVC without epithelial dysplasia (H&E stain, 4x); (B) OVC with epithelial dysplasia (H&E stain, 40x)

In this study 30 cases (67%), which revealed no epithelial dysplasia in H&E stain, showed intact PAS positive basement membrane. Among the remaining 15 cases which revealed epithelial dysplasia in routine H&E stain, 10 cases showed intact PAS positive basement membrane and 5 cases showed discontinuous PAS positive basement membrane and invasion of tumour cells through the basement membrane (Figure 2).

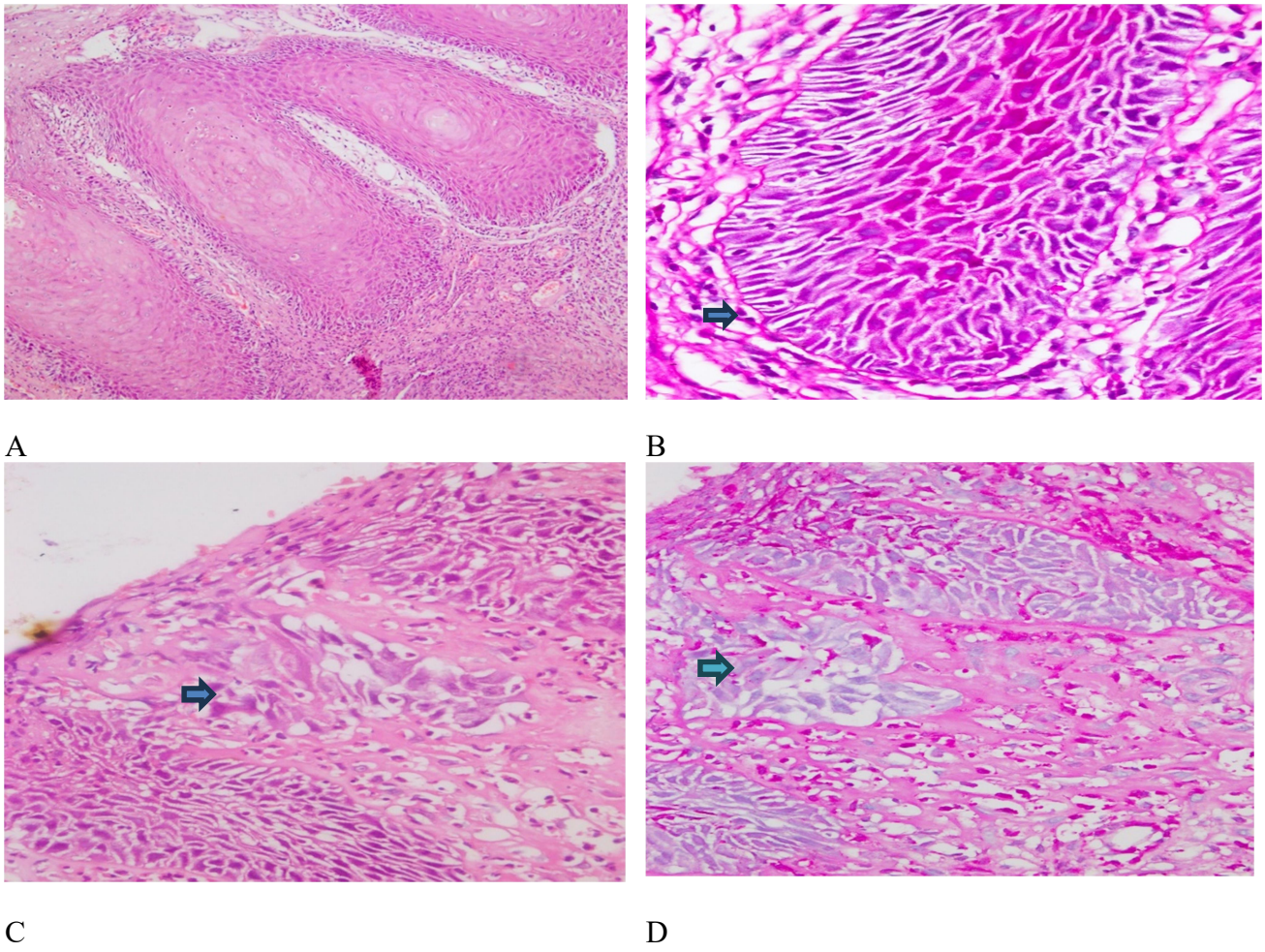

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Figure 2. Photomicrograph of histopathological slides of OVC. (A) OVC without epithelial dysplasia (H&E stain, 20x); (B) PAS positive continuous BM (PAS stain, 40x); (C) OVC with epithelial dysplasia (H&E stain, 40x); (D) PAS positive discontinuous BM and invasion of tumour cells through the BM into the underlying connective tissue stroma (PAS stain, 40x).

Table I: Statistical analysis of invasion through the BM in relation to the presence or absence of epithelial dysplasia in OVC (N=45)

Presence of invasion	OVC with epithelial dysplasia (n=15) n (%)	OVC without epithelial dysplasia (n=30) n (%)	p-value
Yes	5 (33.3)	0	<0.004
No	10 (66.7)	30 (100.0)	

p-value was calculated using Fisher's Exact test.
Values were expressed in frequency.

The table shows that, presence of invasion is significantly higher in OVC cases having the features of epithelial dysplasia.

In this study, 33 cases were followed up after six months to one year during this study period. Thirteen of them underwent subsequent biopsy. Seven cases (15.6%) were diagnosed as invasive squamous cell carcinoma and six cases (13.3%) as oral verrucous carcinoma in their subsequent biopsies. In 20 cases (44.4%) no swelling or white patch reappeared after first excision and they were in good health without any further treatment.

Table II: Statistical analysis of ISCC in subsequent biopsy in relation to the presence or absence of epithelial dysplasia in previously diagnosed cases of OVC (n=33)

Invasive squamous cell carcinoma in subsequent biopsy	Previously diagnosed OVC with the presence of epithelial dysplasia (n=12) n (%)	Previously diagnosed OVC without the presence of epithelial dysplasia (n=21) n (%)	p-value
Yes	7 (58.3)	0	<0.001
No	5 (41.7)	21 (100.0)	

p-value was calculated using Fisher's Exact test.
Values were expressed in frequency.

The table shows that, invasive squamous cell carcinoma in subsequent biopsies is significantly higher in previously diagnosed OVC cases having epithelial dysplasia.

Discussion

Oral verrucous carcinoma is an uncommon variant of well differentiated squamous cell carcinoma with an excellent prognosis. It shows an indolent clinical behaviour with long survival period. It typically lacks the characteristics of epithelial dysplasia and does not metastasize. However, Some OVCs show epithelial dysplasia without any conventional invasive SCC and some OVC have just a minor component of invasive SCC. Tumours with these limited changes are currently very difficult to diagnose and the terminology used varies greatly. A variety of descriptive terms are used by pathologists, including "verrucous carcinoma with focal dysplasia and/or invasion", "focus of squamous cell carcinoma arising within a verrucous carcinoma," "well differentiated squamous cell carcinoma with verrucous features," and "hybrid verrucous carcinoma". Although there is a distinct lack of standard nomenclature, these ‘‘in between’’ type tumors are not uncommon. Oral verrucous carcinoma exhibiting epithelial dysplasia signifies the emergence of a conventional squamous cell carcinoma. If left untreated, this cancerous growth may breach the basement membrane and metastasize.[7] Presence of epithelial dysplasia and/or foci of invasion in OVC indicates worse prognosis and these cases are treated as conventional ISCC. So, earlier detection of epithelial dysplasia and/or invasion through BM in OVC will help the clinician for the proper management of the patient.[10]

In this study, the mean age of the patients was 60.20±9.7 years and females (51.1%) were found affected slightly more than males. In another study done in Bangladesh by Mamun *et al.*, the average age of the patient was 58.9 years and males (53%) was found affected slightly more than females (47%).[4] In this current study right and left buccal mucosa was the most commonly involved site for OVC. Other sites of involvement were lower lip, right and left gingivobuccal sulcus and tongue. In a study, Franklyn *et al.*, also revealed buccal mucosa (56%) as the most common site of involvement by oral

verrucous carcinoma.[11] In this study, majority of the patients presented with an exophytic lesion. Other clinical presentations were ulcerative lesion, ulcero-proliferative lesion and whitish patch. In a study Sonalika and Anand, found exophytic lesion as the most frequent clinical presentation of oral verrucous carcinoma. Ulcero-proliferative lesion and white patch were other clinical presentations of OVC in their study.[12] The exact etiology of OVC is unknown. It is known to have an association with tobacco smoking, betel nut chewing, alcohol addiction and poor oral hygiene. Among the study population of this present study most of the cases had the history of betel nut chewing. Other cases had history of tobacco smoking, both tobacco smoking and betel nut chewing and poor oral hygiene. In one study, Mamun *et al.*, found incidence of betel nut chewing, smoking, and both 46.9%, 20.0% and 13.3% respectively in histopathologically diagnosed oral verrucous carcinoma patients.[4]

Usually OVC lacks features of epithelial dysplasia. But in some cases epithelial dysplasia can be present which is an important prognostic factor that indicates the possibility of transformation into a conventional squamous cell carcinoma. So, evaluation of epithelial dysplasia in oral verrucous carcinoma is of great importance. In this study presence of epithelial dysplasia was observed in 33.3% cases. In their study Sonalika and Anand, found 20% cases showing distinct epithelial dysplasia in oral verrucous carcinoma.[12] In another study Patel *et al.*, found 44% cases of oral verrucous carcinoma having epithelial dysplasia.[9]

PAS stain was done to see the continuity of the PAS positive basement membrane and invasion of tumour cells through the BM. We observed that invasion of tumour cells through the BM was significantly higher in OVC having the features of epithelial dysplasia. Several authors described that as many as 20% cases of oral VC can show coexisting foci of conventional invasive squamous cell carcinoma. Franklyn *et al.*, revealed 13% hybrid OVC in their study.[11] Rekha and Angadi, showed that 15% cases of oral verrucous carcinoma had foci of invasion.[13] Patel *et al.*, revealed 24% cases of hybrid verrucous carcinoma among the histologically diagnosed oral verrucous carcinoma cases.[9]

In this present study, we followed up the cases. We observed that invasive squamous cell carcinoma in subsequent biopsy was significantly higher in previously diagnosed OVC cases having the features of epithelial dysplasia. Rekha and Angadi, showed in their study that 15% cases of oral verrucous carcinoma, which were diagnosed as VC with invasion, all turned out to be SCC on subsequent excisional biopsy.[13] Rath *et al.*, found 11% patients whose histopathology changed from OVC to OSCC in subsequent excisional biopsy.[14]

Conclusion

Epithelial dysplasia in OVC signifies the increased possibility of its progression into conventional invasive squamous cell carcinoma which can invade through the basement membrane and metastasize subsequently. Presence of epithelial dysplasia and/or invasion in OVC also indicates worse prognosis and these cases are treated as conventional ISCC. So, earlier detection of these changes in OVC is of great importance and it will help the clinician in properly managing the patient.

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