

RESEARCH ARTICLE

Association of Syndecan-1 immunoexpression with epithelial dysplasia in oral verrucous carcinoma

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Abstract

Background: Oral verrucous carcinoma (OVC) is an uncommon variant of well-differentiated squamous cell carcinoma with an excellent prognosis. Epithelial dysplasia in OVC is a sign of a poorer prognosis. Syndecan-1 immunoexpression is absent in squamous epithelial dysplasia and squamous cell carcinoma. This study aimed to investigate the association between Syndecan-1 immunoexpression and epithelial dysplasia in oral verrucous carcinoma.

Methods: A cross-sectional study was done at the Department of Pathology, Bangabandhu Sheikh Mujib Medical University, from 2021 to 2023 and included 45 cases of histologically diagnosed OVC. Histopathological variables were assessed and Syndecan-1 immunoexpression was determined. Fisher's exact test was done to examine the relationship between the loss of Syndecan-1 and OVC dysplasia.

Results: Out of 45, histologically diagnosed OVC subjects, 66.7% showed no features of epithelial dysplasia, and 33.3% of cases revealed the presence of epithelial dysplasia in routine hematoxylin and eosin stains. The cases which revealed no epithelial dysplasia showed positive expression of Syndecan-1. Among the cases that revealed epithelial dysplasia, 26.7% showed loss of Syndecan-1 expression in the dysplastic area, and 6.7% showed positive expression of Syndecan-1. The loss of Syndecan-1 expression was significantly ($P < 0.01$) associated with epithelial dysplasia in OVC.

Conclusion: Syndecan-1 immunostain and routine hematoxylin and eosin stain can help detect epithelial dysplasia in oral verrucous carcinoma. Therefore, early detection of epithelial dysplasia in OVC will help the clinician properly manage the patient.

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Key messages

Epithelial dysplasia in oral verrucous carcinoma indicates the increased possibility of transformation into a conventional squamous cell carcinoma. Syndecan-1, also known as CD138, expression of which is absent in epithelial dysplasia of oral verrucous carcinoma and squamous cell carcinoma. Syndecan-1 immunostain, in conjunction with routine hematoxylin and eosin stain, can help to detect epithelial dysplasia in oral verrucous carcinoma.

Introduction

Oral cancer is the sixth most frequent malignancy worldwide [1]. Oral invasive squamous cell carcinomas account for about 90% of reported oral cavity cancers, with a five-year survival rate of around 45% [2]. Oral verrucous carcinoma (OVC) is an uncommon, well-differentiated squamous cell carcinoma variant [3]. It commonly appears in the mucous membrane of the head and neck region, most frequently in the oral cavity and larynx [4]. Risk factors for OVC include alcohol, tobacco, betel nuts, human papillomavirus, and poor oral hygiene [5]. It more commonly affects males over the fifth to sixth decade [4]. Among all primary oral cavity cancers, the reported rate of OVC is 3% worldwide [6]. The prevalence of OVC in Bangladesh is 2.5% [1].

OVC shows grey-white, painless growth that spreads outward and fungates on the oral mucosa. OVC typically lacks the characteristics of epithelial dysplasia. It has a long survival period and better prognosis than conventional squamous cell carcinomas or hybrid verrucous carcinoma. OVC can eventually show epithelial dysplasia, a sign that it is turning into a conventional squamous cell carcinomas. If left untreated, this type of cancer can invade the basement membrane and metastasize [7]. Surgical resection of the primary tumour with adequate margin alone treats OVC, while surgical resection of the tumour with neck dissection and adjuvant radiation therapy if indicated by overall pathologic stage, treats conventional squamous cell carcinomas or OVC with a component of invasive squamous cell carcinomas [8]. Given the differences in treatment protocol and prognosis, it is crucial to diagnose these lesions appropriately [9].

Syndecan-1 is a heparan sulfate proteoglycans, which is also known as CD 138 [10]. It is found in the squamous epithelial cells of various organs, goblet and columnar epithelial cells of the gastrointestinal tract, plasma cells and hepatocytes. It is involved in a number of biological processes such as cellular proliferation, differentiation, adhesion and migration. The downregulation of Syndecan-1 may allow the cells to separate and invade. Keratinocyte differentiation induces the expression of Syndecan-1, which is absent in epithelial dysplasia, squamous cell carcinoma, and OVC, having the features of epithelial dysplasia [11]. Malignant transformation, invasion and metastasis are associated with loss of Syndecan-1 expression [12].

In previous studies, some authors found the presence of epithelial dysplasia in OVC. Significant downregulation of Syndecan-1 expression in epithelial dysplasia was also observed in some studies. However, the expression of Syndecan-1 in OVC in relation to the presence or absence of epithelial dysplasia was not evaluated in previous studies, which we evaluated in our study. The presence of epithelial dysplasia in OVC indicates a worse prognosis and an increased possibility of developing a conventional squamous cell carcinomas if not treated. Detection of epithelial dysplasia in OVC is sometimes difficult when using conventional hematoxylin and eosin stain only. Therefore,

Syndecan-1 immunostain in conjunction with traditional hematoxylin and eosin stain can help early detection of epithelial dysplasia in OVC and assist the clinician in properly managing the patient. The objective of this study was to examine the association between the immunohistochemical expression of Syndecan-1 and epithelial dysplasia in OVC.

Methods

We examined 45 paraffin blocks and hematoxylin and eosin stained slides of histologically diagnosed cases of OVC from the Department of Pathology, Bangabandhu Sheikh Mujib Medical University during 2021-2023. We evaluated hematoxylin and eosin stained sections of each case to see the presence of epithelial dysplasia. 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 [8].

We stained representative sections from the paraffin block with Syndecan-1 antibody using a standard protocol compatible with the DAKO Envision TMFLEX + detection system for immunohistochemistry.

Syndecan-1 immunoreactivity appears as cell membrane staining of squamous epithelial cells. We observed Syndecan-1 immunoreactivity and classified it into two categories: negative/loss of expression and positive expression. Positive expression of Syndecan-1 immunostain in the cell membrane of the squamous epithelial cells of normal oral mucosa was used as positive control and loss of expression of Syndecan-1 immunostain in a diagnosed case of invasive squamous cell carcinoma of oral mucosa was used as negative control.

We coded the participants' paraffin blocks with unidentifiable numbers, such as cases 1 and 2, to prevent bias. Coding was done in every step of data collection. In our study, potential confounding variables were staining processes of hematoxylin and eosin stain and Syndecan-1 immunostain. We were aware of these confounders and addressed them in our research.

Statistical analysis

The statistical analysis was done using SPSS. Descriptive statistics (frequencies and percentages) were used to summarise the patients' data and presented in tables and figures. Fisher's exact test was used to examine the relationship between loss of Syndecan-1 and OVC dysplasia. $P < 0.05$ was considered statistically significant.

Results

In this study, 45 histologically diagnosed OVC cases were included. The mean (standard deviation) age of the patients was 60.2 (9.7) years. Females (51.1%) were affected slightly more than males (48.9%).

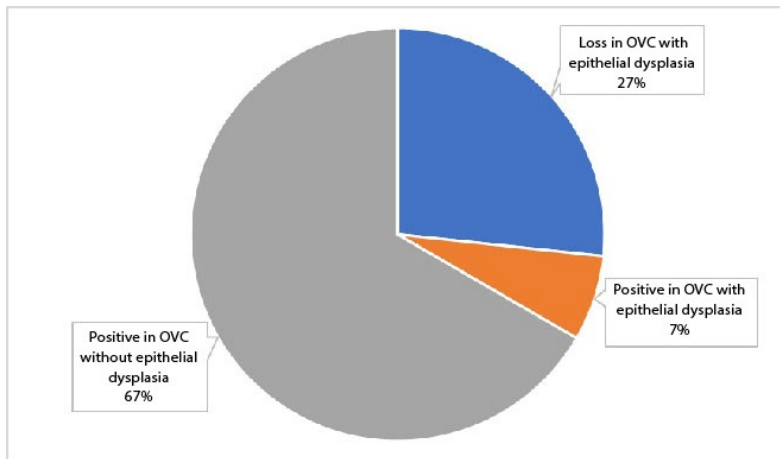


Figure 1 Distribution of the study subjects according to Syndecan-1 expression (n=45). OVC indicates oral verrucous carcinoma.

Right and left buccal mucosa (75.5%) was the most commonly involved site for OVC. Other sites of involvement were the lower lip (11.1%), right and left gingivobuccal sulcus (9.6%) and tongue (4.4%). Most patients presented with an exophytic lesion (51.1%). Other clinical presentations were ulcerative lesions (35.6%), ulcer-proliferative lesions (6.7%) and whitish patches (6.7%). Among the study cases, 44.4% had a history of chewing betel nut, 40% had a history of tobacco smoking, 8.9% had a history of both tobacco smoking and betel nut chewing, and 6.6% cases had poor oral hygiene.

Thirty cases (67%) revealed no epithelial dysplasia in hematoxylin and eosin stain, which showed intact expression of Syndecan-1. Twelve cases (26.7%) revealed loss of Syndecan-1 expression and 3 cases

(6.7%) showed intact expression of Syndecan-1 (Figure 1). Hematoxylin and eosin stain in OVC is shown in Figure 2. In this study, all the cases histologically showed hyperkeratosis, parakeratosis, acanthosis, and occasional papillomatosis and extended downwards with broad pushing strands. In most cases, the keratinocytes appeared well differentiated and showed no features of epithelial dysplasia. Statistical analysis showed that loss of Syndecan-1 immunoexpression was associated with the presence of epithelial dysplasia in OVC (Table 1).

Table 1 Number (%) of subjects with or without epithelial dysplasia (n=45)

Loss of Syndecan-1	OVC ^b with dysplasia (n=15)	OVC ^b without dysplasia (n=30)	P
Yes	12 (80.0)	0	<0.01 ^a
No	3 (20.0)	30 (100.0)	

^aFisher's exact test; ^bOVC indicates oral verrucous carcinoma

Discussion

OVC is a rare variant of well-differentiated squamous cell carcinoma. It has an excellent prognosis, indolent clinical behaviour, and a prolonged survival without distant metastases. It typically lacks the characteristics of epithelial dysplasia. However, Some OVCs show epithelial dysplasia without conventional invasive squamous cell carcinomas, and some OVCs have just a minor component of invasive squamous cell carcinomas. Tumours with these limited changes are difficult to diagnose. OVC exhibiting epithelial dysplasia signifies the emergence of a conventional squamous cell carcinomas. If left untreated, this cancerous growth may breach the basement membrane and metastasize [7]. Therefore, evaluation and early detection of epithelial dysplasia in verrucous carcinoma is very important.

In routine hematoxylin and eosin stain, we found 33.3% of cases revealing one or more features of epithelial dysplasia. Sonalika and Anand histopathologically found a proliferation of well-differentiated squamous epithelium without or with minimal atypia in most of the OVC cases in their study. However, they found that 20% of cases showed distinct epithelial dysplasia [13]. In another study, Patel *et al.* found that 44% of cases of OVC had epithelial dysplasia. They found local recurrence in these cases 2–3 times higher than for OVC without having epithelial dysplasia [8]. Syndecan -1 is a heparan sulfate proteoglycan involved in cellular proliferation, differentiation, adhesion, and migration. Loss of Syndecan-1 expression is associated with malignant transformation, invasion and metastasis [12]. We evaluated Syndecan-1 expression immunohistochemically and found a significant number of cases showing loss of Syndecan-1 expression in dysplastic areas. This study found a statistically significant association between a loss of Syndecan-1 immunoexpression and the presence of epithelial dysplasia in OVC. In their study, Daskalopoulos *et al.* found significant downregulation of Syndecan-1 expression in OVC and invasive

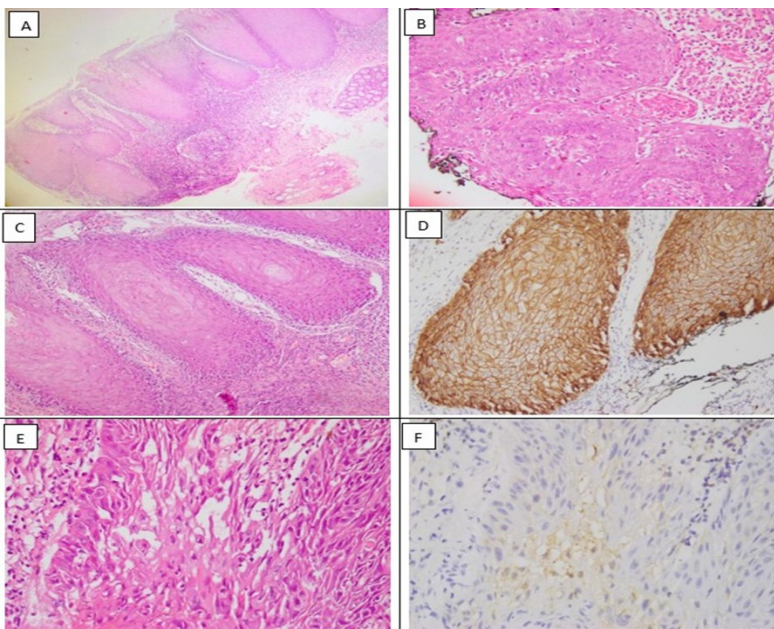


Figure 2 Hematoxylin and eosin stain and Syndecan-1 immunostain in oral verrucous carcinoma: (A) OVC without epithelial dysplasia (4x), (B) OVC with epithelial dysplasia (40x), (C) OVC without epithelial dysplasia (20x), (D) positive Syndecan-1 expression in OVC without epithelial dysplasia (40x), (E) OVC with epithelial dysplasia (40x), and (F) loss of Syndecan-1 expression in OVC with epithelial dysplasia (40x). OVC indicates oral verrucous carcinoma.

squamous cell carcinomas [14]. Soukka *et al.* also found an association between loss of Syndecan-1 immunoexpression and dysplastic changes in oral epithelium in their study [11].

Limitations

The present study has some limitations. We selected the study population from Bangabandhu Sheikh Mujib Medical University only, which may not accurately represent the country. Moreover, the small sample size was a limitation of the present study. As OVC is a rare tumour and our study period was only for two years, we were able to include 45 cases. A larger sample size could be achieved through multicenter study.

Conclusion

In conclusion, a statistically significant loss of Syndecan-1 immunoexpression was found in epithelial dysplasia of OVC. Therefore, the combination of Syndecan-1 immunostain and routine hematoxylin and eosin stain can aid in the detection of epithelial dysplasia in OVC. Epithelial dysplasia in OVC is a significant predictor of the emergence of a conventional squamous cell carcinoma, which, if left untreated, can infiltrate the basement membrane and metastasize subsequently. Therefore, early detection of epithelial dysplasia in OVC can assist the clinician in appropriately managing the patient.

Long-term follow-up of the patients to correlate the expression of this immunomarker with the progression and recurrence of the disease and survival of the patients would be beneficial to establish the role of this biomarker.

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Author contributions

Conception or design of the work; or the acquisition, analysis, or interpretation of data and drafting the work: MA, BPD, IJ, MMA, SGB. *Drafting the work or reviewing it critically for important intellectual content:* MA, BPD, IJ, MMA, SGB. *Final approval of the version to be published:* MA, BPD, IJ, MMA, SGB. *Accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved:* MA.

Conflict of interest

We do not have any conflict of interest.

Data availability statement

We confirm that the data supporting the findings of the study will be shared upon reasonable request.

Supplementary file

None

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