

Detection of Micro-invasion in Sudanese Oral Verrucous Carcinoma Samples Using Syndecan-1 Stain

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ABSTRACT

AIM: Verrucous carcinoma (VC) is a low-grade rare variant of squamous cell carcinoma (SCC). Syndecan-1 (CD138) is a heparan sulfate proteoglycan which participates in cell-to-cell adhesion and cell-matrix interaction. Being misled by the apparent non-aggressive nature of VC, some clinicians and pathologists believe that this tumor is not an aggressive tumor, not realizing the fact that some of these lesions may contain nests or foci of well-differentiated SCC. This study aimed to assess syndecan-1 expression of VC and detection of micro-invasion in VC using syndecan-1 immunohistochemical (IHC) stain.

METHODS: Observational analytical study of 34 paraffin block of VC cases and 24 cases of variable grades of oral epithelial dysplasia. Cases were stained by hematoxylin and eosin (H&E) and then IHC stain for syndecan-1 was applied. Nine paraffin blocks from specimens of normal oral mucosa were used as the reference group for syndecan-1 stain positivity.

RESULTS: In this study, we found that 32 (94.1%) out of 34 of verrucous carcinoma cases showed loss of syndecan-1 expression. Moreover, highly statistically significant association was found between the presence of suggestive micro-invasion in H&E and loss of syndecan-1 expression in micro-invasive area in the same case.

CONCLUSIONS: In conclusion, syndecan-1 stain can be used as a biomarker in detection of micro-invasion in verrucous carcinoma.

KEYWORDS: verrucous carcinoma, syndecan-1, micro-invasion, Sudanese

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Introduction

Oral squamous cell carcinoma (OSCC) represents a major cancer trouble, especially in areas of the world where tobacco is used. It accounts for more than 90% of the malignant neoplasms of the oral cavity.¹ In Sudan, the frequency of oral cancer is 9.4% of all body cancers and verrucous carcinoma (VC) constitutes 3.8%,² and the most common cause of oral cancer is snuff dipping.³ Verrucous carcinoma was first reported by Friedell and Rosenthal⁴ in 1941 and described in 1948 by Lauren V. Ackerman that it represented a unique type of squamous cell carcinoma (SCC). It is usually seen in the oral cavity of elderly men.⁵ Tobacco chewing seems to be a significant cause of oral verrucous carcinoma (OVC).^{6–8} The classic histopathology of VC consists of exophytic growth of well-differentiated stratified squamous epithelium with deep bulbous rete ridges. It exhibits little or no cytological atypia and deep surface invaginations filled with parakeratin or orthokeratin. Its margins show a compressive growth pattern and inflammation of the adjacent stroma with local destruction of connective tissue.^{5,7–9} Oral verrucous carcinoma is a locally invasive tumor which remains for long time without distant metastasis. When invasion occurs it turns into well-differentiated SCC and metastasis can occur if not treated properly.^{10–12} Diagnosis of VC remains a problem to be solved, as the lesion should be

differentiated from low-grade SCC, hence it may contain foci of micro-invasion.^{12–14} Detection of invasion in VC can be difficult in incisional biopsies, and it is difficult to exclude an underlying conventional SCC and often even an adequate biopsy may miss areas of SCC when using conventional hematoxylin and eosin (H&E) staining. Syndecan-1 (CD138) is a member of the family syndecans which are heparan sulfate proteoglycan receptors that are thought to participate in both cell-to-cell adhesion and cell-to-matrix interaction.¹⁵ It is present on the surface of plasma cells and epithelial cells. Its expression is induced during keratinocyte differentiation and reduced in epithelial dysplasia and SCCs.^{15–17} It is degraded through heparanase enzyme and invasion is associated with high level of heparanase on polymerase chain reaction (PCR).¹⁸ Loss of syndecan-1 expression may offer the possibility that cell gets the ability to invade. In this study, immunohistochemical (IHC) staining was performed to detect micro-invasion in VC using syndecan-1 immune stain.

Materials and Methods

Study design

Observational analytical study of 34 paraffin block specimens of oral VC samples retrieved from the archive of the Oral Pathology



Table 1. Predominant histopathological features of VC in H&E examination.

HISTOPATHOLOGICAL PARAMETERS	PREDOMINANT FEATURES	NUMBER OF CASES (%)
Surface keratinization	Thick surface keratinization	31 (91.20)
Epithelium hyperplasia	Presence of hyperplasia	34 (100)
Rete ridges morphology	Broad elongated rete ridges	26 (76.50)
Intra-epithelial keratinization	Presence	26 (76.50)
Basal cytological atypia	Presence of basal cytological atypia	31 (91.20)
Mitotic activity	Increased	24 (60.60)
Epithelial dysplasia	Low grade epithelial dysplasia	18 (52.90)
Basal layer morphology	Altered	25 (73.5)
Sub-epithelial inflammatory reaction	Severe	19 (55.90)
Pushing growth into the underlying tissues	Present	19 (55.90)
Presence of suggestive micro-invasion	Not present	18 (52.90)

Abbreviations: H&E, hematoxylin and eosin; VC, verrucous carcinoma.

Laboratory, Faculty of Dentistry, University of Khartoum in the period January 2006 to June 2014 was performed. In addition, 24 paraffin block specimens of different grades of oral epithelial dysplasia divided using binary system and 9 paraffin block specimens of normal oral mucosa were used as reference groups for syndecan-1 IHC positivity.

Sample preparation

All samples in this study were stained by routine H&E using standard protocol of Harris (H&E) staining. Then, IHC staining with syndecan-1 was applied using Ventana antibody-automated system according to manufacturer's instruction. After staining by syndecan-1, the stain intensity, positivity area, and expression in micro-invasive area were examined.

Interpretation of syndecan-1 staining

For interpretation of syndecan-1 stain, cases were divided into 3 groups: normal oral mucosa taken as a positive reference group, oral epithelial dysplasia group taken as a negative reference, and VC as a test group. In the normal oral mucosa group, the stain showed strong dark brown positive immune reactivity from the basal or suprabasal layer to the prickle cell layer. In the oral epithelial dysplasia group, the stain showed less brown color for negative immune reactivity. The groups are assessed further for stain intensity which was graded for statistical analysis and descriptive purpose into high, high to moderate, moderate, moderate to mild, mild, and negative for both VC and oral epithelial dysplasia groups. Moreover, every slide included internal positive control obtained from normal lymphoid tissue (tonsils) as mentioned to be the best internal positive control by the manufacturer.

Data processing

For the statistical analysis of data variable descriptive analysis is used, Chi-square test is used for comparison of categorical data, and contingency coefficient is used for association.

Results

Microscopic examination of H&E stain

The 34 VC samples were subjected to H&E examination, and the result showed foci suggestive of micro-invasion in 16 (47%) of the samples and 18 (53%) showed no evidence of micro-invasion (Table 1 and Figure 1).

Microscopic examination of syndecan-1 stain

The 34 VC samples and the reference groups were stained with syndecan-1 and examined microscopically. The result showed that 2 (5.9%) of the samples of VC expressed high to moderate syndecan-1 intensity, 25 samples (82.3%) were found with reduced intensity, and 4 samples were negative (Table 2 and Figure 2).

The expression of syndecan-1 in micro-invasive area in VC

A total of 22 samples out of 34 samples of VC show areas of micro-invasion syndecan. Three samples (8.8%) showed mild intensity, whereas 6 samples (17.60%) showed mild positivity to negative and 30 samples (38.20%) showed negative syndecan-1 expression in micro-invasive areas (Table 3).

The comparative finding of the H&E and syndecan-1 stains

The comparative result between the 2 stains for the presence in micro-invasive areas is about 47.1% in H&E, whereas

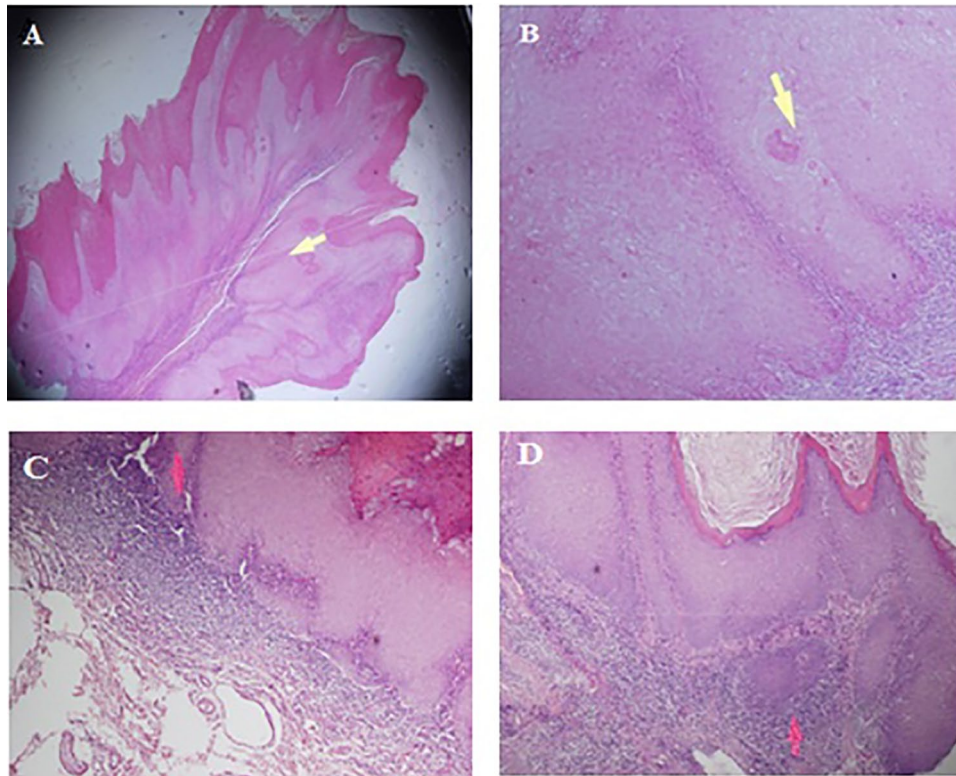


Figure 1. H&E features of VC in patient treated with partial resection of the anterior part of the mandible and lower lip: (A) exophytic hyperkeratotic surface, (B) pushing downward growth and unusual finding of intraepithelial keratin pearls formation, (C) irregular basal layer, and (D) suggestive invasive nests. H&E indicates hematoxylin and eosin; VC, verrucous carcinoma.

Table 2. The result of VC staining by using syndecan-1.

SYNDECAN-1 STAIN INTENSITY	VC SAMPLES, N (%)	NORMAL MUCOSA, N (%)	DYSPLASIA, N (%)
High to moderate	2 (5.9)	9 (100)	3 (12.5)
Moderate to mild	25 (88.2)	0 (0)	21 (87.5)
Negative	4 (11.8)	0 (0)	0 (0)
Total	34 (100)	9 (100)	24 (100)

Abbreviation: VC, verrucous carcinoma.

in syndecan-1 stain was 64.6%. By using the Chi-square test, statistically highly significant association was found between the presence of suggestive micro-invasion in H&E and the syndecan-1 expression in the micro-invasive areas for the VC group. Chi-square was 16.485, and *P*-value was .000. The result is demonstrated in Table 4.

Discussion

Verrucous carcinoma is a low-grade rare variant of squamous cell carcinoma. The VC has been the subject of a continuous debate concerning diagnostic features and mode of treatment as it may show the conventional invasive pattern of SCC with an exo-endophytic growth pattern and may contain foci of micro-invasion.^{14,19,20} Also, the VC might be misdiagnosed, either because the biopsy sample is not adequately representative or because of the difficulty of establishing a diagnosis

based on histopathological features with routine H&E staining due to the presence of tangential cut or unoriented biopsy.²¹ Therefore, early detection of micro-invasion in VC will allow proper management of the tumor, improve the cure rate, and provide good prognosis.²² In this study, H&E examination was performed with histopathological parameters such as surface keratinization, epithelial hyperplasia, and rete ridge morphology. The result revealed that VC could have 2 histological types rather than one type which was described in the previous literature. The first type shows classical histopathological features with bland looking cytological features, and the second shows cytological atypia and micro-invasive foci and has the ability of invasion and subsequently metastases. Our results agreed with Kolokythas et al's²² and Fancher et al's²³ findings, but the percentage was much greater than that mentioned in previous studies by Kolokythas et al,

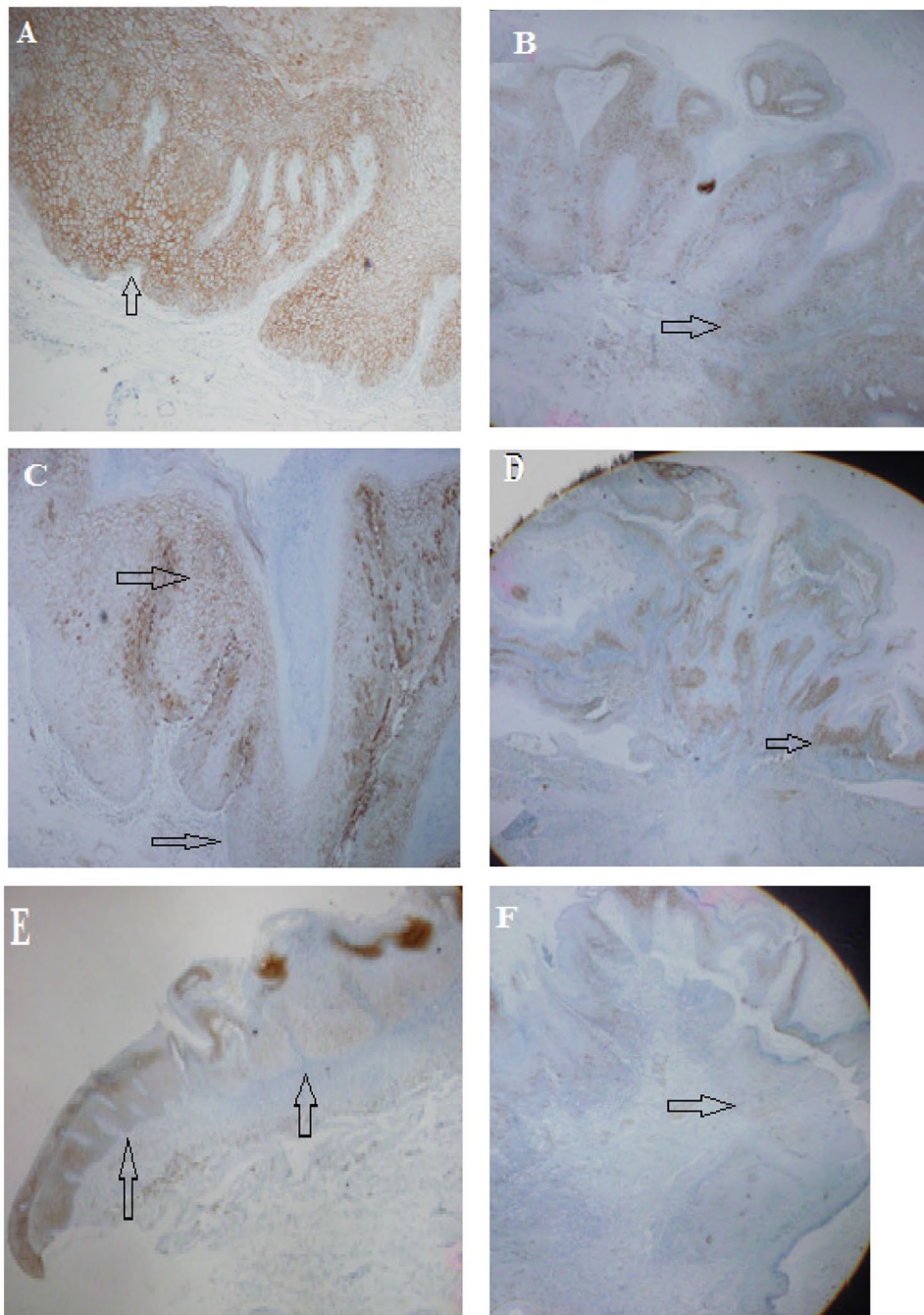


Figure 2. Syndecan-1 expression in VC: (A) high intensity, (B) mild intensity, (c) mild to moderate intensity (note the keratinized layer and keratin plug negative syndecan-1 expression), (D) moderate intensity, (E) loss of syndecan-1 expression in VC area (note the different in intensity between normal epithelium and VC area), and (F) negative expression. VC indicates verrucous carcinoma.

Table 3. Syndecan-1 expression in the micro-invasive area in VC.

PRESENCE OF MICRO-INVASION	NUMBER OF CASES (%)
Mild positivity	3 (8.8)
Mild positive to negative	6 (17.6)
Negative	13 (38.2)
No presence of micro-invasion	12 (35.3)
Total	34 (100)

Abbreviation: VC, verrucous carcinoma.

Medina et al, and Orvidas et al. They indicated that only about 20% of all oral and 10% of all laryngeal VCs have shown foci of micro-invasion or hybrid VC.^{22,24,25} The difference in findings may be due to the small sample size in this study compared with other studies or may be due to the fact that cases of this study specimens are from patient who usually come late seeking treatment. Few previous studies which were performed on syndecan-1 expression in oral dysplastic lesions and OSCC revealed that syndecan-1 is downregulated with increasing degree of oral epithelial dysplasia and its

Table 4. Association of the presence of suggestive micro-invasion in H&E and the expression of syndecan-1 in micro-invasive area in VC.

PRESENCE OF SUGGESTIVE MICRO-INVASION IN H&E	MICRO-INVASION AREA EXPRESSION, N (%)		TOTAL, N (%)	CHI-SQUARE	P-VALUE
	PRESENT WITH LOSS OF EXPRESSION	NOT PRESENT			
No	6 (27.3)	12 (100.0)	18 (52.9)	16.485	0.00
Yes	16 (72.7)	0 (0.0)	16 (47.1)		
Total	22 (100.0)	12 (100.0)	34 (100.0)		

Abbreviations: H&E, hematoxylin and eosin; VC, verrucous carcinoma.

expression becomes very low to negative in oral SCC.^{15,26-28} Hence, in this study different grades of oral epithelial dysplasia were used as negative reference control for the immune stain. The results of this study showed that the total loss in syndecan-1 expression in VC is about 94.1%, which is greater than the oral epithelial dysplastic reference group. These findings are similar to those of Ashok and Babaji.²⁹ This result indicates that syndecan-1 can be used as a marker for early detection of micro-invasion. The presence of micro-invasive areas in syndecan-1 stain was 64.6%, whereas it was about 47.1% on H&E examination. These different findings may be due to the small micro-invasive foci which may not be recognized under H&E examination or being considered as tangential cuts of the rete ridges but on syndecan-1 their detection was easier because syndecan-1 marks the cell membrane of epithelial cells. These findings indicate that loss of syndecan-1 contributes to the invasive ability of VC and is a useful biomarker for invasion detection.

Conclusions

In this study, syndecan-1 showed prominent downregulation in VC samples, and greater loss in the stain intensity was in samples with the presence of micro-invasion areas. The stain positivity areas in most of VC cases were different from positivity areas in the normal oral mucosa control group and similar to the dysplastic control group. These findings indicate that syndecan-1 expression is lost with dysplastic changes in VC. Since previous studies indicated that syndecan-1 have a role in tumor invasion, it can be a useful biomarker for the detection of invasion as the intensity of its expression is changing when the epithelium undergoes dysplastic or neoplastic changes.


Author Contributions

AKBE and AMS planned and directed the project. AKBE wrote the first draft of the manuscript. AKBE, AMS, NIAED, and SBM contributed to the writing of the manuscript, agreed with manuscript results and conclusions and made critical revisions, and approved final version. All authors reviewed and approved the final manuscript.

Ethics Approval and Consent to Participate

This research was approved by the ethical committee of the post graduate Medical & Health studies Board of The Faculty of Dentistry University of Khartoum, Khartoum, Sudan.

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