ORIGINAL RESEARCH

A Comparative Study of Verrucous Hyperplasia and Verrucous Carcinoma of the Oral cavity: Clinicopathological Dilemma Revisited

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Abstract

Background and Objectives: Oral verrucous hyperplasia and verrucous carcinoma are two clinicopathologically distinctive oral verrucous lesions, current understanding of these lesions is perplexing hence it is important to investigate the clinicopathological features of the two verrucous lesions and estimate their relationship. The present study was undertaken with the aim of identifying the pathological features in an attempt to differentiate both lesions.

Materials and Methods: Archives of the department were retrieved for verrucous lesions. 20 cases were included in the study. Each patient was reviewed for clinical details like age, sex, habits, clinical features like site of lesion, clinical diagnosis, presence of associated lesions. Pathological details including both gross and microscopic features were analyzed.

Results: A total of 20 patients included in the study showed a male preponderance with a significant association with tobacco consumption and size of lesion. Majority of the lesions were localized to the buccal mucosa, histopathological features were characteristic of each lesion. Expression of Ki-67, a proliferative marker showed, a statistically significant association between the mean LI of both the lesions.

Conclusions: VH and VC are closely related lesions difficult to clinically differentiate, hence histopathological evaluation plays a pivotal role in establishing a diagnosis. Differentiating the two is essential and it should be kept in mind that VH may transform into VC hence, acting as a potential precancerous lesion.

Keywords: Verrucous, carcinoma, hyperplasia

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Introduction

Verrucous papillary lesions of oral cavity include a spectrum of benign, potentially malignant and malignant lesions and are a diagnostic challenge for the pathologist. Current understanding of these lesions is perplexing. Oral verrucous hyperplasia (VH) and verrucous carcinoma (VC) are two clinicopathologically distinctive oral verrucous lesions, hence it is important to investigate the clinicopathological features of the two verrucous lesions and estimate their relationship[1-3]. Clinically both present as extensive, thick, white plaque, or mass with exophytic verrucous appearance; with no striking characteristic features to distinguish them from each other[1]. Thus, the diagnosis depends on the histopathological characteristics of VH and VC lesions, being distinguished from each other by an exophytic and endophytic growth pattern, respectively. VH was characterized by the hyperplastic epithelium with superficial to adjacent normal epithelium, whereas VC was characterized by a pushing border, invasion of the hyperplastic epithelium into the underlying connective tissue however the basement membrane remains intact[1]. Diagnosing VC accurately is extremely challenging, it has unique histopathologic features. Oral VC is a rare variant of oral squamous cell carcinoma (OSCC), first described by Ackermann, and is also known as Ackermann's tumor[4]. For correct evaluation of these two lesions, an adequate biopsy sample is of utmost importance along with an interaction between the clinician and the pathologist[5,6]. VH and VC often coexist with dysplasia and OSCC[1,3]. Differential diagnosis of these verrucous lesions remains an enigma for the histopathologists either because of the lack of adjacent normal epithelium when a biopsy is performed or because of the improper orientation of the specimen.

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Material and Methods:

The present study was a cross sectional study conducted in the Department of Pathology, Hamdard Institute of Medical Sciences and Research, New Delhi. A total of 20 patients with the diagnosis

of verrucous carcinoma or verrucous hyperplasia were included in the study. The records of the patients with the pathologic diagnoses of VH or VC in the study period extending from 2014 to 2019 were retrieved and reviewed. Each patient was reviewed for clinical details like age, sex, habits, clinical features like site of lesion and clinical diagnosis. Pathological details including both gross and microscopic features were analysed. The haematoxylin and eosin stained sections were examined methodically to establish a diagnosis. Sections were reviewed for projections, degree and type of keratinization, epithelial dysplasia, the width of rete ridges, The diagnoses of VH and VC were made based on criteria recommended in published literature. (Table 1)

Sections were also taken on poly-L-lysine coated slides and subjected to immunohistochemical (IHC) staining for Ki-67, using pre-diluted mouse monoclonal antibody which was ready to use and had been standardized using Ultra View Universal DAB detection kit. Ki-67 positive nuclei were expressed as the percentage of total nuclei counted under 40X magnification. The labeling index for Ki-67 was calculated as following[8].

$$\text{Labeling Index} = \frac{\text{No. of cells showing positive staining} \times 100}{\text{Total No. of cells}}$$

Tissue sections from oral squamous cell carcinoma (OSCC) were taken as positive controls for Ki-67 when distinct nuclear staining was identified

Results:

A total of 20 cases of verrucous lesions of the oral cavity were included in the study out of which eight were VC and 13 VH, with a age range of 28 to 70 years and a mean age of 45.6 yrs. The age wise distribution of cases of verrucous lesion shows majority of the cases seen in the 4th decade of life with 13 cases more than 40 years. (Figure I) A male preponderance was seen (17 males and 3 females with a M:F ratio of 5.6:1. All three female patients were diagnosed as verrucous hyperplasia.

On comparative analysis of the age and lesion, no significant association was seen, similarly no significant association was seen between gender and lesion. A significant association was however seen between tobacco consumption and verrucous lesions. (Table II) The distribution of cases according to site of involvement by verrucous hyperplasia and verrucous carcinoma lesions were analyzed, predominately the lesions were seen in the buccal mucosa comprising of 50% (10/20) of the cases, other sites being gingiva-buccal sulcus, tongue, retro-molar trigone, tonsillar pillar, no significant association was seen (Table II).

Further, to define the differences in lesion size between VH and VC, a comparative analysis was performed, statistically significant difference (p value 0.02) was seen in lesion size. (Table III)

A careful gross and microscopic evaluation can help in distinguishing the clinically indistinct VH and VC lesions. We evaluated the histological characteristics, a comparative depiction is tabulated below. (Table IV) Pattern of keratinization was dominated by hyperkeratosis in both lesions with other patterns being orthokeratosis and parakeratosis. Verrucous projections were predominantly blunt (8/13 cases)in VH followed pointed (3/13) and 15% (2/13) showed both features. Rete pegs were broad in 100% of the VC cases while only 53.8% of the VH showed broad rete pegs.

Acanthosis was commonly seen in both lesions with papillomatosis a prominent feature of VC. Epithelial dysplasia was seen predominantly in VH(69.2%), mild to moderate dyplasia was seen in 22.2% (2/9)and 66.6% (6/9) cases respectively with 1/9 (11.1%) showing features of severe dysplasia. On the other hand, only one case of VC showed mild dysplastic changes. Immunohistochemical staining for Ki-67 a proliferative marker, expressed exclusively in nuclei of proliferating cells, was done. It was positive in all the cases except one case of VH, the LI for each was calculated. (Table V) Intensity of staining for all the cases was strong.

Expression of Ki-67 showed a increase from VH to VC, a statistically significant association was seen between the mean LI of both the lesions. Ki-67 expression in VH was seen predominately in the basal region followed by parabasal and one each in suprabasal and all the layers.

On the other hand, Ki-67 expression in VC predominantly in all layers followed by suprabasal region. Adjacent normal oral epithelium showed predominant expression along the parabasal region and sometimes the basal layer. OSCC taken as control showed increased diffuse and intense staining.

Discussion

The present study attempts to elucidate the clinicopathological characteristics, and assess the relationship between two verrucous lesions, verrucous hyperplasia and carcinoma. Oral verrucous lesions present a diagnostic challenge to pathologists because of their similar characteristic clinical and pathological features.[8] Verrucous hyperplasia is often used to describe a potentially malignant lesion, which is probably part of the spectrum of verrucous carcinoma or

Table I: Criteria for Diagnosis of Verrucous Hyperplasia and Verrucous Carcinoma[7].

Feature	Verrucous hyperplasia	Verrucous carcinoma
Epithelia	Epithelial hyperplasia with parakeratosis or hyperkeratosis and verrucous surface	Verrucous epithelial projections with abundant parakeratin production
Invasion	No invasion of the hyperplastic epithelium into the lamina propria compared with adjacent normal mucosal epithelium	Epithelial overgrowth with wide and elongated rete ridges exhibiting a pushing-border invasion into the underlying connective tissue
Dysplasia	Part of lesions with varying degrees of epithelial dysplasia	With no significant degree of cellular atypia

may be a precursor lesion and it resembles VC both clinically and histopathologically[9]. On the other hand, classical VC is a low-grade variant of squamous cell carcinoma characterized by an exo-/endophytic growth pattern and a "pushing" invasive front. Both lesions resemble clinically and it is almost impossible to distinguish them on that basis hence histopathologic differences between the two act as a benchmark to arrive at a confirmatory diagnosis, most reliable way to separate them on routine hematoxylin–eosin-stained tissue sections is based on distinguishing the exophytic growth pattern of VH, from the endophytic and invasive growth pattern associated with VC[10].

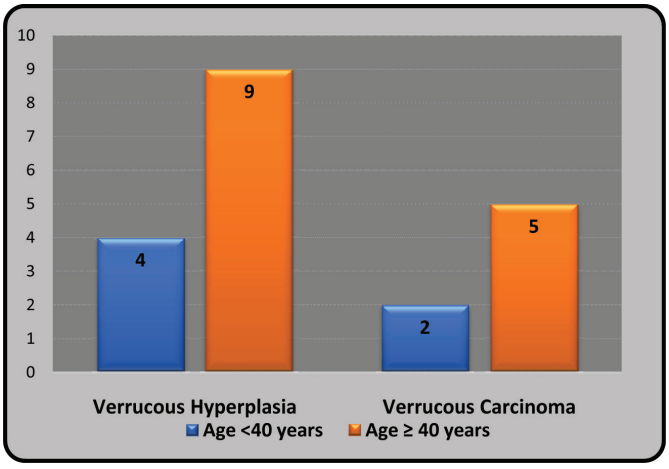

Figure I: Age wise Distribution of Cases according to Diagnosis

Table II: Comparison of clinical and demographic data of the Verrucous Lesion patients

Parameter	Verrucous Hyperplasia (n=13)	Verrucous Carcinoma (n=7)	p value (<0.05)
Mean Age in years			
	52.9	40.4	0.20 (NS)
Gender			
Male	10	7	0.5(NS)
Female	3	0	
Tobacco history			
Present	7	7	0.04 (S)
Absent	6	-	
Site			
Buccal Mucosa	8	2	0.3(NS)
Gingivo-buccal sulcus	3	3	
Tongue	0	1	
Retro-molar Trigone	1	1	
Tonsillar Pillar	1	0	

NS: Not Significant, S: Significant

Table III: Distribution of Verrucous lesions according to size of the lesion

Size of Lesions (in cms)	Verrucous Hyperplasia (n=13)	Verrucous Carcinoma (n=7)	p value (<0.05)
< 2	11	2	0.020(S)
2-4	2	4	
>4	-	1	

The mean age of the patients in the present study did not show any significant association and a higher mean age was observed for VH patients as compared to VC, this was in contrast to a study by Sharma et al who observed a significantly higher age for VC[10] similar to other authors[11,12] thus suggesting VH may be a precursor lesion of VC[11]. However, observations where the elderly population was a more affected group in VH similar to our observations has also been reported[4,13,14].

Demographic analysis revealed a male predominance accounting for 85% of the cases in our study concordant with Franklyn et. al who observed 83% males and 17% females, 5:1

Table IV: Comparison of Histopathological features of Verrucous Lesions

Histopathological features	Verrucous Hyperplasia (n=13)	Verrucous Carcinoma (n=7)
Keratinization		
Orthokeratosis	3 (23%)	1(14.2%)
Parakeratosis	4(30.7%)	2(28.5%)
Hyperkeratosis	6(46.1%)	4(57.1%)
Rete pegs		
Broad	7(53.8%)	7(100%)
Narrow	6(46.1%)	0
Acanthosis	8(61.5%)	4(57.1%)
Papillomatosis	9(69.2%)	5(71.4%)
Epithelial Dysplasia	9(69.2%)	1(14.2%)

Table V: Distribution of cases according to diagnosis and pattern of staining for Ki67

Pattern of Staining for Ki67	Histopathological diagnosis	
	Verrucous Hyperplasia (n=13)	Verrucous Carcinoma (n=7)
Negative	1(7.6%)	-(0%)
Basal	7(53.8%)	1(14.2%)
Parabasal	3(23.0%)	1(14.2%)
Suprabasal	1(7.6%)	2(28.5%)
All layers	1(7.6%)	3(42.8%)
Mean LI±SDKi-67	38.1±5.76	48.2±12.8
Range (Min-Max)	(31.2-49.4)	(44.2-56.6)
p value	0.0223(S)	

LI: Labelling Index, S: Significant

M:F ratio, other authors also report a higher male preponderance in verrucous lesion[2,10,16]. Tobacco consumption was invariably associated with all the cases of VC and 53.8% of VH cases. The etiopathogenesis of OVC is not well established, however, a strong associations with tobacco use has been reported in literature, including inhaled as well as smokeless tobacco, alcohol, and opportunist viral activity associated with human papilloma virus (HPV)[4,6,7,8]. A predominance of male population can be attributed to traditionally males being more likely to display oral habits such as tobacco smoking and betel quid chewing, known risk factors for oral verrucous lesions in an Indian settings. In addition, tumor location was not statistically significant, majority of both VH and VC were localized to buccal mucosa and gingiva buccal sulcus in the present study. A predominant gingivobuccal location has also been reported by Sonalika et.al.[16] while other authors report buccal mucosa as the dominant location[10,17]. Localization of lesion is often attributed to site of placement of tobacco. Further, to define the differences in lesion size between VH and VC a significant difference was seen on lesion size in the present study, this was in concurrence with observations by other authors[10]. Larger size of the lesion seen in VC as compared to VH suggests that increase in size of the lesion favoured a carcinoma.

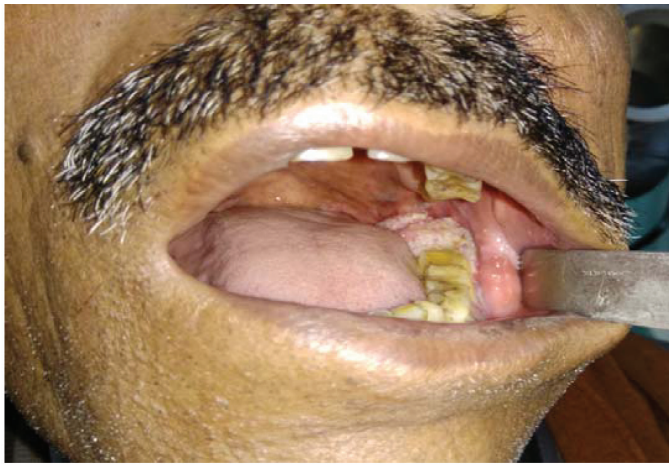

Figure 1: Clinical photograph showing verrucous carcinoma in the left gingivobuccal sulcus

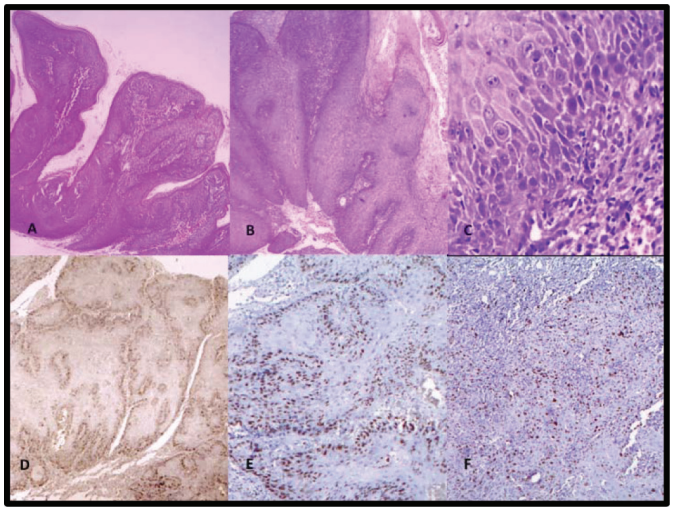

Figure 2:

A. Photomicrograph of verrucous hyperplasia showing blunt surface projections (Low power, 4x, H and E stain).
B. Photomicrograph of verrucous carcinoma showing enlarged bulb like-pushing acanthotic invaginations appearance (Low power, 4x, H and E stain).
C. Photomicrograph of epithelial dyplasia in verrucous hyperplasia (High power, 40x, H & E stain.
D. Photomicrograph showing predominantly basal and focal parabasal nuclear staining of Ki-67 in verrucous hyperplasia. (Low power, 4x, IHC).
E. Photomicrograph of Ki-67 expression in VC seen in all layers focally and predominantly in suprabasal region. (Low power, 4x, IHC).
F. Photomicrograph of Oral Squamous Cell Carcinoma taken as control showed diffuseintense staining (Low power,4x, IHC).

The clinical appearance of these lesions has not generally been well characterised, hence histopathological features play an important role in distinguishing the two entities along with a high degree of clinical suspicion. The term "verrucous" has been used for lesions showing a keratotic exophytic surface composed of sharp or blunt epithelial projections with keratin-filled invaginations (plugging), but without obvious fibrovascular cores[17]. Lesions with a verrucous surface belong to a spectrum extending from verrucous hyperplasia, pseudoepithelial hyperplasia, proliferative verrucous leukoplakia and verrucous carcinoma[18,19].

Various pattern of keratinization have been reported in these lesions, ortho-keratanization is more commonly reported in VH as compared to VC which showed para-keratinization as a dominant feature[20]. However, since the number of cases were limited in the present study a conclusive opinion was not possible. Hyperplastic epithelium was seen to be a constant feature in both lesions with broad rete pegs seen in 100% cases. Verrucous hyperplasia can be differentiated from verrucous carcinoma which exhibits frank downward growth of the epithelial processes below the level of the basement membrane of the adjacent normal epithelium. One of the most reliable ways to separate the histopathological features of the two lesions is to recognize the exophytic growth pattern of oral verrucous hyperplasia from the combined exophytic and endophytic growth pattern associated with a verrucous carcinoma[10,16,21,22]. In our patients, histopathologic appearance was concurrent with those mentioned in the literature.

Cytological atypia, associated with epithelial dysplasia has been often identified as a significant related feature (66%) in OVH[1,23,24]. Epithelial dysplasia was seen in 69.2% of the VH cases in the present study with one case showing severe dyplasia while rest showed mild to moderate cytological changes. In a hospital based follow-up study from Taiwan the malignant transformation rate was estimated at 20% in a cohort of forty-four male subjects with verrucous hyperplasia[21,5]. VH may be associated with irreversible clinicopathologic lesions with considerable potential for evolving into verrucous or squamous cell carcinoma. Human papilloma virus, as a cofactor, may play an important role in some of these lesions[17].

The importance of a properly oriented histopathology section cannot be stressed enough, separation of these lesions is often obscured by small biopsies, poor orientation, and notably, biopsies that fail to demonstrate the lesional margin[21]. Multiple biopsies from multiple sites, is the usual practice due to the numerous disease entities that display verrucous appearance. Most authorities suggest that hyperplasia can be best differentiated from VC in biopsies taken from the margins of the tumor[15].

As stated before, distinguishing the two lesions can be difficult,

they may be clinically identical and at times histologic separation may be made difficult due to specimen size, orientation, and lack of lesional margin. Utilizing immunohistochemical stains can be helpful in identifying the two entities may serve as a useful diagnostic adjunct in difficult cases. We used Ki-67 in an attempt to distinguish VC from VH. This marker has been studied independently in head and neck neoplasia and is known to be significant in the neoplastic process. The labelling index which represents a more sensitive index in comparison to the counting of mitotic figures to determine the cell proliferation, as all active phases of cell cycle can be recognized, showed a increase from VH to VC, a statistically significant association was seen between the mean LI of both the lesions, in concurrence with other studies[2]. Ki67, a marker of proliferative activity is known to express significant staining trends, expression is limited to the basal layer in acanthotic epithelium while it has been demonstrated to be diffuse throughout the entire thickness of the epithelium in invasive squamous carcinomas[26]. Pattern of immunostaining of Ki67 shows basal and suprabasal layer in VH as compared to VC while on the other hand a more diffuse expression was seen in VC, a concurrent finding in the present study and others[2,27]. Other biomarkers such as P53, MMP, E-cadherin, P21, MDM2 have been studied with varying results in differentiating VH and VC[2,10,11].

Conclusion

Although the present study is limited by the small sample size and only single immunohistochemical marker since we are a resource limited center. We would like to conclude that, VH and VC are closely related lesions difficult to clinically differentiate, hence histopathological evaluation plays a pivotal role in establishing a diagnosis. Differentiating the two is essential and it should be kept in mind that VH may transform into VC hence, acting as a potential precancerous lesion. Immunohistochemical stains can be helpful in identifying the two entities and may serve as a useful diagnostic adjunct in difficult cases.

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