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RESEARCH

Angiogenesis, Proliferative Activity and DNA Ploidy in Oral Verrucous Carcinoma: A Comparative Study Including Verrucous Hyperplasia and Squamous Cell Carcinoma

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Abstract Verrucous carcinoma (VC) is a rare and distinct clinicopathologic variant of well-differentiated squamous cell carcinoma (SCC). This study aims to evaluate the histomorphology, proliferative activity, level of angiogenesis, and DNA ploidy of these pathological entities. This was a retrospective-prospective study of 18 cases of verrucous hyperplasia (VH), 41 cases of VC, and 44 cases of SCC. Immunohistochemical analysis for Ki-67 (MIB-1) and CD34 were performed. The tumor proliferative index, endothelial proliferative index and microvascular density were calculated. DNA ploidy was determined using image cytometry. The age range and gender ratio were similar in all three groups. The differences in MIB-1 labeling index (p=0.0001), microvascular density (p=0.01), and endothelial proliferative index (p=0.001) between VC and SCC were found to be statistically significant. A non-significant increasing trend was observed in all of these parameters between VH and VC. On ploidy analysis, 100 % of SCC cases were aneuploid, compared to 39 % of VH and 86 % of VC cases. Our study demonstrates a significant difference in tumor proliferation, microvessel density, and ploidy between VC and SCC while increasing trend between VH and VC. These parameters, along with morphological findings, may be useful in differentiating these entities in small mucosal biopsies.

Keywords Angiogenesis · Proliferative activity · Ploidy · Verrucous carcinoma

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Introduction

The term "verrucous" carcinoma (VC) was coined by Lauren Ackerman in 1948 to denote fine finger-like projections on the surface of a verrucous tumor [1]. VC has been described as a diffuse, papillary, non-metastasizing, well-differentiated malignant neoplasm of squamous epithelium, and is considered to be a low-grade variant of squamous cell carcinoma (SCC). VC is generally found to be associated with tobacco chewing and snuff inhalation. A significant proportion of cases also occurs in individuals without tobacco use [2–4]. Other predisposing factors of VC include chronic inflammatory lesions (lichen planus), consumption of areca nut (betel nut), and infection with human papilloma virus (HPV types 6 and 18) [5–7]. The epidemiology of VC is not clearly known. Studies reported that the incidence of VC varies between 4.5 and 16.08 % of all oral SCC cases [2, 8, 9]. Seventy percent of all cases of VC occur in the oral cavity, making this the most common site of occurrence [10]. Of all oral cavity neoplasms, VC accounts for 3–9 % of cases [2, 3, 11]. Other sites of occurrence include the glans penis, esophagus, trunk, extremities, and scalp [1, 12–14]. Verrucous hyperplasia (VH) resembles VC both clinically and histopathologically, and is frequently mistaken for VC best described by Ackerman and Mc-Gavran [15]. SCC may pursue a more aggressive clinical course, due to a greater tendency for metastatic spread than VC. A complete surgical excision is considered to be the definitive treatment for VC, though recurrence after incomplete surgery is common. VC has an excellent prognosis, with a 3 years survival rate of 94.7 % [16].

Various studies have attempted to differentiate between VC and SCC. Kazunari et al. demonstrated that an increased proliferating cell nuclear antigen (PCNA) labeling index (LI) may contribute to malignant transformation and tumor growth of VC [17]. Theegarten et al. found no significant difference in

the proliferative activity between normal epithelium and VC and Ogawa et al. reported similar PCNA LIs in VCs and SCCs [18, 19]. Angadi et al. studied cyclin D1 expression to distinguish between VC and SCC and found no differences [20]. DNA ploidy studies have furnished evidence that the development of an aneuploid cell from a diploid progenitor cell contributes to aggressive behavior of oral carcinoma. Hemmer and Kraft reported aneuploidy in oral lesions that progressed to VC; however, the role of DNA ploidy in malignant transformation is still a matter of controversy [21].

As the biological behaviors of VH, VC, and SCC vary markedly, to differentiate these 3 entities is important. The present study was undertaken to evaluate the histomorphology, proliferative activity, angiogenesis, and DNA ploidy, and to determine any significant and useful difference between VH, VC, and SCC which can help to differentiate these entities.

Materials & Methods

This was a retrospective and prospective study. A total of 18 cases of histopathologically diagnosed VH, 41 cases of VC and 44 cases of SCC of oral cavity treated at All India Institute of Medical Sciences (AIIMS) and Institute Rotary Cancer Hospital (IRCH) between 2006 and 2011 were included. The cases diagnosed before 2008 were studied retrospectively and those diagnosed after that are prospectively. The histopathological material was retrieved from archives of Department of Pathology, AIIMS. Twenty biopsies from normal appearing oral mucosa from excision specimen were taken as control. Ethical clearance was obtained from institutional review board before the study.

All the specimens obtained by biopsy or during surgery were fixed in 10 % buffered formalin. Paraffin blocks were made and processed into 4 μm thin sections. The slides were stained with hematoxylin and eosin. A detailed histomorphological examination was carried out and the slides were evaluated. The SCC cases were sub-classified according to WHO grading system. The diagnosis of Veruucous carcinoma was made by clinicopathological findings. Presence of verrucous growth on gross finding. Microscopically (I) papillary bulbus projections growing downwards form the surrounding normal mucosa(II) Absence of actual connective tissue invasion (III) Dense chronic inflammatory infiltrate at the tumor stroma interface, (IV) Little or no dysplastic epithelium

All the cases of VH and VC were analyzed as for the following parameters - hyperkeratosis, parakeratosis, orthokeratosis, pushing borders, intensity of inflammation and squamous pearl formation. The above mentioned parameters were graded semiquantitatively as absent (0), mild (1), moderate (2), severe (3).

Immunohistochemistry for CD 34 (IgG1 mouse monoclonal antibody, Bio SB, USA), and Ki-67 (IgG1, mouse monoclonal antibody, MIB-1 clone, Bio SB, USA) were done using streptavidin-biotin peroxidase technique. Each batch was run with an appropriate positive and negative control. Sections of reactive lymph node were used as positive controls for MIB-l and sections of tonsil were taken as positive control for CD34. The negative control was obtained by omitting the primary antibody.

The labeling index (LI) for MIB-1 was calculated as a percentage of immunopositive tumor nuclei per 1,000 cells. One thousand tumor cells were evaluated in different areas of sections (at least 5 representative microscopic fields). The areas where positively stained nuclei were evenly distributed chosen for interpretation and calculation. Vascular endothelial cell and hematogenous cells staining MIB-1 positive were disregarded. Counting was done at a magnification of 400X and an eye piece pinhole was used to facilitate counting.

To estimate angiogenesis, microvascular density (MVD) and endothelial proliferative index (EPI) were measured. For MVD the CD34 immunostained sections were scanned at low magnification (4 X objectives) to locate areas of highest density of microvessels (hot-spots). Minimum of 3 such hotspots were captured at 20X objective using a digital microscope (Model BX50, Olympus Corporation, Japan). Three such images for every case was taken and stored in TIF format. The images of each case were modified by segmentation to obtain only the bright vessel outlines and highlighted endothelial cells either individually or in cluster of any size, against a uniform black (dark) background. The MVD was expressed as number of vessels/ mm^2 (Fig. 1a & b).

For EPI estimation the MIB-1 1 stained slides were counterstained with Periodic Acid Schiff (PAS) stain. As the basement membrane of vessels was highlighted the cells within the vessels were counted to calculate EPI. This procedure hence avoids counting the MIB-1 positive tumor cells. These slides were then examined using 40X objective. A minimum of 100 endothelial cells were counted and the result was expressed as the percentage of positively stained endothelial cell nuclei (Fig. 2a).

DNA Ploidy was measured by image cytometry on fuelgen stained sections. The sections were deparaffinized and hydrolysed in 5 N hydrochloric acid for 1 h at 37°C in an incubator, followed by washing in running tap water for 10 s. The sections were then stained with Schiff's reagent at a pH 1.5 for 1 h. The slides were washed in 3 changes of sulphur dioxide water (SO_2 water) for 10 min each. The feulgen stained slides were analyzed by image analysis (Image pro plus software optimus 6.2, Media Cybernetics Corporation, USA). Images were captured at 400X and nuclei outlined by an automatic or semiautomatic tracing system. A minimum of 100 non-overlapping nuclei of representative cells were traced and integrated optical density (IOD) of the outlined nuclei was measured (Fig. 2b). The data

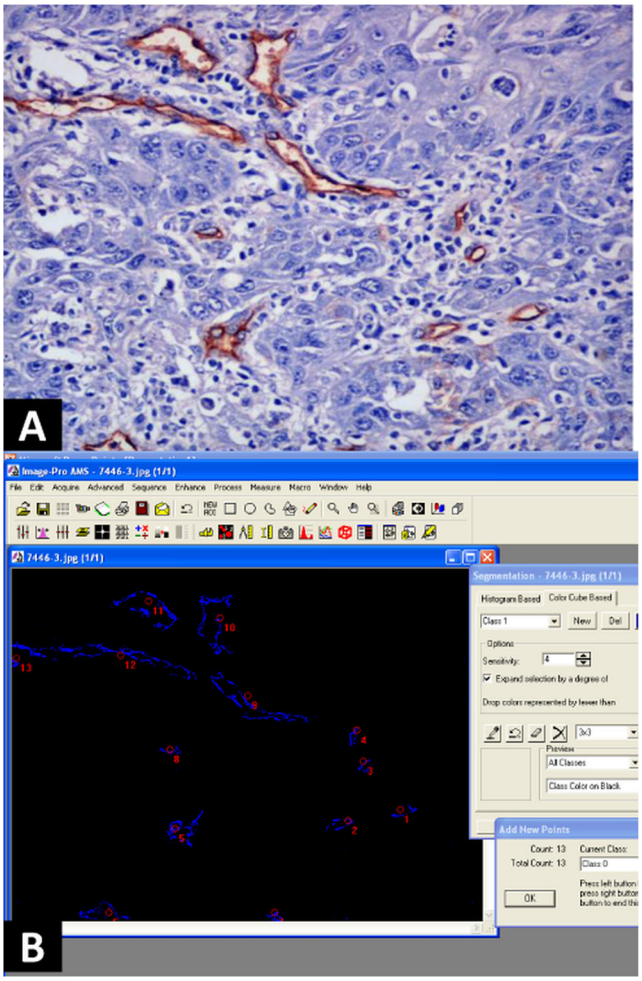

Fig. 1 Immunohistochemistry for CD34 demonstrating a "hotspot" with numerous microvessels (X200). Microvessel density by image analysis system (*blue colour*)

obtained was transported to Microsoft Excel, a histogram was generated and peak value was measured. For each test nucleus, the IOD was converted into a ploidy value using normal 2n ploidy value as reference. Diploid value obtained from nuclei of normal appearing mucosa. When the narrow peak was between 1.8 and 2.2 the case was considered as diploid and when the values were outside this range or multiple peaks occurred, the case was taken to be aneuploid.

Statistical Analysis

All statistical analyses were performed using STATA software, Version 9. ANOVA test was used, post hoc with Bonferroni correction to assess the statistical significance of differences between groups in the MIB-1 LI, MVD and EPI. Chi square test for Ploidy analysis and student t- test for angiogenesis was used.

Results

The patients studied were in the age range of 30 to 80 years with a mean age of 56.5 years, 53.4 and 52.4 years for VH, VC and SCC, respectively. There was a male predominance in each of the 3 groups. The most common site of involvement in all 3 groups was buccal mucosa, followed by tongue and gingivo-buccal sulcus. Out of the 44 cases of VC, 1 occurred in hard palate, 3 in soft palate and 3 in tonsils.

Gross and Microscopy

Cases of SCC primarily presented with ulcerated or an ulceroproliferative growth. VH and VC on the other hand, presented most commonly as fine papillary projections over the mucosa. The squamous cell carcinoma cases were graded according WHO grading system and the majority of the cases were grade 1 [22/44 (50 %)], followed by grade 2 [16/44 (36.3 %)], and grade 3 [6/44 (13 %)] lesions.

There was a significant difference in occurrence of orthokeratosis (p=0.04), pushing border (p=0.001) and squamous pearl formation between VH & VC (p=0.01). These features were seen more frequently in cases of VC.

Tumor Proliferation Index

MIB-1 LI increased as the lesion became higher grade and was highest in SCC, followed by VC, VH and normal

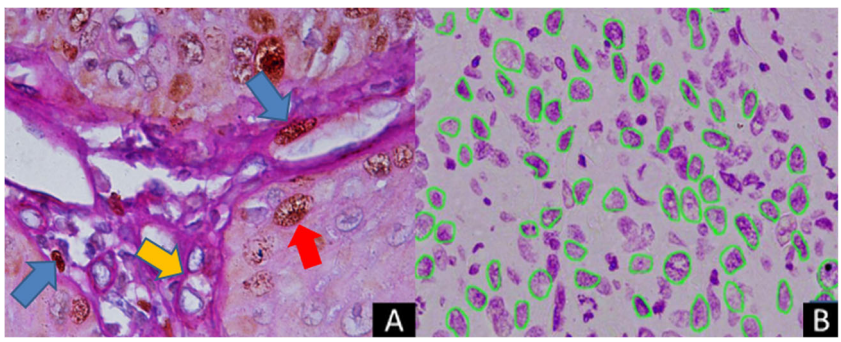

Fig. 2 **a**. PAS- stained section of MIB-1 immunohistochemistry showing proliferating endothelial cell inside the basement membrane. *Blue arrow* MIB labeled endothelial cell, *Yellow arrow* unlabeled endothelial cell and *red arrow* MIB labeled tumor cell. **b** Integral optical density for ploidy by image analysis system (outlined nuclei)

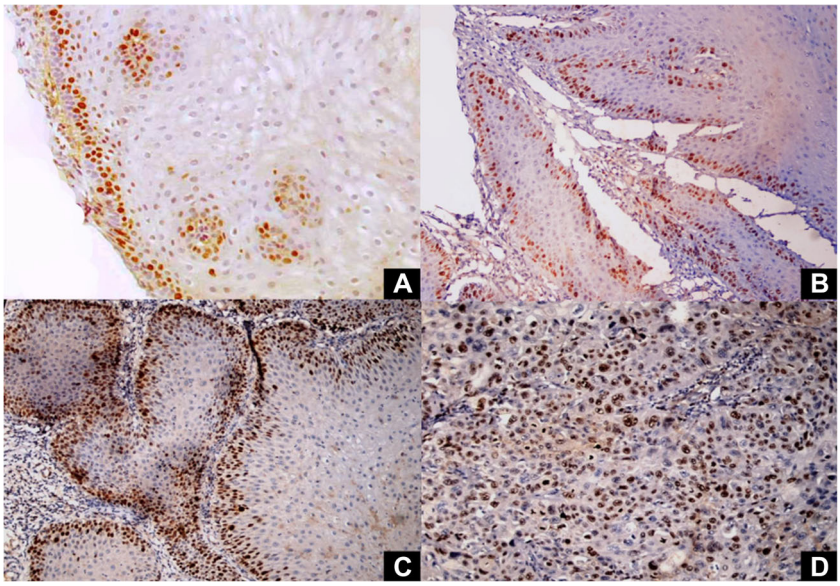

Fig. 3 **a** Normal oral mucosa showing MIB-1 positive cell in basal layer of squamous epithelium (X 200); **b**. verrucous hyperplasia showing MIB-1 positivity mainly in basal layer (X 200); **c**. Photomicrograph demonstrating MB-1 positivity in paracentral tumor cell nuclei in a case of Verrucous Carcinoma (X 200); **d**. MIB-1 staining in Squamous cell carcinoma showing diffuse positivity (X200)

mucosa. The pattern of expression was seen in the basal layer of normal mucosa. In VH and VC, the MIB-1 staining was seen in paracentrally located tumor cells while in SCC the staining was diffuse (Fig. 3a, b, c & d). A significant difference was found in the MIB-1 LI of tumor cells between SCC and VC (p=0.0001). There was an increasing trend of MIB-1 LI from VH to VC but the difference between these 2 groups was not statistically significant (p=0.72) (Fig. 4). On comparing the tumor proliferating index of grade 1 SCC [mean 34.7 % (±10)] and grade 2 SCC [mean 37.3 % (±13.5)] was not found to be statistically significant (p=0.49).

Angiogenesis

Both MVD and EPI were significantly different between VC and SCC. Comparing MVD and EPI between VH and VC like MIB-1 showed an increasing trend but, the difference did not reach statistical significance (Table 1). No correlation was seen between MIB-1 LI of tumour cell and MVD in any of the group (Fig. 5d, e f). There was no significant correlation between MIB-1 LI of tumor cells and EPI (Fig. 5a, b, c). Comparing angiogenesis there was no significant difference (p=0.06) in MVD between grade 1 and grade 2 SCC. The EPI increased from 7.8 % (±2.2) for grade 1 SCC to 8 % (±2.4) for grade 2 SCC, though the difference was statistically insignificant (Fig. 6).

Ploidy

On ploidy analysis, all the cases of SCC were aneuploid compared to 39 % of VH and 86 % of VC. Statistically aneuploidy was more frequent in VC compared to VH (p= 0.001). A significant difference in aneuploidy was also noted between VC and SCC (p=0.03) (Fig. 7).

Distribution of Aneuploid and Diploid Cell Population in SCC, VC & VH

Student's t- test was done to compare angiogenesis between diploid and aneuploid cases. There was a significant difference in MVD and MIB-1 LI of tumor cell between the aneuploid and diploid tumors, the p value being 0.006 and 0.001 (Table 2).

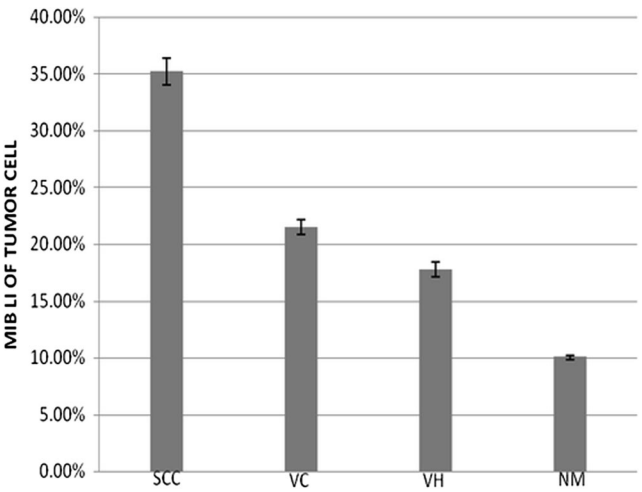

Fig. 4 Comparison of MIB-1 LI of tumor in SCC, VC, VH and NM

Table 1 Microvascular density (MVD) and endothelial proliferative index (EPI) of VH, VC & SCC

	VH (1)	VC (2)	SCC (3)	p-value
MVD(Mean ± SD)	144.8±60.8	165.5±63.8	222.4±59.9	1&2-0.7,2&3- 0.001*
EPI(Mean ± SD)	5.35±2.17	6.04±1.84	8±2.28	1&2-0.79,2&3- .0001*

MVD Microvascular density, *EPI* Endothelial proliferative index, *VC* Verrucous carcinoma, *SCC* squamous cell carcinoma, *VH* Verrucous hyperplasia
*MVD and EPI significant between VC & SCC

Discussion

Most patients in the present study were more than 50 years old with an average age of 52.4 years. This is in concordance with the results of other studies [1, 8, 21, 22] Males outnumbered females in our study with male to female ratio of 9.2:1 in SCC, 7.8:1 in VC and 17: 1 in VH. This is also in accordance with earlier reports in literature, though some studies have shown female predominance [3, 5, 11, 22, 23].

Grossly papillary projections were seen in both VC and VH making clinical distinction difficult. A study of 68 cases by Shear et al. had found that the lesion of VH and VC were clinically similar [24].

The histologic appearance of VC is distinctive and the criteria were originally proposed by Ackerman. In the present study, pushing border was seen more often in the case of VC than VH (p=0.001). Though squamous pearls were usually noted in SCC, 70 % of cases of VC also showed squamous pearl formation in comparison to 27 % in VH. The difference in squamous pearls between VH and VC was statistically significant (p=0.01)

The characteristic histological feature distinguishing VC from VH was the deep bulbous extension of rete ridges into the sub epithelium in addition to the surface projections. In our study, orthokeratosis and parakeratosis were more frequently seen in lesions of VC compared to

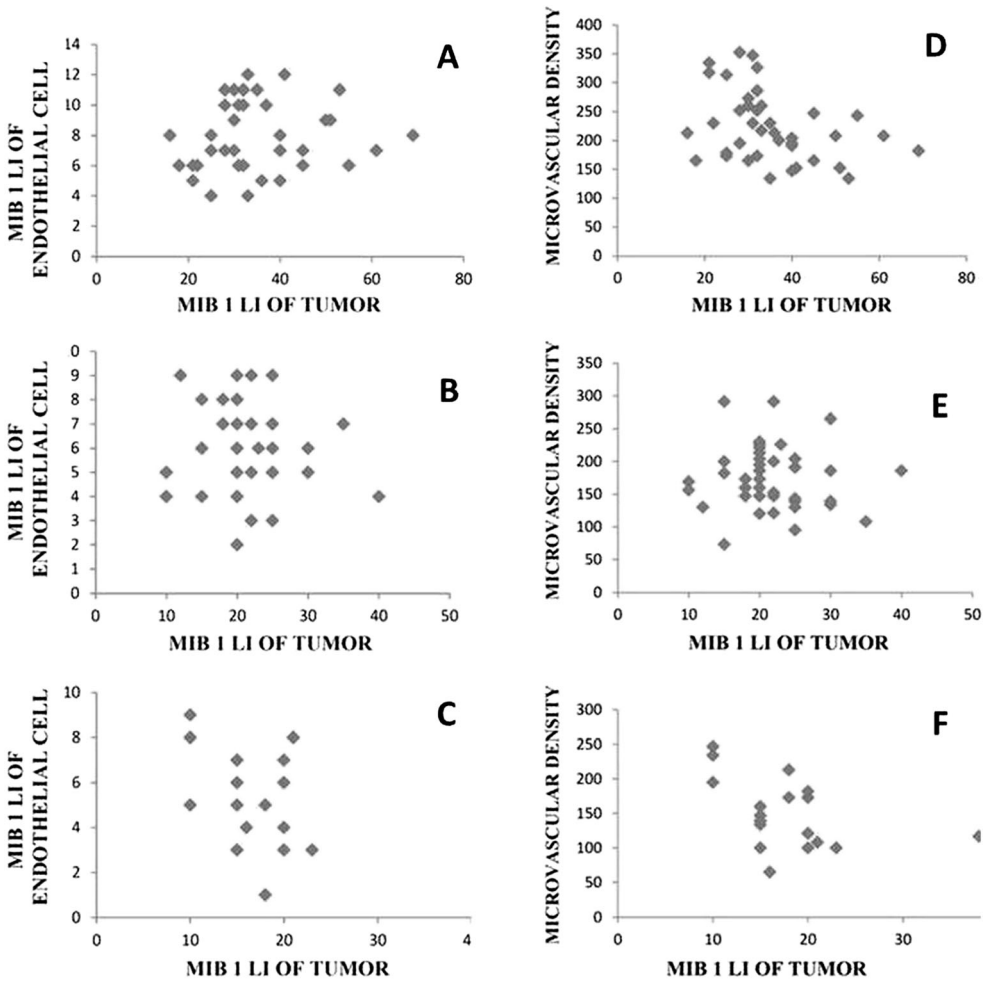

Fig. 5 **a.** Correlation between MIB-1 LI of tumor cells and the EPI in SCC was also not significant (p=0.52 with correlation coefficient of r=0.1), **b.** No significant correlation was seen on comparing MIB-1 LI of tumor cells with the EPI in VC (p=0.51 with correlation coefficient of r=−0.1), **c.** No significant correlation was seen on comparing MIB-1 LI of tumor cells with the EPI in VH (p=0.11 with correlation coefficient of r= 0.39), **d.** No significant correlation was found between MIB-1 LI of tumor cells and MVD in SCC (p=0.02 with correlation coefficient, r=−0.36), **e.** MIB-1 LI of tumor cells did not show any significant correlation with MVD in VC (p=0.73 with correlation coefficient of r= −0.05), **f.** There was no significant correlation between MIB-1 LI of tumor cells and MVD in VH (p=0.03 with correlation coefficient, r=−0.5)

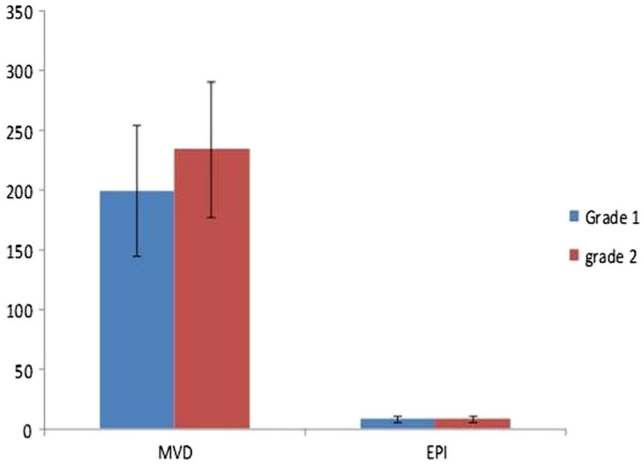

Fig. 6 Comparison of angiogenesis between grade 1 and grade 2 SCC

VH, accompanied by dense inflammatory infiltrate around the rete ridges (Fig. 8a, b, c).

Folkman first reported reduction of tumor growth by interfering angiogenesis [25, 26]. Many parameters have been studied to evaluate angiogenesis in solid tumors, including microvascular density (MVD), microvessel area and proliferation of endothelial cells. We used MVD and EPI as marker of angiogenesis. The total field area evaluated was 0.23 mm^2, as the significance of MVD of tumor decreases when total field area examined is below 0.19 mm^2 [27].

CD34 was used to highlight endothelial cell instead of more frequently used CD31, to avoid cross reactivity with plasma cells. VC and VH, both show numerous lymphoplasmacytic infiltration around rete ridges and hence an antibody which does not stain the inflammatory cell is desirable [28]. Hannen et al. also demonstrated that CD34 is more suitable for oral specimens [29].

Earlier studies have showed that metastasis is influenced by increase in MVD. It is also an indirect prognostic factor for many tumors like breast, non small cell carcinoma lung, and melanoma [30–32]. In comparison, oral SCC has shown conflicting results with MVD in

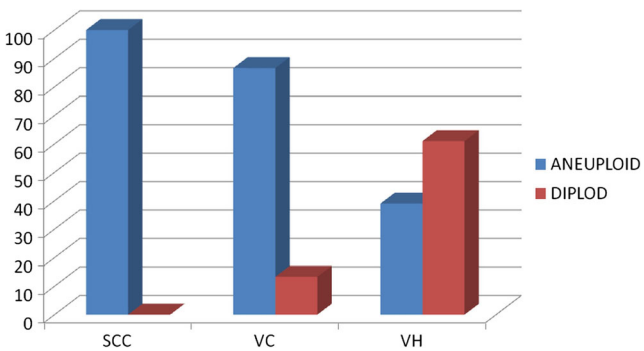

Fig. 7 DNA ploidy distribution in SCC, VC and VH

different studies. There is significant difference in MVD between lip SCC and rest of the buccal mucosa SCC [33]. A recent study by Et Rouby et al. highlighted difference in MVD between VC and SCC [34]. A study by Abbas et al. also brings out difference in MVD between normal, dysplastic and SCC of oral mucosa [35]. This is in concordance with the results of the present study. Mashhadiabbas et al. showed that lymphovascular density (LVD) is significantly correlated to matrix metalloproteinase 10 (MMP 10) expression which helps in invasion and metastasis of oral SCC [36]. Since angiogenesis has been considered as a poor prognostic factor in many solid tumours, we hypothesize that the less aggressive biologic behaviour of VC may be due to lower MVD compared to SCC. This however needs to be confirmed in further studies.

A study by Matsumoto showed strong positivity of endothelial cell growth factor (VEGF) in the tumor cells and platelet-derived endothelial cell growth factor both the tumor cells and the stromal cells of metastatic VC. Helenisa et al. also showed the significant correlation of VEGF and MVD in oral SCC [34]. Many solid tumors also responded to anti-angiogenesis therapy [37]. The application of anti-angiogenic strategies in treatment of oral SCC seems to hold a promise for useful targeted cancer therapy.

It has already been proven that MIB-1 LI is one of the better prognostic indicators in various tumors. Maclusky et al. showed a significant increase in MIB-1 LI as lesion progresses from normal mucosa to SCC in oral cavity [38]. A few studies in literature have compared MIB-1 LI in VC and SCC. We also found significant difference in MIB-1 LI of tumor cells between SCC and VC (p= 0.001). Though there was an increasing trend in MIB- 1 LI from VH to VC, the difference was not statistically significant (p=0.57). A significant difference was noted between normal mucosa and VH (p=0.0001). This finding is in agreement with the previous studies by Sakuri et al. where they found significant difference in tumor proliferative activity of SCC and VC, however Ogawa et al. contradicted this finding in their study [17, 19].

In our study, the pattern of expression of MIB-1 was found to be distinct in each group. Normal mucosa showed MIB-1 positive cells mainly in basal cells of squamous epithelium. Positive cells were observed in VH, mainly near the basement membrane. Positivity was more paracentrally located in VC, whereas diffuse positivity was observed in SCC. Flow cytometry is more accurate but less sensitive in oral specimens because of inflammation, as it is difficult to isolate the malignant cell. In contrast, imagecytometry allows visual inspection and accurate identification of dysplastic cells. Earlier studies have shown that ploidy analysis is a useful method in detecting the premalignant lesions of the oral mucosa. Exfoliative cytology combined with DNA imagecytometry has been

Table 2 Correlation between ploidy and other parameter

Ploidy		Mean ± SD	CI for means	*P* value
MVD (Vessels/ 0.23 mm^2)	Aneuploid	192.4±67.4	177–206	0.006
	Diploid	143±63.2	110–175	
EPI	Aneuploid	6.8±2.37	6.3–7.3	0.1
	Diploid	6±2	4.9–7.2	
MIB 1 LI of tumor cell	Aneuploid	27±11.8		0.001
	Diploid	14±5.9		

shown to be useful in predicting the clinical behavior of oral mucosal lesions [39].

The occurrence of aneuploid cell population was significantly higher (p=0.001) in VC (86.6 %) compared to VH (39 %) in our study. This may support the hypothesis that VH is a precursor lesion of VC, as aneuploid cases of VH may progress to VC. This however, could not be confirmed in the present study. Hemmer et al. showed that oral lesions, which progressed to VC, revealed DNA aneuploidy in the lesional tissue, which was maintained throughout the complete follow-up period. They further concluded that aneuploidy is a common marker for both SCC and VC [21]. A study by Ng et al. showed that significant difference in ploidy between normal epithelium and high risk premalignant oral epithelial lesions [40]. Similar results were also observed in another follow up study by Kahn et al., who observed aneuploidy in a large proportion (88 %) of the biopsies [41]. This finding possibly supports the view that aneuploid tumors behave more aggressively compared to diploid tumors, however, follow up studies would be necessary to substantiate the observation. Significantly higher MVD (p=0.006) and MIB-1 LI of tumor cell (p=0.001) was observed in aneuploid cases compared to those in diploid cell population. This finding is in concordance with the previous reports of aneuploidy being common marker for SCC and VC [21]. There is a significant correlation between MVD and ploidy in our study however EPI doesn't correlate. In the study of head and neck squamous cell carcinoma Kljianienko et al., found no significant relationship

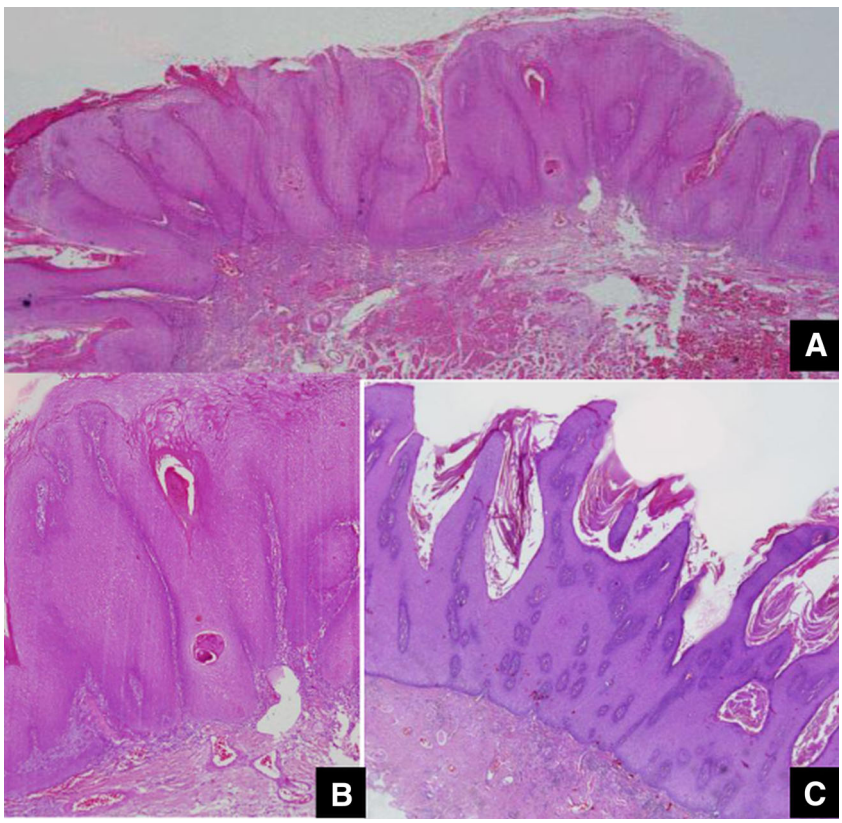

Fig. 8 **a.** Photomicrograph of Verrucous carcinoma showing papillaryprojections and pushing border (H&E 10x). **b**. Verrucous carcinoma showing bulbous rete pegs with pushing border. (H&E, X100) **c.** Upward papillary projections of epithelial cells with minimal connective core and verrucous surface in case of verrucous hyperplasia (H&E, X100)

between angiogenesis and Ploidy [42]. Csisti et al. showed a significant relation between the above 2 parameters in colon cancer [43].

Conclusion

This study evaluated the combined factors of angiogenesis, proliferative index and DNA ploidy in verrucous hyperplasia, verrucous carcinoma and squamous cell carcinoma for the first time, to the best of our knowledge. Our results indicated that there is significant difference between SCC and VC while an increasing trend in above mentioned parameters as the disease progresses from verrucous hyperplasia to carcinoma. Whether this difference can be utilized in differentiation of these entities on small biopsies needs to be evaluated in future studies.

Conflict of Interest None

Source of Funding None

References

1. Ackerman LV (1948) Verrucous carcinoma of oral cavity. Surgery 23:670–81
2. McCoy JM, Waldron CA (1981) Verrucous carcinoma of the oral cavity: a review of 49 cases. Oral Surg 52:623–9
3. Eisenberg E, Rosenberg B, Krutchkoff DJ (1985) Verrucous Carcinoma: a possible viral pathogenesis. Oral Surg Oral Med Oral Pathol 59:52–7
4. Goethals PL, Harrison EG, Devine KD (1963) Verrucous squamous cell carcinoma of oral canity. Am J Surg 106:845–51
5. Gassenmaier A, Hornstein OP (1988) Presence of papilloma virus DNA in benign and precancerous oral leukoplakias and squamous cell carcinoma. Dermatologica 176:224–33
6. Trivedy CR, Craig G, Warnakulasuriya S (2002) The oral health consequences of chewing areca nut. Addict Biol 7:115–25
7. Fujita S, Senba M, Kumatori A et al (2008) Human papillomavirus infection in oral verrucous carcinoma: genotyping analysis and inverse correlation with p53 expression. Pathobiology 75:257–64
8. Medina JE, Dichtel W, Luna MA (1984) Verrucous squamous carcinoma of the oral cavity: a clinicopathological study of 104 cases. Arch Otolaryngol 110:437–40
9. Rekha KP, Angadi PV (2010) Verrucous carcinoma of the oral cavity: a clinico-pathologic appraisal of 133 cases in Indians. Oral Maxillofac Surg 14:211–8
10. Kraus FT, Perz-mesa C (1966) Verrucous carcinoma clinical and pathologic study of 105 cases involving oral cavity, larynx genitalia. Cancer 19:26–33
11. Fonts EA, Greenlaw RH, Rush BF et al (1969) Verrucous squamous cell carcinoma of the oral cavity. Cancer 23:152–9
12. Ali Sbai M, Balti W, Dhahak S et al (2009) Buschke Lowenstein tumor: unusual bilateral localization. Tunis Med 87:627–9
13. Pattee SF, Bordeaux J, Mahalingam M et al (2007) Verrucous carcinoma of the scalp. J Am Acad Dermatol 56:506–17
14. Chiheb S, Bouziane K, Azzouzi S et al (2010) Verrucous carcinoma of the toe. Ann Dermatol Venereol 137:169–70
15. Ackerman LV, McGavran (1958) Proliferating benign and malignant potential lesion of the oral cavity. J Oral Surg 16:400–13
16. Kang CJ, Chang JT, Chen TM et al (2003) Surgical treatment of oral verrucous carcinoma. Chang Gung Med J 26:807–12
17. Sakurai K, Urade M, Takahashi Y et al (2000) Increased expression of c-erb- 3 protein and proliferating cell nuclear antigen during development of verrucous carcinoma of oral mucosa. Cancer 89: 2597–605
18. Theegarten D, Barfuss A, Gellirich NC et al (1997) Expression of proliferation markers PCNA and MIB1 in verrucous squamous epithelial carcinoma of oral cavity. Mund Kiefer Gesichtschir 1:133–6
19. Ogawa A, Fukuta Y, Nakajima T et al (2004) Treatment results of oral verrucous carcinoma and its biological behavior. Oral Oncol 40:793–7
20. Angadi PV, Krishnapillai R (2007) Cyclin D1 expression in oral squamous cellcarcinoma and verrucous carcinoma: correlation with histological differentiation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 103:e30–5
21. Hemmer J, Kraft K (2000) High resolution DNA flow cytometry in oral verrucous carcinoma. Oncol Rep 7:433–35
22. Fitzpatrick SG, Neuman AN, Cohen DM et al (2012) The clinical and histologic presentation of gingival squamous cell carcinoma: a study of 519 cases. Oral Surg Oral Med Oral Pathol Oral Radiol 114:509–15
23. Winn DM, Blot WV, Shy CM et al (1981) Snuff dipping and oral cancer among women in the Southern United States. N Engl J Med 304:745–9
24. Shear M, Pindborg JJ, Odont D (1980) Verrucous hyperplasia of the oral mucosa. Cancer 46:1855–62
25. Folkman J (1995) Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med 1:27–31
26. Folkman J (1971) Tumor angiogenesis: therapeutic implications. N Engl J Med 285:1182–6
27. Weidner N, Semple JP, Welch WR et al (1991) Tumor angiogenesis and metastasis- correlation in invasive breast carcinoma. N Engl J Med 3(324):1–8
28. Vermeulen PB, Gasparini G, Fox SB et al (1996) Quantification of angiogenesis in solid human tumor: an international consensus on methodology and criteria of evaluation. Eur J Cancer 32: 2472–84
29. Hannen EMJ, Riediger D (2004) The quantification of angiogenesis in relation to metastatic oral cancer: a review. Int J Oral Maxillofac Surg 33:2–7
30. Lörincz T, Tóth J, Szendröi M et al (2005) Microvascular density of breast cancer in bone metastasis: influence of therapy. Anticancer Res 25:3075–81
31. Macchiarini P, Fontanini G, Hardin MJ et al (1992) Relation of neovascularisation to metastasis of non-small-cell lung cancer. Lancet 340:145–46
32. Srivastava A, Laidler P, Davies RP et al (1988) The prognostic significance of tumor vascularity in intermediate-thickness (0.76–4.0 mm thick) skin melanoma. A quantitative histologic study. Am J Pathol 133:419–23
33. Oliveira-Neto HH, Gleber-Netto FO, de Sousa SF et al (2012) A comparative study of microvessel density in squamous cell carcinoma of the oral cavity and lip. Oral Surg Oral Med Oral Pathol Oral Radiol 113:391–8
34. El-Rouby DH (2010) Association of macrophages with angiogenesis in oral verrucous and squamous cell carcinomas. J Oral Pathol Med 39:559–64
35. Abbas NF, Labib El-Sharkawy S, Abbas EA et al (2007) Immunohistochemical study of p53 and angiogenesis in benign and preneoplastic oral lesions and oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 103:385–90

36. Mashhadiabbas F, Mahjour F, Mahjour SB et al (2012) The immunohistochemical characterization of MMP-2, MMP-10, TIMP-1, TIMP-2, and podoplanin in oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol 114:240–50
37. Matsumoto Y, Ishiko O, Nishimura S et al (2000) Angiogenesis in metastatic verrucous carcinoma of the uterine cervix. Oncol Rep 7: 1079–82
38. Macluskey M, Chandrachud LM, Pazouki S et al (2000) Apoptosis, proliferation, and angiogenesis in oral tissues: possible relevance to tumour progression. J Pathol 191:368–75
39. Maraki D, Boecking A, Pomjanski N et al (2006) Verrucous carcinoma of the buccal mucosa: histopathological, cytological and DNA-cytometric features. J Oral Pathol Med 35:633–5
40. Ng SP, Mann IS, Zed C et al (2012) The use of quantitative cytology in identifying high-risk oral lesions in community practice. Oral Surg Oral Med Oral Pathol Oral Radiol 114:358–64
41. Kahn MA, Dockter ME, Hermann-Petrin JM (1994) Proliferative verrucous leukoplakia. Four cases with flow cytometric analysis. Oral Surg Oral Med Oral Pathol 78:469–75
42. Klijanienko J, el-Naggar AK, de Braud F et al (1995) Tumor vascularization, mitotic index, histopathologic grade, and DNA ploidy in the assessment of 114 head and neck squamous cell carcinomas. Cancer 75:1649–56
43. Cristi E, Perrone G, Toscano G et al (2005) Tumour proliferation, angiogenesis, and ploidy status in human colon cancer. J Clin Pathol 58:1170–4