



Utility of Microvessel Density (MVD) by CD34 in Different Histological Grades of Oral Squamous Cell Carcinoma

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Oral squamous cell carcinoma is one of the leading causes of cancer related death and is preventable. Different grade at which a carcinoma presents determines the treatment modality and prognosis of the disease. Microvessel density measures formation of new vessels around tumor mass and is very important criteria for tumor metastasis and disease spread. Immunohistochemistry provide very definitive measures to determine the micro vessel density. Our study showed a positive correlation between Microvessel density and tumor size and dysplasia.

Keywords: Oral squamous cell carcinoma; CD 34; microvessel density.

1. INTRODUCTION

Oral squamous cell carcinoma (OSCC) has the prevalence of more than 90% of all oral cancers [1]. It is the most common in males and the third

most common carcinoma in females [2]. OSCC and adenocarcinoma are two common subtypes of carcinoma of the oral cavity. Carcinomas are malignant neoplasms, arising from epithelial cells and can be derived from any of the three germ

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layers. Despite considerable upgradation in conscientious technique & meticulous implementation of radical dissection with/without adjuvant chemoradiotherapy, half of the total patients usually die within one year of diagnosis & 24.3% are alive three years following the complete surgical resection of carcinoma [3], bio-informatics, as well as systems biology, have disentangled the complex anomalous signalling network in oral squamous cell carcinoma. Angiogenesis is also one such factor aiding in tumor growth and metastasis [4]. The survival rate of patients with oral squamous cell carcinoma has not significantly increased in the last few years. Chief risk factors for OSCC are smoking, alcohol consumption, and chronic irritation like dental carries, overuse of mouthwash chewing tobacco, and betel quid. The survival rate without recurrence of OSCC is fifty-six percentage [5].

Angiogenesis: This is the process of new blood vessels and capillaries formation from the pre-existing ones & is pivotal for orderly progression and expansion of the organism. Abnormal increase and decrease in angiogenesis are important in different pathological conditions like tumor growth, progression, and spread [6].

Neo vascularisation provides a channel for nutrients supply to tumor cells and provides a pathway for tumor cells to travel inside blood vessels in circulation. New proliferating capillaries have non-functional leaky basement membranes. This makes them more accessible to tumor cells rather than mature vessels [7].

For ten years, microvessel density, an additional and important marker of tumor angiogenesis. It identifies the patients at high risk of recurrence more precisely than other classical indicators, particularly in some of the node-negative patients [8].

The levels of the angiogenic inhibitors and activators factors control the angiogenic switch as well as the angiogenic activity of tumors including breast cancer. Adapted from Hanahan [9].

Microvessel density (MVD) assessment is the most commonly used technique to quantify intratumoral angiogenesis in breast cancer by counting small and tortuous vessels in the tumor tissue by immunohistochemical staining using antibodies such as CD34, CD31, CD105, and Von-Willebrand factor (Factor VIII) that are

specific for vessel endothelium. It was first developed by Weidner et al. in 1991 and uses pan endothelial immunohistochemical staining of blood microvessels [mainly with Factor VIII antigen, CD31, PECAM-1, or CD34; integrin alpha v beta 3, CD105, or type IV collagen].⁸ Increased MVD is reported to be associated with advanced pathological stage and poor prognosis of the disease in breast, lung, colon, stomach, prostate, and bladder cancers, and malignant melanomas [10].

This study will utilize the grading system of squamous cell carcinoma and compare the microvessel density evaluated by CD34 with various histological grades of OSCC by Weidner's criteria [11].

2. MATERIALS AND METHODS

The present study is of observational, analytical and cross-sectional type and was carried out in two years (1st August 2019 to 31st July 2021) in the divisions of histopathology and immunohistochemistry of the Department of Pathology, Jawaharlal Nehru Medical College (J.N.M.C) and Acharya VinobhaBhave Rural Hospital (A.V.B.R.H) in co-ordination with the Departments of General Surgery, Otorhinolaryngology (ENT), A.V.B.R.H and departments of Oral Pathology and Oral & Maxillofacial Surgery, Sharad Pawar Dental College (S.P.D.C), Sawangi (Meghe), Wardha, Maharashtra, India.

Cases confirmed on histopathological examination as oral squamous cell carcinoma were operated upon and the resected specimens were received in the Departments of Oral Pathology and General Pathology in 10 % neutral buffered formalin solution. The resected specimens were kept for overnight fixation.

The specimens were subjected to routine tissue processing. After the processing was complete, routine H & E staining was carried out.

Evaluation of microvessel density by CD34 IHC is calculated by the method by Weidner : [11]

- First, we identified the area of the highest microvessel density ("hotspot") by scanning the whole tumor section at low power.
- Each microvessel was counted of the high field in 200 fields in an adequate area

(0.74 mm per field using 40 objective lenses and 10 oculars).

- Endothelial cells or clusters separated from the adjacent vessel but stained will be counted as single microvessel, even if no vessel lumen.

3. OBSERVATION AND RESULTS

The present analytical, cross-sectional and prospective study came across 30 cases of oral squamous cell carcinoma and was carried out in the Department of Pathology, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha for a duration of 2 years (July 2019 to August 2021).

The main aim of this study was to develop an understanding of the relationship between the microvessel density evaluated by CD34 and the histological grading of oral squamous cell carcinoma.

All the results obtained on histopathological, histochemical, and immune histochemical evaluations were carefully noted, recorded in a well-tabulated master chart, and interpreted. Statistical analysis was done by descriptive and inferential statistics using chi-square test, student's unpaired "t" test, regression analysis. The software used in the analysis was SPSS 22.0 version, GraphPad Prism 6.0 version, and EPI-INFO 6.0 version, and $p < 0.05$ was considered as the level of significance (S).

For this purpose, all the 30 cases of OSCC confirmed on histopathological examination were graded. After determining the grade, histochemical and immunohistochemical evaluation of the tumor was carried out respectively. The cell proliferative markers employed in this study were CD34 for determining the histochemical and immunohistochemical profile of oral squamous cell carcinoma respectively.

Cases have been divided into two groups male and female. Again based on site of lesion percentage is determined. Maximum cases were from GB sulcus 9(30.0%). The least number of cases were noted in lower lip 3(10.0%). Male 23(76.67%) has a higher prevalence than female 7(23.3%). When cases were divided into different gradings of carcinoma, the maximum was well-differentiated carcinoma 19(63.3%) and least were poorly differentiated carcinoma 2(6.7%). The only place poorly differentiated carcinoma was found was GB sulcus. Also 9/30 times carcinoma was present in GB sulcus followed by buccal mucosa as shown in Fig. 1.

As the groups were divided age-wise the number of patients less than 30 years was quite less than 2(6.67%) as compared to higher age group patients. Also, the age group between 30 To 50 years has the same number of patients as more than 50 years age group. Thus as the age increases the chances of getting OSCC is found to be increased as shown in Fig. 2.

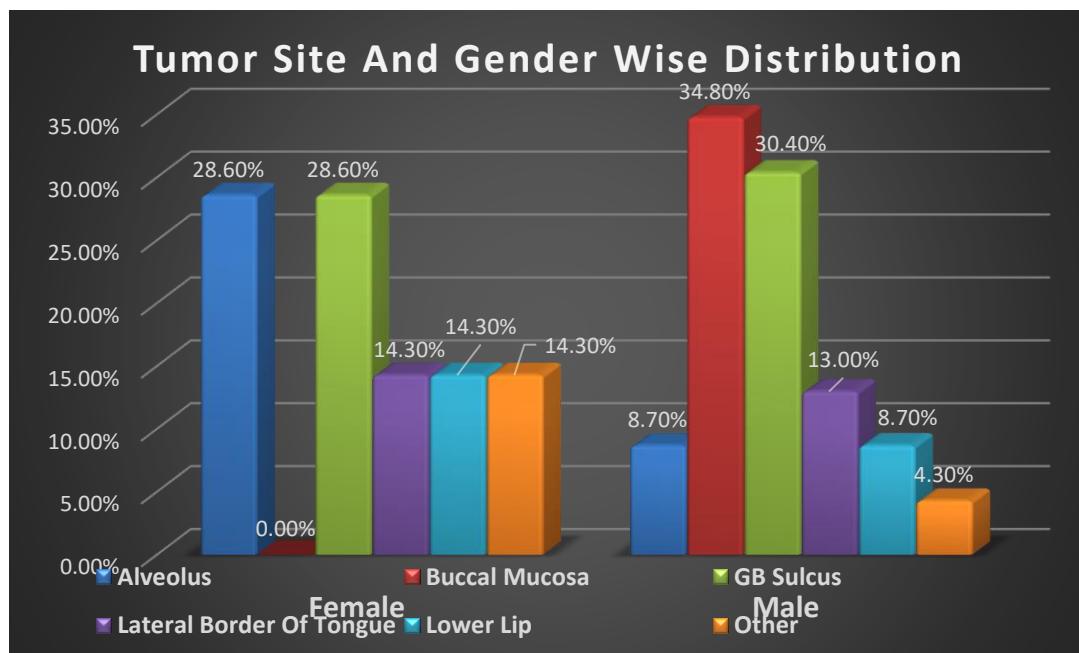


Fig. 1.

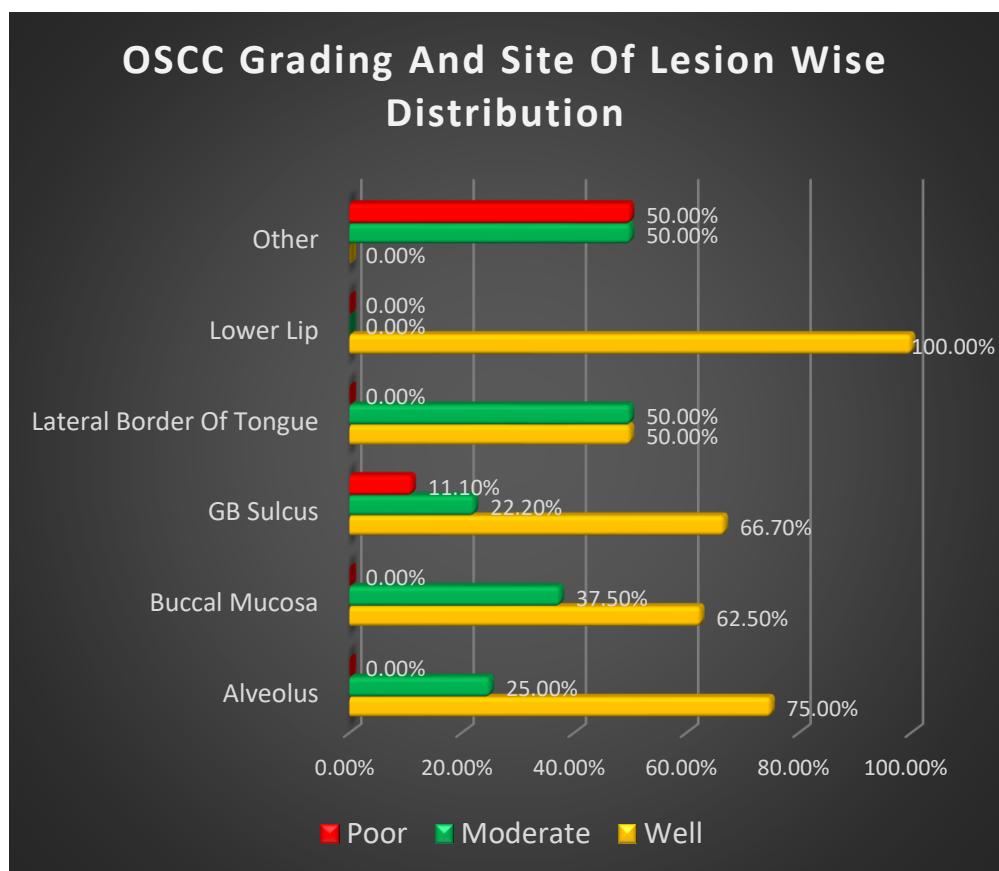


Fig. 2.

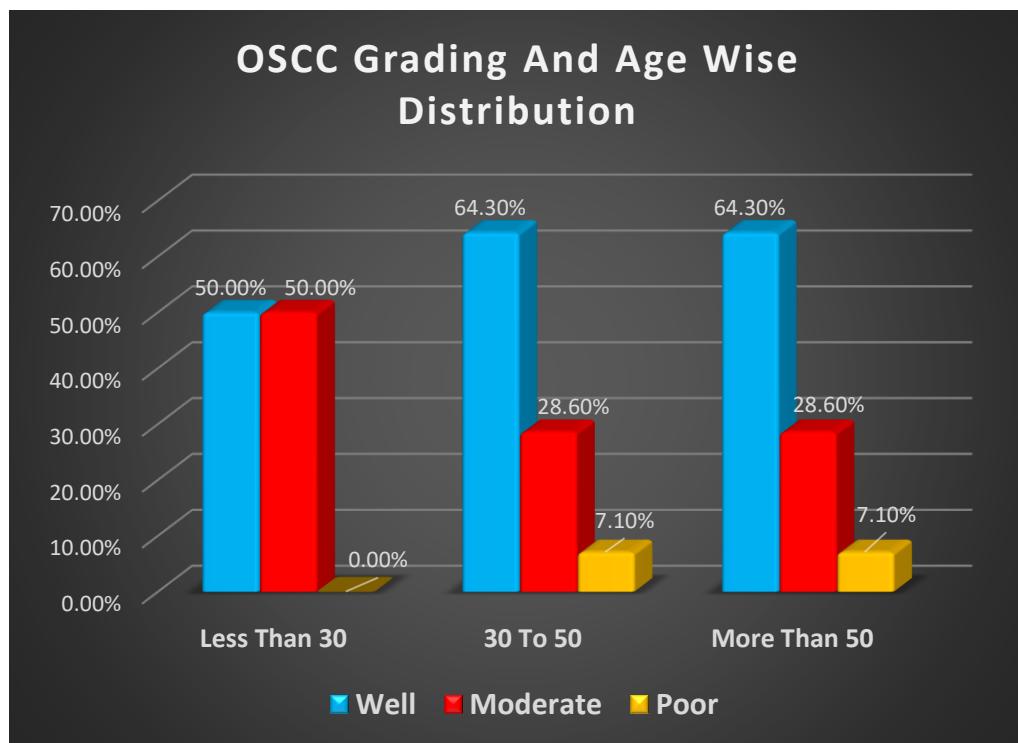


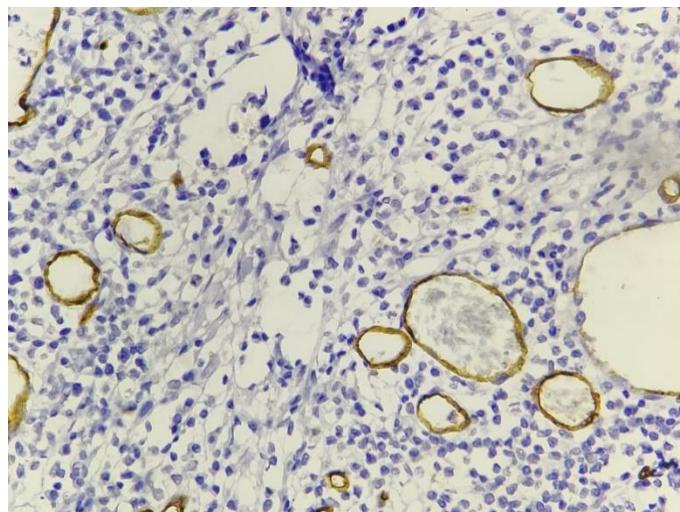
Fig. 3.

Table 1. Correlation between OSCC grade and Weidner score

OSCC GRADE	Weidner Score			Total	Pearson Correlation & p-value
	0 TO 5	5 TO 15	More Than 15		
Well	19(100.0%)	0(0.0%)	0(0.0%)	19(100.0%)	r = 0.89
Moderate	1(11.1%)	6(66.7%)	2(22.2%)	9(100.0%)	&
Poor	0(0.0%)	1(50.0%)	1(50.0%)	2(100.0%)	p-value < 0.001
Total	20(66.7%)	7(23.3%)	3(10.0%)	30(100.0%)	

No relation was found between lymphatic spread of tumor and degree of differentiation ($p= 0.604$, $\chi^2 = 1.068$).

There has been positive correlation between microvessel density and OSCC grade ($r = 0.89$, $p\text{-value} < 0.001$)

**Plate 1. CD34 positive in moderately differentiated squamous cell carcinoma**

4. DISCUSSION

Anaklmaron et al conducted a retrospective study entitled “ Increase of mast cell and tumor angiogenesis in oral squamous cell carcinoma” to elaborate the correlation between tumor angiogenesis in 26 cases of oral squamous cell carcinoma, 6 cases of oral premalignant dysplasia, 10 cases of oral hyperkeratosis, and 6 cases of normal oral mucosa by immunohistochemistry. The density of microvessel increase along with the progression of the disease.

The microvascular count was significantly higher in oral squamous cell carcinoma than in hyperkeratosis and normal mucosa [12].

A study was conducted by Siddharth Pandit et al, entitled “Immunohistochemical analysis of angiogenesis by CD34 and mast cells by toluidine blue in different grades of oral squamous cell carcinoma” on 50 cases of control along with 14 cases of each grade of Oral Squamous Cell Carcinoma, sections were

stained for immunohistochemistry for CD34 and toluidine for mast cells. The mean MVD was higher in different grades as compared to normal mucosa. Intergroup comparison of increase in MVD between different grades of OSCC was not found to be highly statistically significant. Pearson’s correlation between MVD and MCD revealed a linear increase in MVD as the MCD increased, suggestive of a positive correlation [13].

Roland Sedivy et al (2003) in their study of 28 patients attempted to determine how the density of blood and lymphatic microvessels were related to primary OSCC and the clinical course of the disease. The expression of podoplanin, CD-34, and VEGF-C was determined and compared with the long-term clinical and pathological data of the patients. It was found that lymphatic vessel density and microvessel density were significantly higher than in the control tissues. They concluded that VEGF-C expression in OSCC triggers lymphatic angiogenesis resulting in an increased risk of cervical lymph node metastasis [14].

Tiziana Martone et al (2004) in their study of 127 patients with squamous cell carcinoma using immunohistochemistry using monoclonal antibodies against CD34 and CD105 (endoglin) analyzed for the first time the clinical relevance of microvessel density in a large series of primary squamous cell carcinomas using anti CD34 and anti CD105 monoclonal antibodies. Their data provided evidence that CD105 MVD is an independent predictor of survival either in all patients and in the subset of node-negative patients, thus suggesting that it may help to select patients with more aggressive diseases who may benefit from targeted therapies [15].

Tan Z et al, carried out a study of 45 cases being divided into well-differentiated squamous cell carcinoma, moderately differentiated squamous cell carcinoma, poorly differentiated squamous cell carcinoma, morphometric analysis showed that microvessel density is slightly lower in well-differentiated as compared to moderately differentiated squamous cell carcinoma and poorly differentiated squamous cell carcinoma. ($p < 0.001$). The keratin pearl score was slightly higher in well-differentiated squamous cell carcinoma as compared to moderately differentiated squamous cell carcinoma, Pearson correlation has shown that a significant negative correlation between keratin pearl and microvessel density ($r = -0.805$ $p = <0.001$) [16].

Vijay Wadhwan et al carried a study of 10 cases of each well-differentiated squamous cell carcinoma, moderately differentiated squamous cell carcinoma, and poorly differentiated squamous cell carcinoma. And morphometrically analyzed for MND, MVA, and TVA. 10 cases were taken as control, student t-test was applied, and the result have shown a significant difference between MVD, TVA, MVA when poorly differentiated squamous cell carcinoma was compared with normal mucosa, well-differentiated squamous cell carcinoma and moderately differentiated squamous cell carcinoma. However, when compared between well-differentiated between well-differentiated squamous cell carcinoma and moderately differentiated squamous cell carcinoma, the difference in MVD, TVA and MVA were there but not statistically significant [17].

NooshinMohtasham et al (2010) in their study on 42 specimens of OSCC and 22 specimens of dysplastic oral mucosa using immunohistochemical staining, showed a significant increase in mast cell count and MVD

between normal oral mucosa and epithelial dysplasia, normal oral mucosa, and OSCC, epithelial dysplasia, and OSCC. But there were no statistically significant differences in MCC and MVD between low and high-grade OSCC [18].

In our study based on the site of lesion, the percentage is determined. Maximum cases were from GB sulcus 9(30.0%). The least number of cases were noted in lower lip 3(10.0%). Male 23(76.67%) has a higher prevalence than female 7(23.3%). When cases were divided into different gradings of carcinoma, the maximum was well-differentiated carcinoma 19(63.3%) and least were poorly differentiated carcinoma 2(6.7%). The only place poorly differentiated carcinoma was found was GB sulcus. Also maximum times carcinoma was present in GB sulcus followed by buccal mucosa. Also, the groups are divided age-wise the number of patients less than 30 years was quite less than 2(6.67%) as compared to higher age group patients. Also, the age group between 30 To 50 years has the same number of patients as more than 50 years age group. Thus as the age increases the chances of getting OSCC are found to be increased.

There has been positive correlation between microvessel density and degree of differentiation ($r = 0.89$, p -value < 0.001). Also, we found positive correlation between tumor size and CD 34 expression in our study with $r = 0.712$ & p -value < 0.001 .

5. CONCLUSION

- The present study concludes a positive correlation between the histopathological grades of oral squamous cell carcinomas and microvessel proliferative markers on and immunohistochemistry (CD 34 expression).
- It implies that these microvessel proliferative markers provide a supportive cue to the clinician and the histopathologist to predict the rate of proliferation and helps to understand the biological behavior of the tumor along with clinic pathological parameters like tumor stage, grade, lymph node involvement, etc.
- These biomarkers (CD 34) help to differentiate between different grades of oral squamous cell carcinoma. They help to bridge the gap that arises due to inter-observer variability while diagnosing different grades of oral squamous cell carcinoma.

- The study also concludes that these biomarkers can prove to be dependable adjuncts along with histopathological grading of oral squamous cell carcinoma which is the gold standard method of diagnosis.

6. SCOPE AND LIMITATIONS

6.1 Scope

Immunohistochemical analysis in detecting oral squamous cell carcinoma can be used as an adjunct in the grading of oral squamous cell carcinoma in correlation with the grading system and can also assess the refractoriness of the tumor mass to the chemotherapeutic agents.

6.2 Limitation

1. Interobserver variability.
2. Outer observer variability.
3. Technical errors while processing can influence the interpretation of immunostaining.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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