Original Article

Role of cell proliferation and vascularity in malignant transformation of potentially malignant disorders

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Abstract

Background: Significant increase in cell proliferation and vascularity occurs during the transition from normal oral mucosa through differing degrees of dysplasia to oral squamous cell carcinoma (OSCC).

Aims: To evaluate the cell proliferation and vascularity in potentially malignant disorders and OSCC.

Settings and Design: Proliferating cell nuclear antigen (PCNA), vascular endothelial growth factor (VEGF) and CD34 were quantified immunohistochemically (IHC) using anti-PCNA, anti-VEGF and anti-CD34 antibody.

Materials and Methods: A total of 60 archival specimens included 10 oral lichen planus, 10 oral leukoplakia, 10 oral submucous fibrosis and 30 OSCC (well differentiated, moderately differentiated and poorly differentiated), and also, 10 normal oral mucosa as control group were taken. PCNA, VEGF and CD34 expression was assessed in relation to the localization and area of IHC-stained cells.

Statistical Analysis: One-way analysis of variance test and *post hoc* least significant difference test were assessed for statistical significance.

Results: Cell proliferation and vascularity appeared to increase gradually with disease progression.

Conclusion: Upregulation of cell proliferation and vascularity indicates their possible role in malignant transformation of potentially malignant disorders.

Keywords: Cell proliferation, microvessel density, vascularity

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INTRODUCTION

The information obtained in the clinical and histopathological examinations is not always satisfactory for the diagnosis and prognosis of potentially malignant disorders. Therefore, more specific methods are used to allow the measurement of the cellular alterations by means of cellular and tissue markers. Several markers have been used to provide additional information about malignant transformation in potentially malignant disorders, including angiogenesis and cell proliferation markers, which have long been used in the study of cancer and are the focused of this study.[1]

At present, angiogenesis is considered an essential process in tumor development. Angiogenesis, the formation of new blood vessels, is crucial to the growth, invasion and metastasis of a tumor.[2] Tumor angiogenesis, like the physiological one, is the process of creating new blood vessels starting from the already existing ones, either by

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recruiting precursor endothelial cells or by multiplying the endothelial cells of the already existing capillaries.[3]

Cell proliferation is a biological process vitally important to all living organisms due to its role in the growth and maintenance of tissue homeostasis. The control of this important process is completely dysregulated in cancer, and the assessment of cell proliferation activity in tumors has become a common tool used by histopathologists to provide useful information for diagnosis, clinical behavior and therapy.[1]

The present study has been proposed to assess some aspects of the angiogenesis and cell proliferation processes based on vascular endothelial growth factor (VEGF), CD34 and proliferating cell nuclear antigen (PCNA) expression in oral lichenplanus (OLP), oral leukoplakia (OL), oral submucous fibrosis (OSF) and oral squamous carcinoma (OSCC).

MATERIALS AND METHODS

Tissuesamples

Sixty formalin-fixed paraffin-embedded archival biopsies of 10 OLP (GroupI), 10 OL (GroupII), 10 OSF (GroupIII) and 30 OSCC (10 well-differentiated SCC[WSCC], 10 moderatelydifferentiated SCC [MSCC] and 10 poorlydifferentiatedSCC [PSCC] [GroupIV]) were obtained from the Department of Oral Pathology and Microbiology, Kamineni Institute of Dental Sciences, Narketpally.Ten cases of normal control group (Group V) were also included.

Histopathological and immunohistochemical analysis

All tissue biopsies were sectioned at 3μm thickness and taken onto a poly-L-lysine-coated glass slide, and further, immunohistochemistry (IHC) procedure was performed to detect VEGF, CD34 and PCNA expression. Sections were deparaffinized followed by rehydration and antigen retrieval was carried out. Thereby, sections were incubated with peroxidase block to block the endogenous peroxidase activity which was followed by protein block, primary antibody, post primary antibody, polymer and substrate chromogen application and finally counterstained with Mayer's hematoxylin and mounted.

Staining was performed as per the IHC staining protocol. The presence of brown-colored end product at the site of target antigen was indicative of positive immunoreactivity.

PCNA expression was evaluated on the basis of number of positively stained cell; expression of PCNA was designated as positive (>5% of cells were stained) and negative (<5% of cells stained). Three high-power fields (×40) were selected from the stained sections to determine the stained cells per 100 counted cells in the basal and parabasal layers as positive and negative.

VEGF expression was quantified according to the area of staining in the connective tissue under low-power view (×10). The area of staining was scored as 0, no stained cells in any microscopic field, 1, <25% of tumor cells stained positively, 2, 25-50% of tumor cells stained positively, 3, 50-75% of tumor cells stained positively and 4, >75% of tumor cells stained.

CD34 expression was assessed as microvessel density, and the assessment was carried out at the level of endothelial cells lining the blood vessels by their brown cytoplasmic staining in the connective tissue. Microvessel density in areas showing the highest density of staining determined by low-power view (×10) was selected, and then, under three high-power view (×40), the number of CD34-positive endothelial lined blood vessels was counted.

Statistical analysis

SPSS (Statistical package for social sciences) is a software package used for statistical analysis. IBM SPSS Statistics for Windows, Version 22.0. (Armonk, NY: IBM Corp). The significance of the results obtained from the control and study group was statistically analyzed by Chi-squared test and one-way analysis of variance (ANOVA) test, and multiple comparisons between the groups were assessed for statistical significance using *post hoc* least significance difference (LSD) test.

RESULTS

The cell proliferation determined by PCNA expression was based on nuclear staining per 100 counted cells in

Table 1: Descriptive analysis between the groups stained with proliferating cell nuclear antigen antibody

Group	*n*	Mean	SEM	Median	Mode	SD	Range	Minimum	Maximum
OLP	10	0.6000	0.16330	1.0000	1.00	0.51640	1.00	0.00	1.00
OSF	10	0.8000	0.13333	1.0000	1.00	0.42164	1.00	0.00	2.00
OL	10	0.7000	0.15275	1.0000	1.00	0.48305	1.00	0.00	1.00
OSCC	30	0.9667	0.3333	1.0000	1.00	0.18257	1.00	0.00	1.00
Normal	10	0.5000	0.166	0.5000	0.00	0.52705	1.00	0.00	1.00

SEM: Standard error of the mean, SD: Standard deviation, OLP: Oral lichen planus, OL: Oral leukoplakia, OSF: Oral submucous fibrosis, OSCC: Oral squamous cell carcinoma

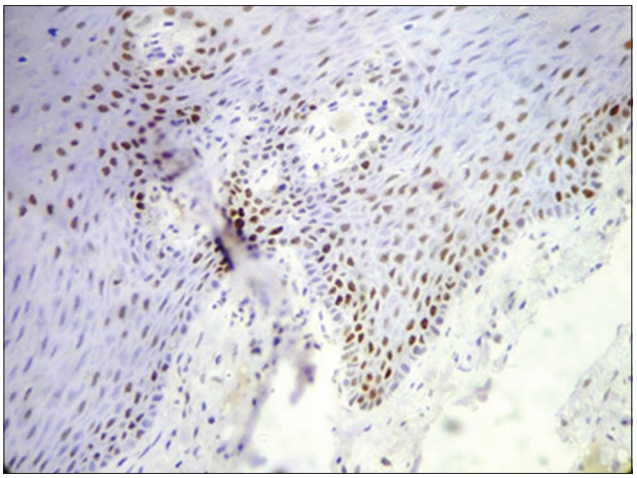


Figure 1: Anti-proliferating cell nuclear antigen antibody staining in oral lichen planus

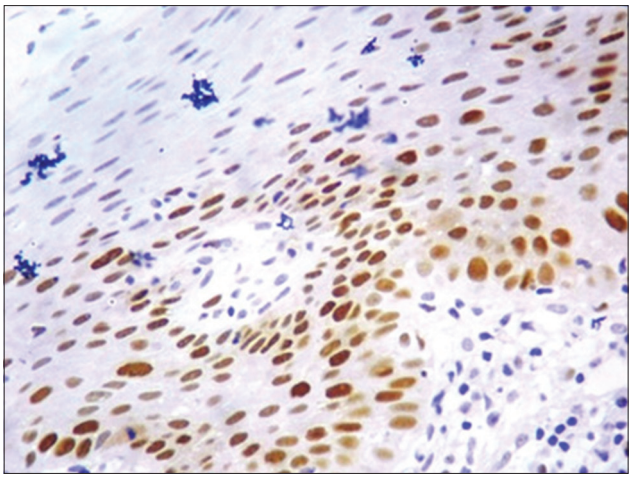


Figure 2: Anti-proliferating cell nuclear antigen antibody staining in oral submucous fibrosis

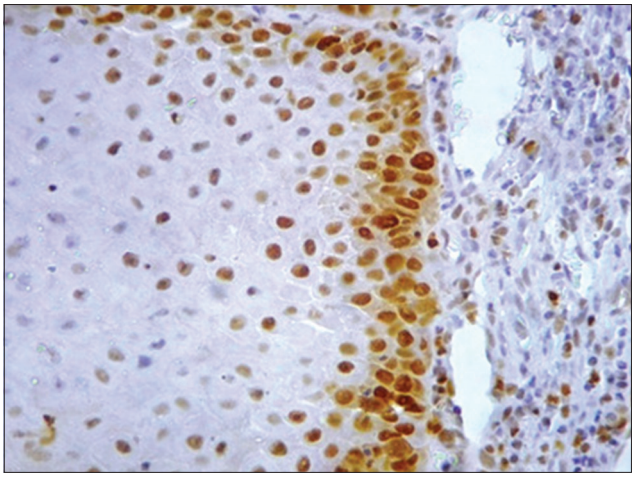


Figure 3: Anti-proliferating cell nuclear antigen antibody staining in oral leukoplakia

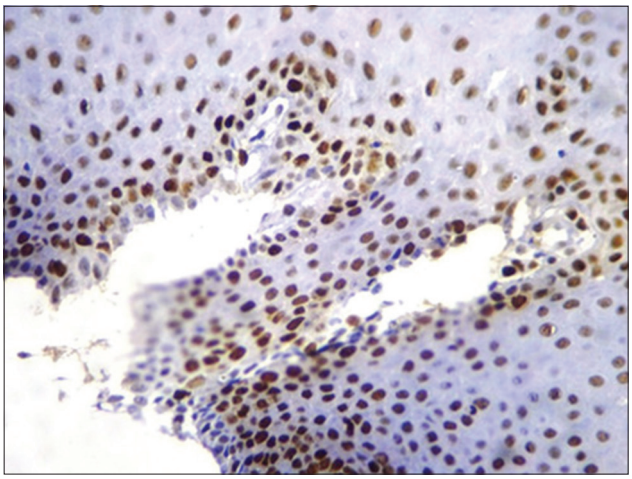


Figure 4: Anti-proliferating cell nuclear antigen antibody staining in well-differentiated squamous cell carcinoma

Table 2: Chi-square test table between the groups stained with proliferating cell nuclear antigen antibody

Group	PCNA		Significance
	0, *n* (%)	1, *n* (%)	
OLP	4 (40.0)	6 (60.0)	0.035
OSMF	2 (20.0)	8 (80.0)	
OL	3 (30.0)	7 (70.0)	
OSCC	1 (3.3)	29 (96.7)	
Normal	5 (50.0)	5 (50.0)	

PCNA: Proliferating cell nuclear antigen, OLP: Oral lichen planus, OL: Oral leukoplakia, OSCC: Oral squamous cell carcinoma, OSMF: Oral submucous fibrosis

the basal and parabasal layer when viewed under three high-power views (×40) [Figures 1-7]. Based on the Chi-squared test among the study groups, normal group showed only 50% expression of PCNA, whereas OLP and OL groups showed 60% and 70% expression, respectively, while OSF showed 80% and OSCC showed majority (96.7%) expression. This difference in the expression was statistically significant ($P = 0.035$) [Tables 1 and 2, Graph 1]. The positivity for expression of PCNA in WSCC, MSCC and PSCC was 90%, 100% and 100%, respectively. Within the OSCC group, PCNA expression determined using Chi-squared test showed no statistical significance ($P = 0.355$) [Tables 3 and 4, Graph 2].

VEGF expression was confirmed by the presence of brown-stained cytoplasm in the connective tissue when viewed under low-power view (×10) [Figures 8-15]. The data assessed for significance between the groups using one-way ANOVA showed statistical significance ($P = 0.000$). Multiparametric *post hoc* LSD test was done between the study groups, and there was a statistical significance between the groups, but the results were not statistically significant ($P = 0.068$) between OL and normal oral mucosa group [Tables 5-7]. The data assessed for significance within the OSCC using one-way ANOVA and multiparametric *post hoc* LSD test showed statistical significance [Tables 8-10, Graphs 3 and 4].

Table 3: Descriptive analysis within the oral squamous cell carcinoma group stained with proliferating cell nuclear antigen antibody

Group	*n*	Mean	SEM	Median	Mode	SD	Range	Minimum	Maximum
WSCC	10	0.9000	0.10000	1.0000	1.00	0.31623	1.00	0.00	1.00
MSCC	10	1.0000	0.00000	1.0000	1.00	0.00000	0.00	1.00	1.00
PSCC	10	1.0000	0.00000	1.0000	1.00	0.00000	0.00	1.00	1.00

SEM: Standard error of the mean, SD: Standard deviation, WSCC: Well-differentiated squamous cell carcinoma, MSCC: Moderately differentiated squamous cell carcinoma, PSCC: Poorly differentiated squamous cell carcinoma

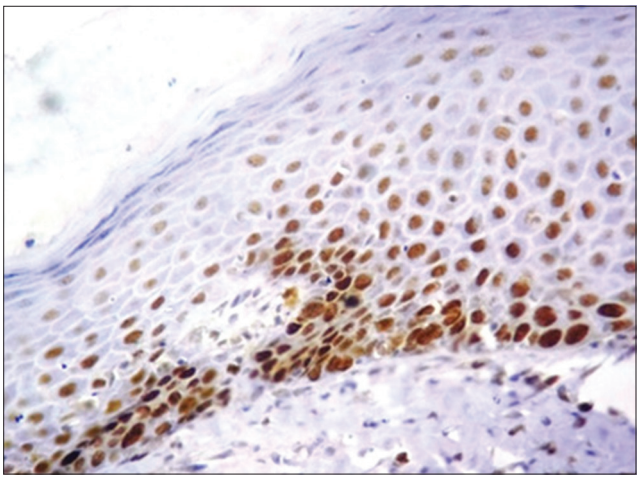


Figure 5: Anti-proliferating cell nuclear antigen antibody staining in moderately differentiated squamous cell carcinoma

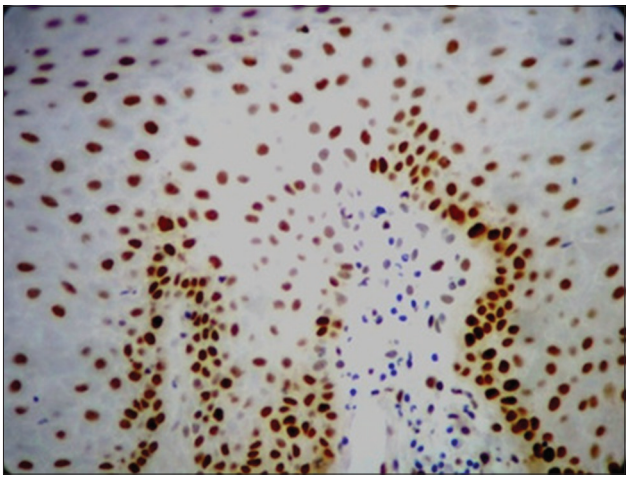


Figure 6: Anti-proliferating cell nuclear antigen antibody staining in poorly differentiated squamous cell carcinoma

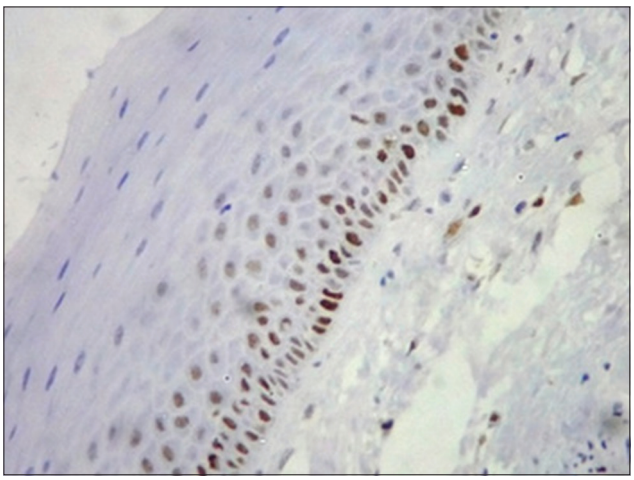


Figure 7: Anti-proliferating cell nuclear antigen antibody staining in normal oral mucosa

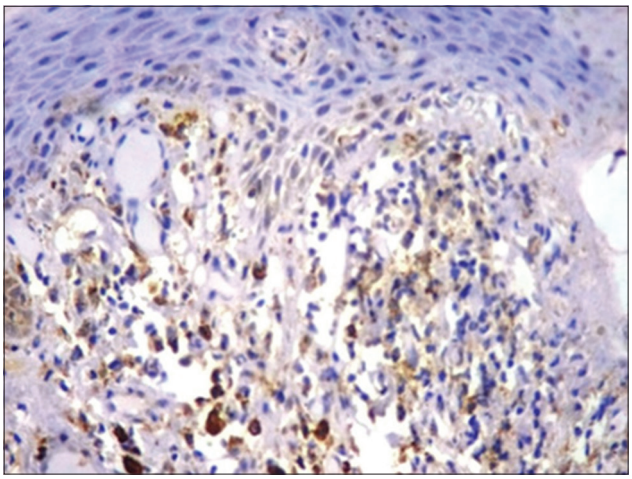


Figure 8: Anti-vascular endothelial growth factor antibody staining in oral lichen planus

Microvessel density was based on the CD34-positive endothelial cells lining the blood vessel in the connective tissue; at first, three microscopic fields of highest neovascularization under low-power view (×10) were selected and then counted under high-power view (×40) [Figures 16-21]. One-way ANOVA was performed for significance between the groups and $P = 0.000$ was considered statistically significant. Multiparametric *post hoc* LSD test done between the study groups also was statistically significant [Tables 11-13]. The data assessed for significance within the OSCC using one-way ANOVA and multiparametric *post hoc* LSD test showed statistical significance [Tables 14-16, Graphs 5 and 6].

DISCUSSION

The results from the present study indicate a significant upregulation of VEGF, CD34 and PCNA expression during the transition from normal oral mucosa through OLP, dysplasia, OSF and OSCC. An overall increase in mean scores from normal to OLP, OL, OSF and different grades of OSCC was similar to other studies.[2-20]

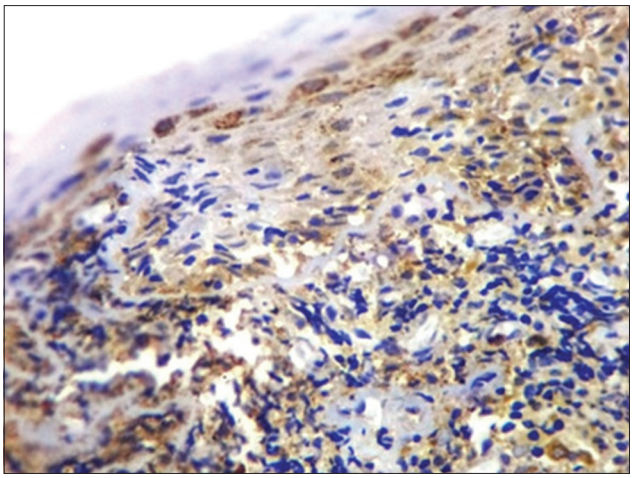


Figure 9: Anti-vascular endothelial growth factor antibody staining in oral submucous fibrosis

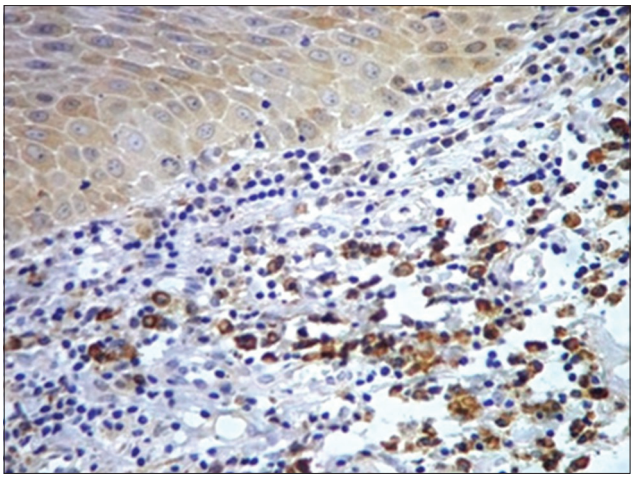


Figure 10: Anti-vascular endothelial growth factor antibody staining in oral leukoplakia

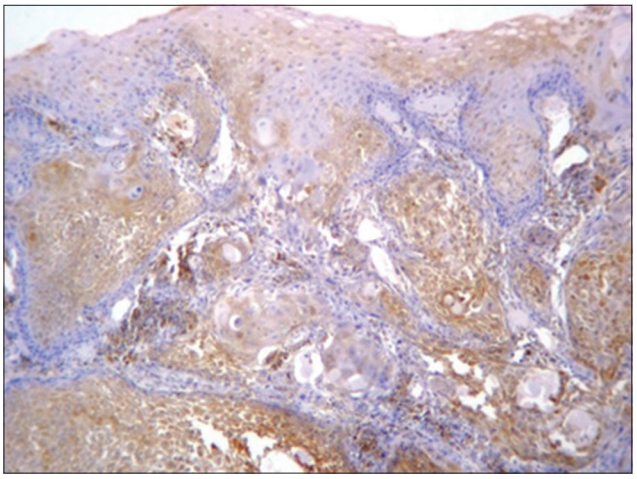


Figure 11: Anti-vascular endothelial growth factor antibody staining in oral leukoplakia

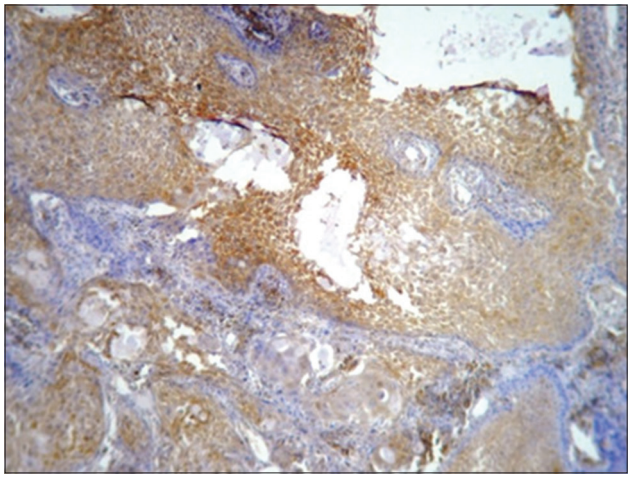


Figure 12: Anti-vascular endothelial growth factor antibody staining in well-differentiated squamous cell carcinoma

Table 4: Chi-squared test table within the oral squamous cell carcinoma groups stained with proliferating cell nuclear antigen antibody

Group	PCNA		Significance
	0, *n* (%)	1, *n* (%)	
WSCC	1 (10.0)	9 (90.0)	0.355
MSCC	0	10 (100.0)	
PSCC	0	10 (100.0)	

PCNA: Proliferating cell nuclear antigen, WSCC: Well-differentiated squamous cell carcinoma, MSCC: Moderately differentiated squamous cell carcinoma, PSCC: Poorly differentiated squamous cell carcinoma

Previous studies[2-20] included most but not all the parameters as in the present study, providing evidence of variation in anti-VEGF, anti-CD34 and anti-PCNA antibody staining. However, few studies[2-7,12,15,18-20] have not shown statistically significant results among the parameters considered.

A probable explanation could be that changes in the proliferative capacity may be an early consequence of carcinogen exposure and simultaneous field cancerization, a phenomenon that could occur before the appearance of morphologically apparent hyperplasia or dysplasia. It is generally accepted that increased proliferation is associated with more advanced lesions and that the distribution of proliferating cells in tissue may tell us more about the regulatory mechanism that becomes dysfunctional during the multi step process of carcinogenesis.[21] Along with cell proliferation, at present, angiogenesis is considered an essential process in oral cancer development. Significance of angiogenesisis because the exact quantification of tumor vessels is useful for assessing the lesion prognosis and metastasization ability.[3]

In OLP, an increase in proliferation might be related to there lease of cytokines and inflammatory mediators from injured keratinocytes or inflammatory cells following immunological reactions. This increase may result in

Table 5: Descriptive analysis between the groups stained with vascular endothelial growth factor antibody

Group	*n*	Mean	SEM	Median	Mode	SD	Range	Minimum	Maximum
OLP	10	1.4000	0.16330	1.0000	1.00	0.51640	1.00	1.00	2.00
OSM	10	2.4000	0.16330	2.0000	2.00	0.51640	1.00	2.00	3.00
OL	10	1.9000	0.10000	2.0000	2.00	0.31623	1.00	1.00	2.00
OSCC	30	3.4333	0.10376	3.0000	3.00	0.56832	2.00	2.00	4.00
Normal	10	1.0000	0.00000	1.0000	1.00	0.00000	0.00	1.00	1.00

SEM: Standard error of the mean, SD: Standard deviation, OLP: Oral lichen planus, OL: Oral leukoplakia, OSCC: Oral squamous cell carcinoma, OSM: Oral submucous fibrosis

Table 6: One-way analysis of variance table between the groups stained with vascular endothelial growth factor antibody

	Sum of squares	df	Mean square	*F*	Significant
Between groups	64.076	4	16.019	69.109	0.000
Within groups	15.067	65	0.232		
Total	79.143	69			

Table 7: Multiple comparisons *post hoc* least significant difference test table between the groups stained with vascular endothelial growth factor antibody

Group (I)	Group (J)	Mean difference (I–J)	SE	Significant	95% CI	
					Low lower bound	Upper bound
OLP	OL	−0.50000	0.21531	0.023	−0.9300	−0.0700
	OSF	−1.00000	0.21531	0.000	−1.4300	−0.5700
	OSCC	−2.03333	0.17580	0.000	−2.3844	−1.6822
	Normal	0.40000	0.21531	0.068	−0.0300	0.8300
OL	OLP	0.50000	0.21531	0.023	0.0700	0.9300
	OSF	−0.50000	0.21531	0.023	−0.9300	−0.0700
	OSCC	−1.53333	0.17580	0.000	−1.8844	−1.1822
	Normal	0.90000	0.21531	0.000	0.4700	1.3300
OSF	OLP	1.00000	0.21531	0.000	0.5700	1.4300
	OL	0.50000	0.21531	0.023	0.700	0.9300
	OSCC	−1.03333	0.17580	0.000	−1.3844	−0.6822
	Normal	1.40000	0.21531	0.000	0.9700	1.8300
OSCC	OLP	2.03333	0.17580	0.000	1.6822	2.3844
	OL	1.53333	0.17580	0.000	1.1822	1.8844
	OSCC	1.03333	0.17580	0.000	0.6822	1.3844
	Normal	2.43333	0.17580	0.000	2.0822	2.7844
Normal	OLP	−0.40000	0.21531	0.068	−0.8300	0.0300
	OL	−0.90000	0.21531	0.000	−1.3300	−0.4700
	OSF	−1.40000	0.21531	0.000	−1.8300	−0.9700
	OSCC	−2.43333	0.17580	0.000	−2.7844	−2.0822

OLP: Oral lichen planus, OL: Oral leukoplakia, OSF: Oral submucous fibrosis, OSCC: Oral squamous cell carcinoma, CI: Confidence interval, SE: Standard error

Table 8: Descriptive analysis within the oral squamous cell carcinoma group stained with vascular endothelial growth factor antibody

Group	*n*	Mean	SEM	Median	Mode	SD	Range	Minimum	Maximum
WSCC	10	2.9000	0.10000	3.0000	3.00	0.31623	1.00	2.00	3.00
MSCC	10	3.4000	0.16330	3.0000	3.00	0.51640	1.00	3.00	4.00
PSCC	10	4.0000	0.00000	4.0000	4.00	0.00000	0.00	4.00	4.00

WSCC: Well-differentiated squamous cell carcinoma, MSCC: Moderately differentiated squamous cell carcinoma, PSCC: Poorly differentiated squamous cell carcinoma, SEM: Standard error of the mean, SD: Standard deviation

Table 9: One-way analysis of variance table within the oral squamous cell carcinoma group stained with vascular endothelial growth factor antibody

	Sum of squares	df	Mean square	*F*	Significant
Between groups	6.067	2	3.033	24.818	0.000
Within groups	3.300	27	0.122		
Total	9.367	29			

a suitable ground for malignant transformation.[22] As an autoimmune disease with inflammatory origin and chronic progression, OLP satisfies all the prerequisites of hypoxia which is essential for angiogenesis. If angiogenesis is increased, it leads to more recruitment and retention of lymphocytes or inflammatory infiltrate or progression of disease or recurrence of lesions. Inflammatory infiltrates in turn can progress to carcinogenesis.[9]

In OL, accumulation of mutations in growth regulatory genes may result in an increased proliferative activity.[23]

Table 10: Multiple comparisons *post hoc* least significant difference test table within the oral squamous cell carcinoma group stained with vascular endothelial growth factor antibody

Group (I)	Group (J)	Mean difference (I–J)	SE	Significant	95% CI	
					Lower bound	Upper bound
WSCC	MSCC	−0.50000	0.15635	0.004	−0.8208	−0.1792
	PSCC	−1.10000*	0.15635	0.000	−1.4208	−0.7792
MSCC	WSCC	0.50000	0.15635	0.004	0.1792	0.8208
	PSCC	−0.60000	0.15635	0.001	−0.9208	−0.2792
PSCC	WSCC	1.10000	0.15635	0.000	0.7792	1.4208
	MSCC	0.60000	0.15635	0.001	0.2792	0.9208

CI: Confidence interval, SE: Standard error, WSCC: Well-differentiated squamous cell carcinoma, MSCC: Moderately differentiated squamous cell carcinoma, PSCC: Poorly differentiated squamous cell carcinoma

Table 11: Descriptive analysis between the groups stained with CD34 antibody

Group	*n*	Mean	SEM	Median	Mode	SD	Range	Minimum	Maximum
OLP	10	17.2000	0.69602	16.5000	15.00	2.20101	6.00	15.00	21.00
OSM	10	32.8000	1.15277	32.5000	30.00	3.64539	11.00	28.00	39.00
OL	10	23.8000	0.66332	24.0000	22.00	2.09762	7.00	20.00	27.00
OSCC	30	51.766	1.49842	51.0000	50.00	8.20716	35.00	35.00	70.00
Normal	10	7.4000	0.37118	7.5000	6.00	1.17379	3.00	6.00	9.00

SEM: Standard error of the mean, SD: Standard deviation, OLP: Oral lichen planus, OL: Oral leukoplakia, OSCC: Oral squamous cell carcinoma, OSM: Oral submucous fibrosis

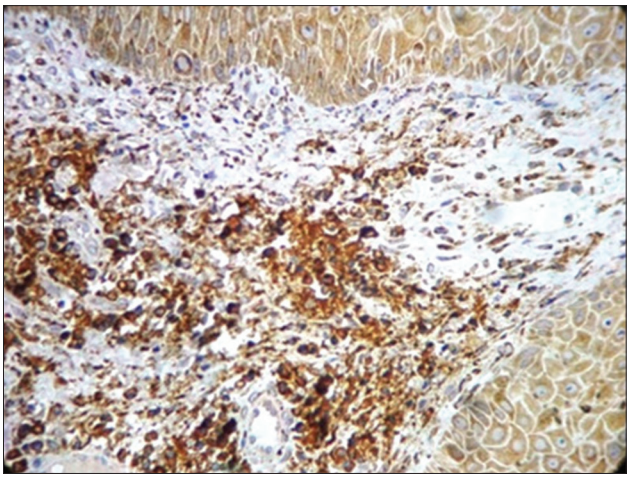


Figure 13: Anti-vascular endothelial growth factor antibody staining in moderately differentiated squamous cell carcinoma

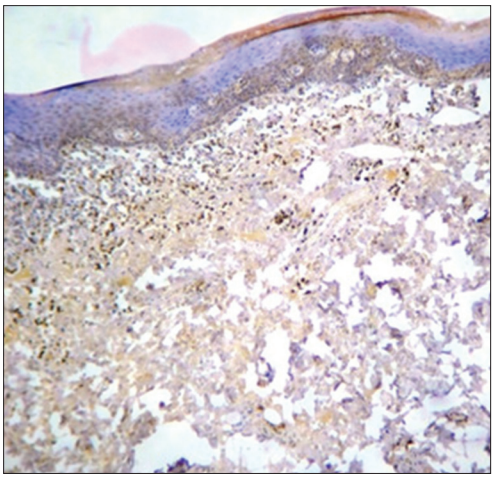


Figure 14: Anti-vascular endothelial growth factor antibody staining in poorly differentiated squamous cell carcinoma

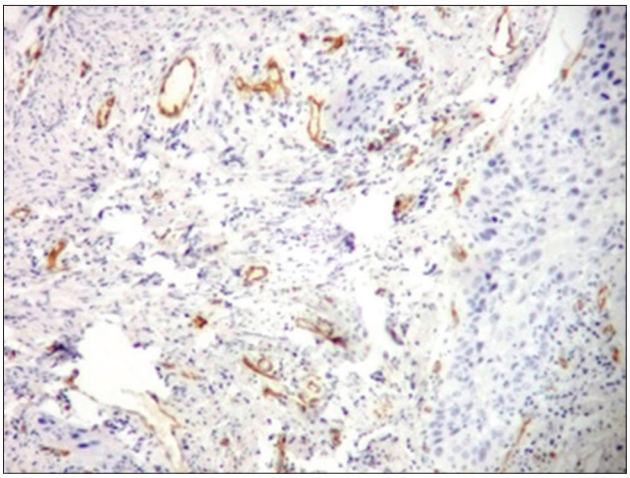


Figure 15: Anti-vascular endothelial growth factor antibody staining in normal oral mucosa

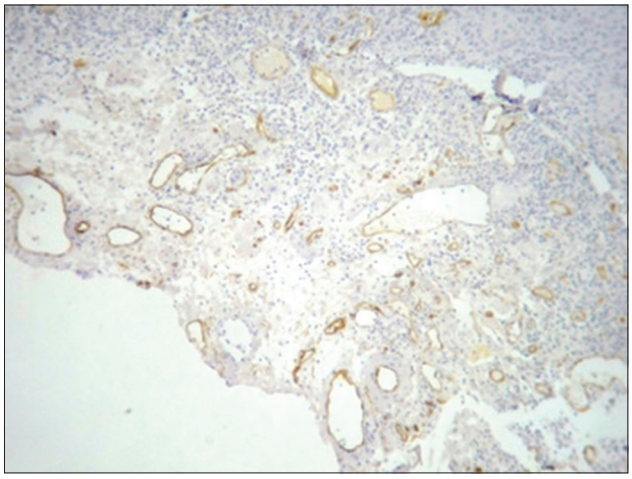


Figure 16: Anti-CD34 antibody staining in oral lichen planus

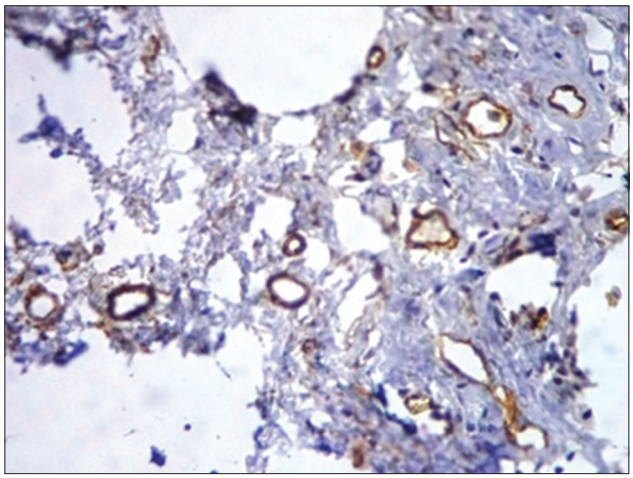


Figure 17: Anti-CD34 antibody staining in oral lichen planus

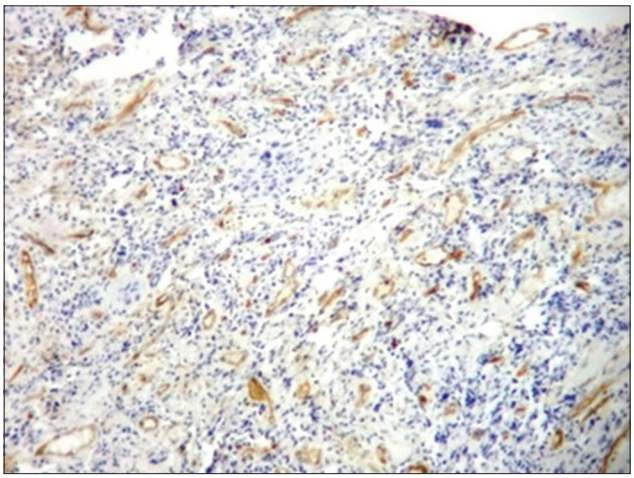


Figure 18: Anti-CD34 antibody staining in well-differentiated squamous cell carcinoma

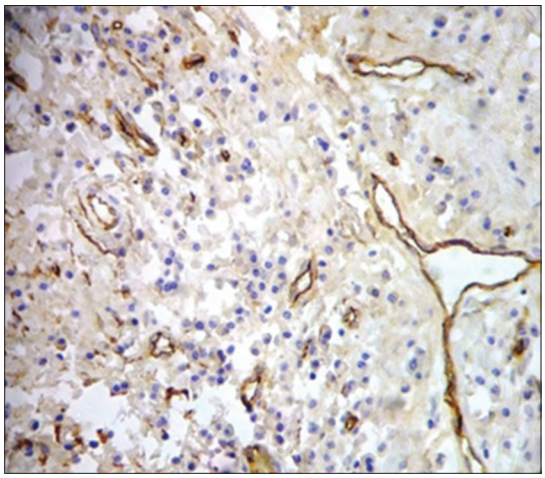


Figure 19: Anti-CD34 antibody staining in moderately differentiated squamous cell carcinoma

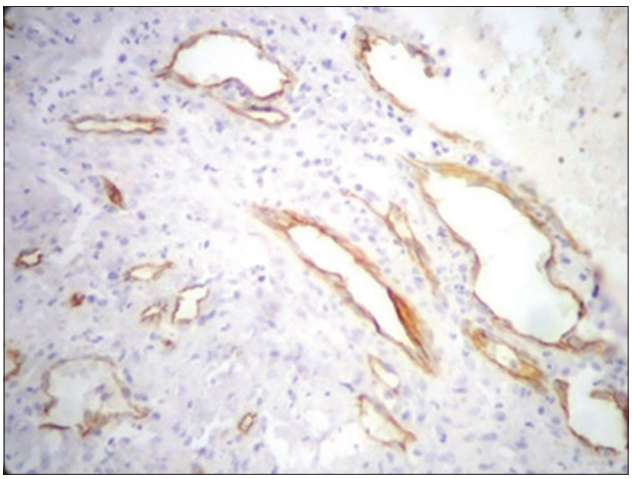


Figure 20: Anti-CD34 antibody staining in poorly differentiated squamous cell carcinoma

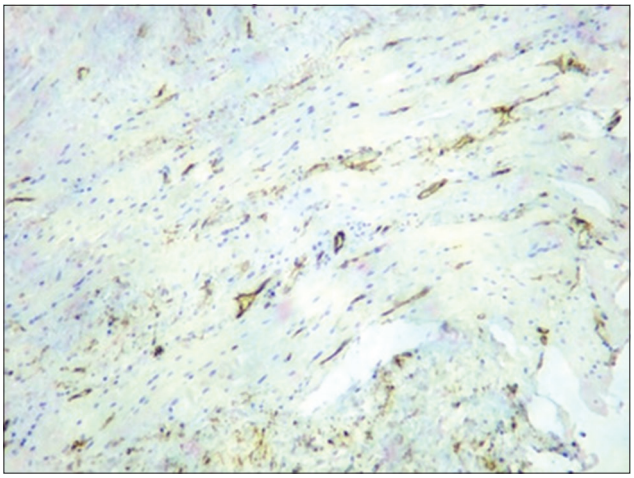
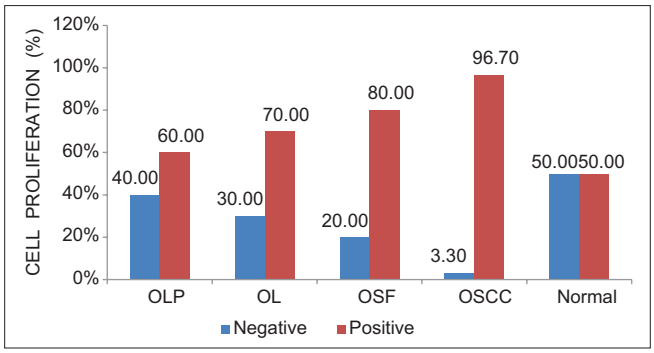


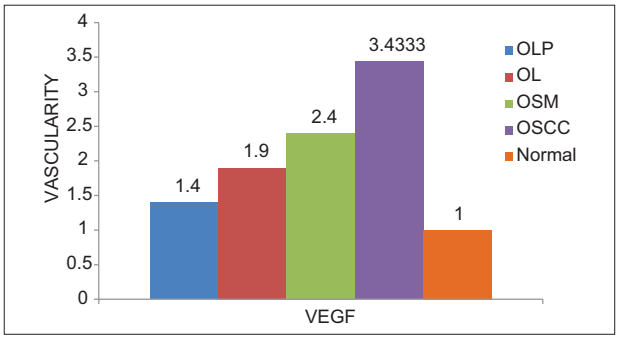
Figure 21: Anti-CD34 antibody staining in normal oral mucosa

As cells transform from normal to dysplastic, the balance between proangiogenic and antiangiogenic factors is altered and the dysplastic epithelial cells themselves acquire transient angiogenic properties. Thereby, shifting to angiogenic phenotype occurs as early as mild dysplasia.[12] As appearance of OSCC is gradually preceded by epithelial dysplasia,[13] a gradual increase of VEGF in OL is considered to satisfy the criteria of a potentially malignant disorder progressing into a malignancy.

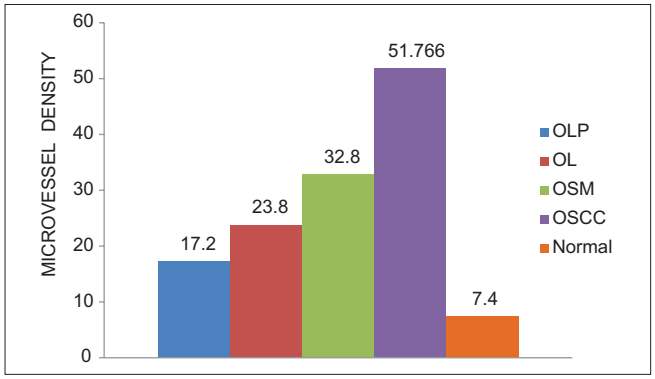
In OSF, the increased cell proliferation could be induced by direct stimulation from the mitogen-like compounds contained in areca quid or by there generative proliferation after cell death. Secondly, as PCNA is associated with DNA excision repair, PCNA expression may also increase after DNA damage is induced by areca quid components.[15] As the stroma becomes more and more hyalinized due to progressive deposition and cross-linkage of mature collagen bundles, the tissue suffers resultant ischemia/hypoxia due to physical and biochemical effects of the process. Pursuing further the pathological mechanism, the tissue tries to cope up with hypoxia by actively promoting neovascularization as



Graph 1: Comparison of cell proliferation between study groups with proliferating cell nuclear antigen. OLP: Oral lichen planus, OL: Oral leukoplakia, OSM: Oral submucous fibrosis, OSCC: Oral squamous cell carcinoma



Graph 3: Comparison of vascularity between study groups with vascular endothelial growth factor. OLP: Oral lichen planus, OL: Oral leukoplakia, OSM: Oral submucous fibrosis, OSCC: Oral squamous cell carcinoma

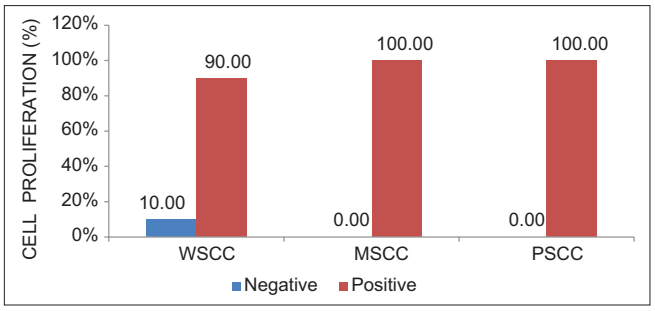


Graph 5: Comparison of microvessel density between study groups with CD34. OLP: Oral lichen planus, OL: Oral leukoplakia, OSM: Oral submucous fibrosis, OSCC: Oral squamous cell carcinoma

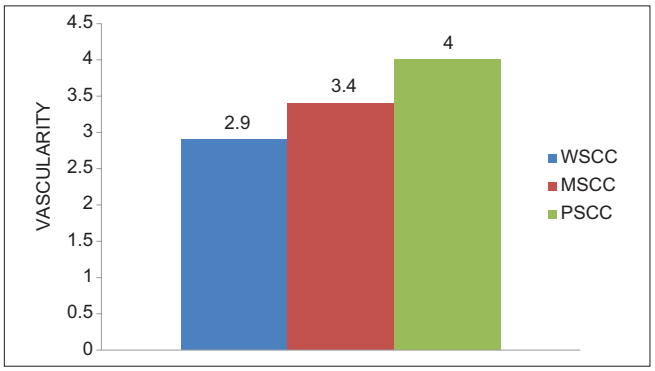
Table 12: One-way analysis of variance table between the groups stained with CD34 antibody

	Sum of squares	df	Mean square	*F*	Significant
Between groups	20419.219	4	5104.805	153.010	0.000
Within groups	2168.567	65	33.363		
Total	22587.786	69			

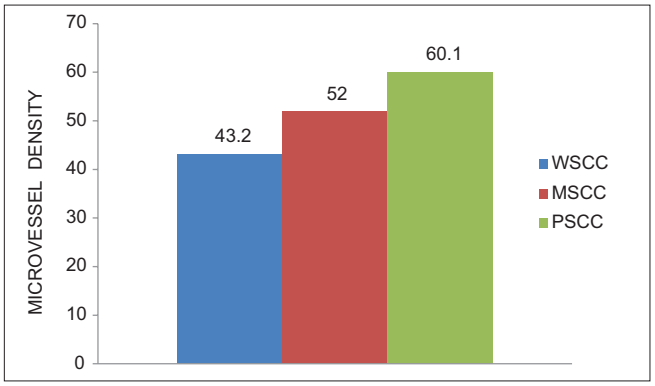
an adaptive response on the part of the mucosa in survival of the atrophic epithelium.[24]



Graph 2: Comparison of cell proliferation within oral squamous cell carcinoma study group with proliferating cell nuclear antigen. WSCC: Well-differentiated squamous cell carcinoma, MSCC: Moderately differentiated squamous cell carcinoma, PSCC: Poorly differentiated squamous cell carcinoma



Graph 4: Comparison of vascularity within oral squamous cell carcinoma study group with vascular endothelial growth factor. WSCC: Well-differentiated squamous cell carcinoma, MSCC: Moderately differentiated squamous cell carcinoma, PSCC: Poorly differentiated squamous cell carcinoma



Graph 6: Comparison of microvessel density within oral squamous cell carcinoma study group with CD34. WSCC: Well-differentiated squamous cell carcinoma, MSCC: Moderately differentiated squamous cell carcinoma, PSCC: Poorly differentiated squamous cell carcinoma

In OSCC, the correlation between PCNA and cell proliferation is probably because of the PCNA involvement in DNA repair which is active and ongoing function so that it might be upregulated in nonproliferating cells.[25] The increase in VEGF expression within the OSCC group supports the idea that VEGF is involved in increasing vascularity with

Table 13: Multiple comparisons *post hoc* least significant difference test table between the groups stained with CD34 antibody

Group (I)	Group (J)	Mean difference (I–J)	SE	Significant	95% CI	
					Lower bound	Upper bound
OLP	OL	−6.60000*	2.58312	0.013	−11.7588	−1.4412
	OSF	−15.60000*	2.58312	0.002	−20.7588	−10.4412
	OSCC	−34.56667*	2.10911	0.000	−38.7788	−30.3545
	Normal	9.80000*	2.58312	0.013	4.6412	14.9588
OL	OLP	6.60000*	2.58312	0.013	1.4412	11.7588
	OSF	−9.00000*	2.58312	0.001	−14.1588	−3.8412
	OSCC	−27.96667*	2.10911	0.000	−32.1788	−23.7545
	Normal	16.40000*	2.58312	0.004	11.2412	21.5588
OSF	OLP	15.60000*	2.58312	0.000	10.4412	20.7588
	OL	9.00000*	2.58312	0.001	3.8412	14.1588
	OSCC	−18.96667*	2.10911	0.001	−23.1788	−14.7545
	Normal	25.40000*	2.58312	0.012	20.2412	30.5588
OSCC	OLP	34.56667*	2.10911	0.000	30.3545	38.7788
	OL	27.96667*	2.10911	0.012	23.7545	32.1788
	OSF	18.96667*	2.10911	0.001	14.7545	23.1788
	Normal	44.36667*	2.10911	0.000	40.1545	48.5788
Normal	OLP	−9.80000*	2.58312	0.014	−14.9588	−4.6412
	OL	−16.40000*	2.58312	0.013	−21.5588	−11.2412
	OSF	−25.40000*	2.58312	0.000	−30.5588	−20.2412
	OSCC	−44.36667*	2.10911	0.001	−48.5788	−40.1545

*: Significant at 0.05, OLP: Oral lichen planus, OL: Oral leukoplakia, OSF: Oral submucous fibrosis, OSCC: Oral squamous cell carcinoma, CI: Confidence interval, SE: Standard error

Table 14: Descriptive analysis within the oral squamous cell carcinoma group stained with CD34 antibody

Group	*n*	Mean	SEM	Median	Mode	SD	Range	Minimum	Maximum
WSCC	10	43.2000	1.35647	44.0000	44.00	4.28952	14.00	35.00	49.00
MSCC	10	52.0000	0.68313	51.5000	50.00	2.16025	6.00	49.00	55.00
PSCC	10	60.1000	1.87646	60.5000	65.00	5.93390	20.00	50.00	70.00

SEM: Standard error of the mean, SD: Standard deviation, WSCC: Well-differentiated squamous cell carcinoma, MSCC: Moderately differentiated squamous cell carcinoma, PSCC: Poorly differentiated squamous cell carcinoma

Table 15: One-way analysis of variance table within the oral squamous cell carcinoma group stained with CD34 antibody

	Sum of squares	df	Mean square	*F*	Significant
Between groups	1428.867	2	714.433	36.777	0.000
Within groups	524.500	27	19.426		
Total	1953.367	29			

Table 16: Multiple comparisons *post hoc* least significant difference test table within the oral squamous cell carcinoma group stained with CD34 antibody

Group (I)	Group (J)	Mean difference (I–J)	SE	Significant	95% CI	
					Lower bound	Upper bound
WSCC	MSCC	−8.80000*	1.97109	0.003	−12.8443	−4.7557
	PSCC	−16.90000*	1.97109	0.000	−20.9443	−12.8557
MSCC	WSCC	8.80000*	1.97109	0.002	4.7557	12.8443
	PSCC	−8.10000*	1.97109	0.001	−12.1443	−4.0557
PSCC	WSCC	16.90000*	1.97109	0.000	12.8557	20.9443
	MSCC	8.10000*	1.97109	0.004	4.0557	12.1443

*: Significant at 0.05, CI: Confidence interval, SE: Standard error, WSCC: Well-differentiated squamous cell carcinoma, MSCC: Moderately differentiated squamous cell carcinoma, PSCC: Poorly differentiated squamous cell carcinoma

disease progression.[2] This could be supported by the fact that VEGF secreted by tumor cells does not stimulate growth directly but leads to increased growth and permeability of endothelial cells, and as vascular permeability increases, microvessels in tumor environment may become leaky, thereby making them more penetrable by tumor cells.[12]

CONCLUSION

Cell proliferation and angiogenesis can be considered a paramount for the assessment of the behavior of potentially malignant disorder. Infact, the malignant transformation of a potentially malignant disorder

can be predicted based on cell proliferation rate and degree of vascularity. In turn, therapies that focus on targeting various molecules and pathways involved in cell proliferation and vascularity may provide better control of the progression of potentially malignant disorders to malignancies.

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Conflicts of interest

There are no conflicts of interest.

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