

Expression of Vascular Endothelial Growth Factor in Oral Invasive Squamous Cell Carcinoma

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ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) is the predominant malignancy of the oral cavity and remains a major health burden in South Asia, where tobacco-related exposures are highly prevalent. Vascular endothelial growth factor (VEGF) plays an important role in tumor angiogenesis, growth, and progression. **Aim of the study:** This study aimed to evaluate VEGF immunohistochemical expression, staining pattern, intensity, and proportion of VEGF-positive tumor cells in histopathologically confirmed invasive OSCC. **Methods Materials:** This cross-sectional observational study was conducted at the Department of Pathology, Rajshahi Medical College, Bangladesh, from September 2018 to August 2020, involving 50 histopathologically confirmed cases of invasive OSCC. Formalin-fixed, paraffin-embedded tissue sections were evaluated histologically and graded as I, II, or III. VEGF immunohistochemistry was assessed based on expression score, staining pattern, intensity, and the proportion of positive tumor cells. Data were analyzed using SPSS version 26.0. **Results:** Among 50 patients with OSCC, the mean age was 52.96 ± 11.19 years, and 56.0% were female. Tobacco exposure was highly prevalent, with smoking reported by 100% of males and 92.9% of females. Buccal mucosa was the most common tumor site, 46.0%, while Grade I tumors predominated, 60.0%. VEGF expression was positive in 86.0% of cases, including strong positivity in 38.0%. Combined cytoplasmic and membranous staining predominated (69.8%), while 46.0% of tumors showed VEGF positivity in at least 25% of cells. **Conclusion:** OSCC predominantly affected middle-aged adults with substantial tobacco exposure, most commonly involving the buccal

mucosa. VEGF expression was detected in most tumors, frequently with moderate-to-strong staining, suggesting its potential utility as an adjunctive marker of tumor angiogenesis.

Keywords: Oral squamous cell carcinoma; Vascular endothelial growth factor; Immunohistochemistry; Tumor angiogenesis; Tobacco exposure.

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INTRODUCTION

Among malignant tumors of the oral cavity, oral squamous cell carcinoma (OSCC) constitutes approximately 90% of cases and remains an important cause of cancer-related morbidity and mortality worldwide [1,2]. Globally, the burden has continued to increase despite advances in diagnostic imaging, surgery, radiotherapy, systemic therapy, and supportive care [2,3]. Using Global Burden of Disease data, recent analyses showed increases in oral cancer incidence, mortality, and disability-adjusted life-years over recent decades, with particularly substantial burdens in Asian populations [3,4]. By 2022, oral cavity cancer accounted for approximately 42% of all lip, oral cavity, and pharyngeal cancers worldwide, while some of the highest incidence rates occurred in South-Central Asia [1]. Within South Asia, the high prevalence of tobacco smoking, smokeless tobacco, betel quid, and areca-nut consumption contributes substantially to the disproportionate burden of oral malignancy [4-6]. In Bangladesh, oral cancer represents an important public health concern, with recent tertiary-care evidence showing that betel quid containing tobacco was used by 61.2% of surgically treated patients and that

more than half presented with stage IV disease [7]. Alongside these observations, recent Bangladeshi case-control evidence demonstrated a strong association between smokeless tobacco and oral cancer, with an adjusted odds ratio of 8.78, further emphasizing the importance of tobacco-related carcinogenesis in this population [8]. Across broader evidence syntheses, smokeless tobacco has consistently been associated with markedly elevated oral cancer risk, particularly in South and South-East Asian populations [6,9]. Clinically, OSCC can cause considerable impairment of mastication, swallowing, speech, facial appearance, and overall quality of life, while cervical lymph-node metastasis and recurrence remain major determinants of prognosis [2,10]. Despite the accessibility of the oral cavity for clinical examination and biopsy, delayed presentation remains frequent, particularly in resource-constrained settings [7,10]. Consequently, increasing attention has been directed toward biological markers that may complement conventional histopathological evaluation and improve understanding of tumor behavior. At the biological level, sustained angiogenesis is fundamental to malignant growth, providing oxygen and

nutrients while facilitating invasion and metastatic dissemination [11,12]. Under hypoxic conditions in the tumor microenvironment, vascular endothelial growth factor, particularly VEGF-A, acts as a major pro-angiogenic mediator, promoting vascular proliferation via VEGF receptors and downstream signaling pathways [11,13]. Through VEGF-mediated signaling, tumor cells may acquire enhanced proliferative, invasive, and metastatic potential, while angiogenesis may also interact with epithelial-to-mesenchymal transition and therapeutic resistance [11,13]. Recent experimental evidence further indicates that OSCC cells can express VEGF-A, VEGFR-1, and VEGFR-2 and may use both autocrine and paracrine signaling to support tumor growth [14]. Consistent with these mechanisms, increased VEGF expression has been demonstrated during progression from premalignant oral lesions to invasive carcinoma, with stronger expression associated with poorer differentiation and nodal involvement [12]. More recently, VEGF-A gene expression and serum concentrations were shown to be higher in patients with OSCC than in controls, with levels increasing across higher tumor grades

and stages [15]. Similarly, immunohistochemical evaluation has demonstrated significantly greater VEGF expression in OSCC than normal oral mucosa, particularly among poorly differentiated tumors [16]. Nevertheless, evidence on VEGF expression in OSCC remains heterogeneous, and clinicopathological data from Bangladesh, particularly from sites outside major centers in Dhaka, are limited. Because geographic differences in tobacco exposure, tumor sites, histopathological characteristics, and disease presentation may influence tumor biology, locally generated pathological evidence is needed. Accordingly, the present study aimed to evaluate the immunohistochemical expression, staining pattern, intensity, and proportion of VEGF-positive tumor cells in histopathologically confirmed invasive OSCC patients at Rajshahi Medical College, Bangladesh.

METHODS & MATERIALS

A cross-sectional observational study was conducted in the Department of Pathology, Rajshahi Medical College, Rajshahi, Bangladesh, from September 2018 to August 2020. The study population comprised patients with oral tumors received at the Department of Pathology during the study period and histopathologically diagnosed as invasive oral squamous cell carcinoma. A total of 50 eligible cases were included in the study.

Patients with histologically confirmed invasive squamous cell carcinoma involving the oral cavity or adjacent oropharyngeal sites and having adequate representative tissue for histopathological and immunohistochemical evaluation were included. Cases with inadequate tissue, extensive tissue necrosis, poorly preserved specimens, or incomplete essential clinical and pathological information were excluded. Relevant demographic and clinical information, including age, sex, occupation, tobacco use history, and anatomical site of the tumor, was recorded on a structured data collection sheet. Tissue specimens were fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin. Sections were prepared and stained with hematoxylin and eosin for histopathological examination. Tumors were evaluated microscopically and classified by histopathological differentiation into Grades I, II, and III. Representative paraffin-embedded tissue blocks containing viable tumor were selected for immunohistochemical assessment of vascular endothelial growth factor (VEGF). For immunohistochemistry, tissue sections were deparaffinized, rehydrated, subjected to appropriate antigen retrieval, and incubated with an antibody directed against VEGF. Immunoreactivity was assessed in tumor cells based on the presence, distribution, and intensity of staining. VEGF expression was categorized

as negative (−), weakly positive (+), positive (++), or strongly positive (+++). The staining pattern was recorded as cytoplasmic, membranous, or combined cytoplasmic and membranous. Staining intensity was further classified as no staining, slight yellowish staining, moderate brown-yellow staining, or maximal dark-brown staining. The proportion of VEGF-positive tumor cells was grouped as 0%, 1–<25%, 25–50%, and >50%. Data were entered, checked for completeness, and analyzed using SPSS (V. 26.0). Categorical variables were expressed as frequencies and percentages, while continuous variables were summarized using mean and standard deviation. Ethical principles regarding confidentiality and use of patient-related information were maintained throughout the study.

RESULTS

Among the 50 participants, the mean age was 52.96 ± 11.19 years. The largest proportion was aged 41–50 years (32.0%), followed closely by those aged 51–60 years (30.0%). Females represented 56.0% of the study population, whereas males accounted for 44.0%. Regarding occupation, housewives constituted the largest group, 56.0%, while business and other occupations each accounted for 14.0% (Table I).

Table I
Sociodemographic characteristics of the study participants (n=50).

Variables	Frequency (n)	Percentage (%)
Age (years)		
≤40	7	14.00
41–50	16	32.00
51–60	15	30.00
61–70	9	18.00
71–80	3	6.00
Mean ± SD	52.96 ± 11.19	
Sex		
Male	22	44.00
Female	28	56.00
Occupation		
Housewife	28	56.00
Service holder	4	8.00
Business	7	14.00
Day labour	4	8.00
Others	7	14.00

A high prevalence of tobacco exposure was observed among both male and female participants. Smoking tobacco was reported by all male participants (100%) and by

92.9% of females. Similarly, smokeless tobacco use was present in 86.4% of males and 89.3% of females, indicating substantial

tobacco exposure across both sexes (Table II).

Table II

Sex-wise distribution of smoking and smokeless tobacco use among the study participants.

Tobacco-use variable	Status	Frequency (n)	Percentage (%)
Male participants			
Smoking tobacco	No	0	0
	Yes	22	100
Use of smokeless tobacco	No	3	13.6
	Yes	19	86.4
Female participants			
Smoking tobacco	No	2	7.1
	Yes	26	92.9
Use of smokeless tobacco	No	3	10.7
	Yes	25	89.3

With respect to tumor location, the buccal mucosa was the most frequently affected site, accounting for 46.0% of cases. Tumors of the tongue represented 10.0%, while the

palatine tonsil, floor of the mouth, and retromolar area each contributed 8.0%. Less frequent sites included the lip and alveolar mucosa (6.0% each), followed by the

gingiva and vallecular area (4.0% each) (*Table III*).

Table IIIDistribution of study participants according to primary tumor site (*n*=50).

Tumor site	Frequency (n)	Percentage (%)
Buccal mucosa of oral cavity	23	46
Tongue	5	10
Palatine tonsil	4	8
Floor of mouth	4	8
Lip	3	6
Alveolar mucosa	3	6
Retromolar area	4	8
Gingiva	2	4
Vallecular area	2	4
Total	50	100

Histopathological assessment showed that Grade I tumors predominated, comprising 60.0% of all cases. Grade II tumors were

identified in 28.0%, whereas Grade III tumors accounted for 12.0%. Thus, lower histopathological grades were more

frequently observed in the study population (*Table IV*).

Table IVDistribution of study participants according to histopathological tumor grade (*n*=50).

Histopathological grade	Frequency (n)	Percentage (%)
Grade I	30	60
Grade II	14	28
Grade III	6	12
Total	50	100

Regarding VEGF immunohistochemical expression, strongly positive staining was most common, occurring in 38.0% of cases, followed by positive staining (26.0%) and weakly positive staining (22.0%); negative expression was observed in 14.0% of cases.

Among VEGF-positive cases, combined cytoplasmic and membrane staining predominated (69.8%), compared with cytoplasmic staining alone (16.2%), and membrane staining alone (14.0%). Moderate brown-yellow staining was the

most frequent intensity (32.0%), followed by maximal dark-brown staining (30.0%), slight yellowish staining (24.0%), and no staining (14.0%) (*Table V*).

Table V

VEGF immunohistochemical expression, staining pattern, and staining intensity among the study participants.

Variables	Frequency (n)	Percentage (%)
VEGF score		
Negative (–)	7	14
Weakly positive (+)	11	22
Positive (++)	13	26
Strongly positive (+++)	19	38
VEGF staining pattern		
Cytoplasmic staining	7	16.2
Membrane staining	6	14
Both cytoplasmic and membrane staining	30	69.8
VEGF staining intensity		
No staining	7	14
Slight staining, yellowish	12	24
Moderate staining, brown-yellow	16	32
Maximal staining, dark brown	15	30

Based on the proportion of VEGF-positive tumor cells, the largest group had 1–<25% positive cells, representing 40.0% of cases.

Positivity in 25–50% of tumor cells were observed in 28.0%, while more than 50% positivity was observed in 18.0%. No

VEGF-positive cells were detected in the remaining 14.0% of cases (*Table VI*).

Table VI
Distribution of study participants according to the percentage of VEGF-positive tumor cells (n=50).

Percentage of positive cells	Frequency (n)	Percentage (%)
0%	7	14
1–<25%	20	40
25–50%	14	28
>50%	9	18
Total	50	100

DISCUSSION

In the present study, oral invasive squamous cell carcinoma predominantly affected middle-aged and older adults, with a mean age of 52.96 ± 11.19 years; 62.0% of patients were between 41 and 60 years. Comparable age distributions have been reported in South Asian populations. In an Indian series of 130 patients, most OSCC cases occurred during the fifth to seventh decades, while another study reported a median age of 60 years [17, 18]. A recent systematic review also demonstrated greater tobacco exposure among OSCC patients older than 45 years than among younger patients [19]. The age pattern observed here may reflect cumulative exposure to tobacco-related carcinogens and the prolonged multistep process of oral carcinogenesis. Interestingly, females constituted 56.0% of the participants, in contrast to the male predominance commonly reported in OSCC. Male proportions of 53.0% and approximately 75% have been documented in Indian cohorts, while regional epidemiological data consistently show higher oral-cancer incidence among men [17,18,20]. This difference may be explained by local exposure patterns, particularly the widespread use of smokeless tobacco among Bangladeshi women. Indeed, 89.3% of females in the present study used smokeless tobacco, while 92.9% reported smoking tobacco. Recent Bangladeshi evidence found smokeless tobacco to be strongly associated with oral cancer, with an adjusted odds ratio of 8.78; notably, the association was stronger among women (OR 14.33) than men (OR 5.29) [8]. Regarding tobacco exposure, smoking was reported by 100% of males and 92.9% of females, while smokeless tobacco use occurred in 86.4% and 89.3%, respectively. These figures exceed the 89% overall tobacco exposure reported by Dwivedi et al. [18]. Nevertheless, the direction of association is consistent with regional evidence. A Sri Lankan study reported a 2.93-fold higher OSCC risk among smokers and a 4.26-fold increase with tobacco-containing betel quid, while concurrent smoking and betel chewing increased risk approximately 15-fold [21]. Such substantial exposure supports tobacco-related field carcinogenesis as an important underlying mechanism in the current population. With

respect to anatomical distribution, the buccal mucosa accounted for 46.0% of tumors, followed by the tongue at 10.0%. This distribution is characteristic of South Asian OSCC, where chewing tobacco, betel quid, and related products are retained in prolonged contact with the buccal mucosa. The study by Rai and Ahmed similarly identified the buccal mucosa as the most common site, while Dwivedi et al. reported involvement of the tongue and buccal mucosa in 38% and 36%, respectively [17,18]. Likewise, buccal mucosa carcinoma has been described as the predominant oral cavity malignancy in the Indian subcontinent, with important biological and prognostic differences from tongue carcinoma [22,23]. Histopathologically, Grade I carcinoma predominated in the present study, accounting for 60.0%, followed by Grade II (28.0%) and Grade III (12.0%). Similar findings were reported by Rai and Ahmed, in which 66 of 130 cases (50.8%) were well differentiated [17]. Likewise, Padma et al. observed well-differentiated tumors in 49.5% of 198 cases [24]. Although lower-grade morphology predominated, histological differentiation remains clinically important; poorer differentiation has been associated with recurrence, lymph node involvement, invasive features, and reduced survival [24,25]. Consequently, the 12.0% of Grade III tumors identified here may represent a particularly high-risk subgroup requiring careful staging and follow-up. A major finding was the high frequency of VEGF immunoreactivity: 86.0% of tumors showed some degree of VEGF positivity, including strong positivity in 38.0%, positive in 26.0%, and weak in 22.0%. This is close to the 88% VEGF-receptor overexpression reported by Pianka et al. [26]. The study by Kim et al. also demonstrated increased VEGF expression in invasive OSCC, with significantly greater expression associated with poorer differentiation and larger tumor size [27]. Similarly, elevated VEGFA transcription has been reported in advanced T3–T4 tumors, metastatic lymph nodes, and stage III–IV OSCC [28]. More recently, significantly higher VEGF expression has been demonstrated in OSCC than normal oral mucosa, particularly in poorly differentiated tumors [16]. Regarding cellular localization, combined cytoplasmic and

membranous VEGF staining predominated (69.8%), and moderate-to-maximal staining intensity was observed in 62.0% of tumors. Furthermore, 46.0% of cases contained VEGF-positive cells in at least 25% of the tumor, including 18.0% with >50% positivity. Such extensive expression supports an active angiogenic microenvironment. Positive cytoplasmic VEGFA expression has previously been associated with poorer differentiation, while VEGF-related signaling has also been linked to tumor size, nodal metastasis, distant metastasis, and disease-specific survival [26,29,30].

LIMITATIONS

The study was limited by its single-center cross-sectional design, relatively small sample size, and lack of longitudinal follow-up. Associations of VEGF expression with tumor stage, lymph-node metastasis, recurrence, treatment response, and survival could not be evaluated. In addition, immunohistochemical assessment may have been affected by subjective interpretation and the absence of standardized quantitative VEGF scoring.

CONCLUSION

Overall, oral squamous cell carcinoma in this cohort predominantly affected middle-aged adults, with buccal mucosa as the most frequent tumor site and substantial tobacco exposure among both sexes. Histologically, Grade I tumors were most common, while VEGF expression was detected in the majority of cases, often with moderate-to-strong staining. Clinically, these findings support a potential role of VEGF as an adjunctive biomarker of tumor angiogenesis, although larger prospective studies are required to establish its prognostic significance.

RECOMMENDATIONS

Further multicenter prospective studies with larger sample sizes are recommended to validate VEGF expression patterns in oral squamous cell carcinoma and to determine their associations with tumor stage, lymph node metastasis, recurrence, treatment response, and survival. Standardized VEGF immunohistochemical scoring should be used to improve comparability across studies. Strengthening tobacco-control

interventions, early oral screening among high-risk individuals, and timely histopathological evaluation may also contribute to earlier detection and improved clinical outcomes.

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CONFLICT OF INTEREST

None declared

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee.

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