RESEARCH ARTICLE

Editorial Process: Submission:03/07/2025 Acceptance:08/14/2025 Published:08/23/2025

Cross-Talk Between Hypoxia-Inducible Factors in Driving the Pathology of Oral Squamous Cell Carcinoma: An Immunohistochemical Analysis of the Role of Hypoxia-Inducible Factors and Cancer-Associated Fibroblasts

Indu M[1]*, Beena VT[2], Moinak Banerjee[3], Latha Mary Cherian[4], Aiswarya Menon P[5], Sajitha PS[6]

Abstract

Background: Tumor hypoxia refers to reduced oxygen levels in tumor tissues, and the transcription factors of cellular response to hypoxia are hypoxia-inducible factors (HIFs). Although the altered expression of HIFs has been identified in many malignancies, their role in oral squamous cell carcinoma (OSCC) is still debatable. Cancer-associated fibroblasts (CAF) are part of the tumor microenvironment; however, the effects of hypoxia on CAFs require further investigation. **Objectives:** This observational study aimed to evaluate the *HIF -1α and HIF - 2α* expression in OSCC compared to normal oral mucosal tissues (NOM) using immunohistochemistry. Further, the study aimed to analyze the association of 1α and 2α isotypes of HIF with the clinicopathologic features of OSCC and their relationship with CAFs in tumor tissues. **Materials and Methods:** Immunostaining of *HIF -1α and HIF - 2α*, and alpha-smooth muscle actin (α- SMA; as a marker for CAFs) was performed on 50 OSCC and 50 NOM samples. Analysis of variance, chi-square test, and Mann–Whitney U test were used to analyze the data. A P value < 0.05 was considered significant. **Results:** *HIF -1α and HIF - 2α*, and α-SMA expressions were significantly higher in OSCC samples than in NOM ($P < 0.001$). The expressions of *HIF-1α* and *HIF-2α* were higher in histologically more differentiated tumor cells; however, the association with histologic grading was not significant. α-SMA exhibited a positive correlation with the expression of 1α and 2α isotypes of HIF. **Conclusions:** The elevated nuclear and stromal immunostaining of HIFs in OSCC substantiates their role in the pathogenesis of OSCC. The correlation between HIFs and α-SMA indicates an influence of hypoxia in inducing CAFs in oral cancer.

Keywords: Alpha-smooth muscle actin- cancer-associated fibroblast- hypoxia- hypoxia-inducible factor

Asian Pac J Cancer Prev, **26 (8)**, 3027-3034

Introduction

Oral cancer is the sixteenth most prevalent cancer globally. According to Globocan 2022, lip and oral cancer is the most common cancer in males in the Indian population [1]. A hypoxic environment is one of the major hallmarks of cancer, which typically represents less than 1% O_2.

Solid tumors are more prone to hypoxia due to their abnormal vascular architecture and an imbalance between oxygen requirement and supply [2]. Both tumor cells and cells in the tumor microenvironment (TME) are affected by hypoxia. Cancer-associated fibroblasts (CAFs), endothelial cells, adipocytes, immune cells, etc., are part of the TME [3]. CAFs demonstrate both pro- and anti-tumorigenic effects. However, the mechanisms by which hypoxic cancer cells affect CAFs remain incompletely understood [4]. The hypoxic response is mediated by hypoxia-inducible factors (HIFs), which are heterodimer proteins comprising α and β subunits (HIF-α and HIF-β). HIF-α has different isoforms, such as *HIF-1α*, *HIF-2α*, and HIF-3α [2]. Of these, *HIF-1α* and *HIF-2α* are two important isoforms in mammals [5]. *HIF-1α* and *HIF-2α* exhibit temporospatial variations in their expressions and have overlapping but independent roles in tumor hypoxia [6]. Altered expression of HIF isotypes has been identified in many tumors; however, the association of HIF isotypes with different tumor types is not similar [7-10]. In oral

*[1]Department of Oral Pathology, Govt Dental College Thrissur, Kerala, India. [2]Directorate of Medical Education, Thiruvananthapuram, Kerala, India. [3]Human Molecular Genetics Laboratory, Rajiv Gandhi Centre for Biotechnology (RGCB), Thycaud Post, Poojappura, Thiruvananthapuram, Kerala 695014. India. [4]Department of Oral Pathology, Govt Dental College Kottayam, Kerala, India. [5]Consultant Oral Pathologist, Kerala, India. [6]Department of Public Health Dentistry, Government Dental College, Kozhikode, Kerala, India. *For Correspondence: indumohan2011@gmail.com*

cancer and head and neck carcinoma, the association of HIF with clinicopathologic features and prognosis remains controversial [10-14].

This study aimed to evaluate the immunohistochemical expression of *HIF-1α* and *HIF-2α* in oral squamous cell carcinoma (OSCC) and normal oral mucosal tissue (NOM). Additionally, the study aimed to analyze the association of hypoxia with the clinicopathologic features of OSCC and its relation to CAFs in tumor tissues.

Materials and Methods

Patients and tissue samples

In this observational study, formalin-fixed paraffin-embedded (FFPE) tissue samples of OSCC (n = 50) were retrieved from the archival collection of biopsy specimens at the Department of Oral Pathology, Government Dental College, Kottayam. The demographic and clinical data were recorded. As controls, normal oral mucosal tissue samples (NOM, n = 50) were collected from patients undergoing operculectomy and periodontal surgeries. Informed consent was obtained from these patients. The study received approval from the Institutional Ethics Committee. Hematoxylin and Eosin (H&E)-stained slides were reviewed using a double-headed bright field microscope (Labomed LX 500, Labo America Inc., CA, USA) to assess histopathologic features. The highest density tumor budding (TB) area was identified in the sections at low magnification (10×). TB was counted at high magnification (40×) at the tumor-invasive front [15]. The intensity of TB (budding index) was classified as follows [16]:

A. High-intensity TB: ≥5 TBs in the field

B. Low-intensity or no TB: <5 TBs or no detectable TB

Immunohistochemistry

Serial sections with 5 µm thickness were prepared from the tissue blocks and mounted on positively charged slides (Auto frost adhesion micro slides, Cancer Diagnostics Inc). Immunohistochemistry was performed using the following primary antibodies: rabbit monoclonal *HIF-1α* antibody (clone EP118, BioGenex), rabbit polyclonal *HIF-2α* antibody (BioGenex), and mouse monoclonal smooth muscle actin (SMA) antibody (1A4, Quartett). The tissue sections were immunostained according to the manufacturer's instructions. Lung cancer and renal cell carcinoma tissue served as positive controls for *HIF-1α* and *HIF-2α*, respectively. For *α-SMA*, vascular smooth muscle acted as an internal positive control. Primary antibodies were excluded from staining to prepare the negative controls.

Evaluation of slides

Two oral pathologists independently assessed the IHC slides at 40× magnification. The following parameters were used to assess the immunopositivity of HIFs: A = staining intensity; B = percentage of stained cells. An immune reactive score (IRS) was obtained by multiplying the scores of A and B. IRS was categorized as follows: a) Negative = 0–1, b) Mild = 2–3, c) Moderate = 4–8, and d) Strong = 9–12 [17]. The mean labeling index (MLI) for *HIF-1α* and *HIF-2α* was calculated using the formula [14]:

(Number of positive cells / Total number of cells per field) x 100

The *α-SMA* score was categorized as follows: a) Score 0 = no CAFs, b) Score 1 (scanty) = a few scattered CAFs, c) Score 2 (focal) = concentrated and irregular distribution of CAFs, and d) Score 3 (abundant) = concentrated, extensive, and continuous distribution of CAFs surrounding the tumor. *α-SMA* scores 0 and 1 were classified as low expression, whereas scores 2 and 3 were classified as high expression [18].

Statistical analysis

Data analysis was performed using SPSS Version 25.0 (IBM Corp., Armonk, NY). Mann–Whitney U test was used to compare the mean IRS and MLI for HIFs between groups. The site of HIF positivity between groups was compared by the chi-square test. The proportions of SMA positivity were compared by Fisher's exact test. Immunostaining among groups was compared by the analysis of variance test. P value < 0.05 was considered statistically significant. Inter-examiner variability was tested using Kappa statistics.

Results

The present study compared the immunostaining of *HIF 1α*, *HIF-2α*, and *α-SMA* in OSCC and NOM. Among OSCC cases, 40% of the patients were aged <60 years, and 60% were aged ≥60 years; 66% were males, and 34% were females. The demographic and clinicopathologic findings of OSCC cases are shown in Table 1.

Expression of hypoxia markers

HIFs immunopositivity was observed as brown staining of the cytoplasm and/or nucleus. Expression of both hypoxia markers was significantly higher (mean IRS) in OSCC than in NOM (p <0.001 for both *HIF-1α* and *HIF-2α*). Moreover, the MLI for *HIF-1α* and *HIF-2α* was significantly higher in OSCC than in NOM (p <0.001 for both *HIF-1α* and *HIF-2α*) (Table 2). Kappa analysis showed almost perfect interobserver agreement for *HIF-1α* and *HIF-2α*

Well-differentiated SCC (WDSCC) and moderately differentiated SCC (MDSCC) had greater mean IRSs of *HIF-1α* and *HIF-2α* expression than poorly differentiated SCC (PDSCC) (Graph 1); however, the difference was not significant (p = 0.143 and 0.289 for *HIF-1α* and *HIF-2α*, respectively). The sites of expression of both hypoxia markers differed significantly (p <0.001) between OSCC and NOM; OSCC showed greater nuclear positivity for both HIF isotypes than NOM. Absolute cytoplasmic expression of HIF isotypes was predominantly observed in WDSCC and MDSCC; however, the difference in the site of expression among different histologic grades of OSCC was not significant (p = 0.190 and 0.279 for *HIF-1α* and *HIF-2α*, respectively). The most common IRS category of *HIF-1α* in OSCC was the moderate type (76%), and that of *HIF-2α* expression was the mild type (58%). The

Table 1. The Demographic and Clinicopathological Features of OSCC Cases

Characteristics of OSCC cases		Percentage distribution % (n)	
Age	≥60 years	60%	(n= 30)
	<60 years	40 %	(n= 20)
Gender	Male	66 %	(n= 33)
	Female	34 %	(n= 17)
Family history of any cancer	No	94%	(n= 47)
	Yes	6 %	(n = 3)
Family history of oral cancer	No	98 %	(n = 49)
	Yes	2%	(n =1)
History of tobacco habit	No	14 %	(n = 7)
	Yes	86 %	(n = 43)
History of the alcohol habit	No	52 %	(n= 26)
	Yes	48 %	(n= 24)
History of systemic diseases	No	74 %	(n= 37)
	Yes	26 %	(n = 13)
Food habit	Vegetarian	2%	(n=1)
	Non- vegetarian	98 %	(n= 49)
	Buccal mucosa	34 %	(n=17)
Site of lesion	Tongue	34 %	(n= 17)
	Floor of mouth	4 %	(n= 2)
	Palate	6 %	(n = 3)
	Alv.mucosa/gingiva	14 %	(n= 7)
	Retromolar region	8%	(n= 4)
Size of lesion	≤ 2cm	36 %	(n = 18)
	> 2 to ≤4	48 %	(n = 24)
	> 4 cm	16 %	(n= 8)
Lymph node involvement	No	50 %	(n = 50)
	Yes	50 %	(n = 50)
Type of lesion	Ulcer	22 %	(n= 11)
	Ulceroproliferative	70%	(n= 35)
	White/red patch	8 %	(n= 4)
Histopathologic grading	WDSCC	18%	(n= 9)
	MDSCC	76 %	(n = 38)
	PDSCC	6 %	(n= 3)
Presence of Necrosis	No	74 %	(n =37)
	Yes	26 %	(n= 13)
	Mild	2 %	(n=1)
Inflammation	Moderate	40 %	(n = 20)
	Severe	58 %	(n= 29)
Invasion of adjacent structures	No	60 %	(n= 30)
	Yes	40 %	(n = 20)
Tumor budding	Low	36 %	(n= 18)
	High	64 %	(n = 32)

prevalent IRS category of *HIF-1α* expression in WDSCC and MDSCC was the moderate type, while in PDSCC, it was the mild type (Figure 1).

A few specimens showed stromal expression of HIF isotypes along with epithelial positivity. OSCC showed a higher stromal positivity of both hypoxia markers than NOM, but a significant difference was observed only for *HIF-1α* (p = 0.039) (Figure 1). Similarly, different histologic grades of OSCC showed a significant difference in *HIF-1α* stromal expression (p = 0.042) but not in *HIF-2α* stromal expression (p = 0.117). Factors such as age, gender, habit history, and family history of cancer and clinicopathologic features such as size, site, type of lesion, lymph node involvement, TB, degree of inflammation, and necrosis were not associated with the degree of *HIF-1α* and *HIF-2α* expression in OSCC (Supplementary file 1a, 1b).

α-SMA staining

α-SMA expression was significantly higher in OSCC compared to NOM (p < 0.001) and increased significantly with the grade of OSCC (p = 0.026). The control group exhibited negative or scanty *α-SMA* expression. *α-SMA* was expressed in focal, scanty, and abundant patterns in OSCC. The scanty pattern was the most common in WDSCC, while the focal pattern was more frequently observed in MDSCC and PDSCC (Figure 2).

α-SMA expression was significantly higher in lesions that invaded adjacent structures (p = 0.002) Factors such as age, gender, habit history, and family history of cancer and clinicopathologic features such as size, site, type of lesion, lymph node involvement, TB, degree of inflammation, and necrosis were not associated with the degree of *α-SMA* expression in OSCC (Supplementary file 2). Kappa analysis showed almost perfect interobserver agreement for *α-SMA*. The difference in the expression of HIF isotypes and *α-SMA* between NOM and different grades of OSCC was found to be significant. (Supplementary file 3). A moderate positive correlation was observed between *HIF-1α* and *HIF-2α*, *HIF-1α* and *α-SMA*, and *HIF-2α* and *α-SMA* (Table 3).

Discussion

The present study showed significantly higher expression of *HIF-1α* and *HIF-2α* in OSCC compared to NOM, with minimal staining in NOM. Similar observations have been reported between OSCC and NOM [12, 19]. The present study observed a higher MLI for *HIF-2α* than for *HIF-1α*. In addition, a lower mean IRS for *HIF-2α* than for *HIF-1α* in OSCC was noted. Differences in the immunostaining intensity of *HIF-1α* and *HIF-2α* may be the cause of variations between MLI and IRS values. Interestingly, a recent study has also reported that MLI for *HIF-2α* was significantly higher than that for *HIF-1α* [10] The most common IRS category for *HIF-1α* in OSCC was moderate, whereas for *HIF-2α*, it was mild. Another study also noted intense expression of *HIF-2α* only in 13% of OSCC cases, with most cases showing mild to moderate intensity [14].

In this study, mixed cytoplasmic and nuclear expression was the most common pattern for HIFs in OSCC (*HIF-1α*, 64%; *HIF-2α*, 48%). In contrast, absolute cytoplasmic expression was the most common pattern in NOM (*HIF-1α*, 70%; *HIF-2α*, 82%). The nuclear positivity of HIFs was higher in OSCC. Among OSCC grades, PDSCC showed the greatest nuclear expression, though the difference was not significant. El-Sayed et al. in 2015 stated that nuclear expression is strongly related

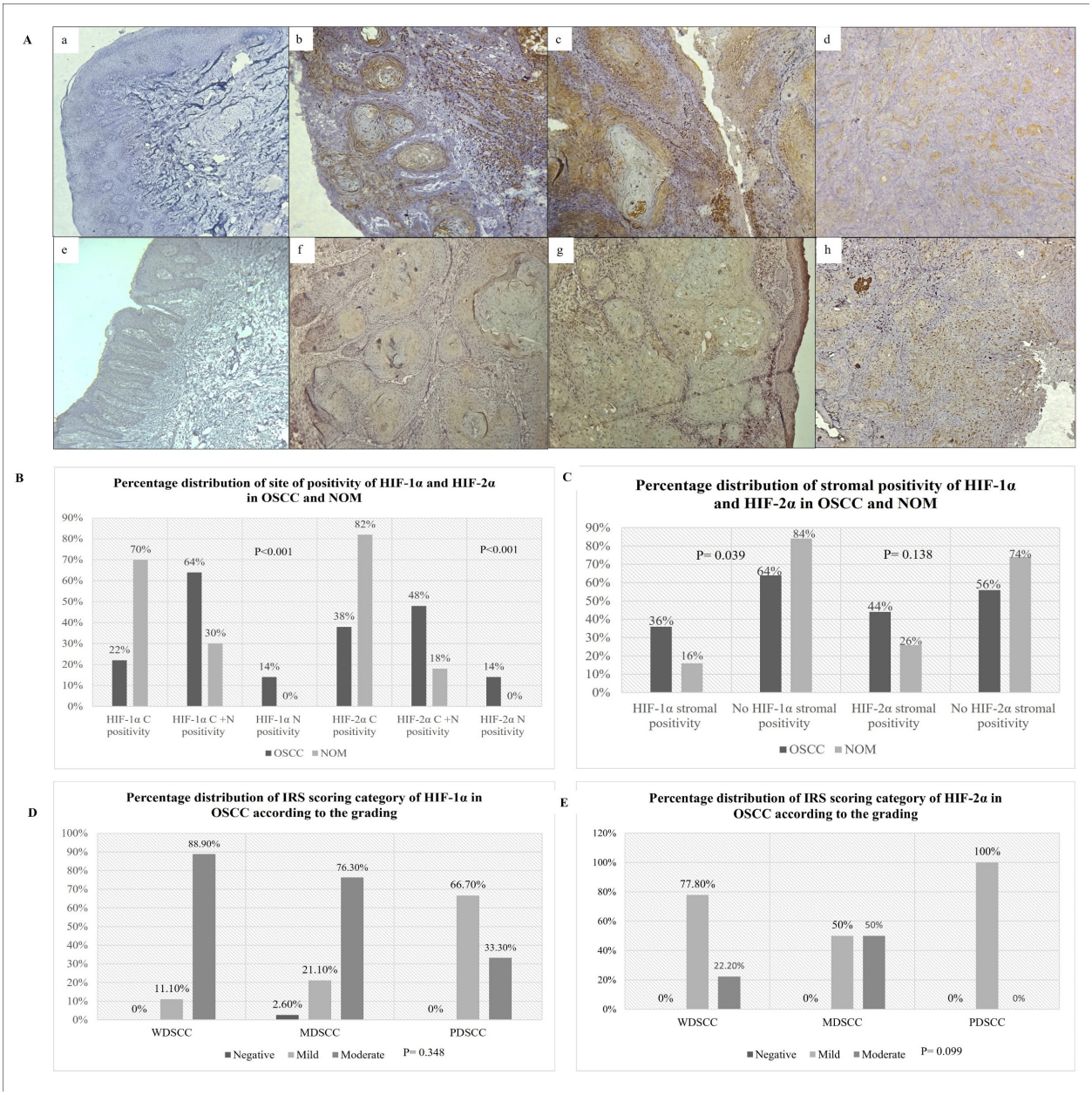


Figure 1. *HIF-1α* and *HIF-2α* Expression in OSCC and NOM. Fig 1: A: Photomicrographs showing immunohistochemistry staining of HIF-1α and HIF-2α at 10x a) HIF-1α in NOM, 10×. b) HIF-1α in WDSCC, c) HIF-1α in MDSCC, d) HIF-1α in PDSCC, e) HIF-2α in NOM, f) HIF-2α in WDSCC, 10×, g) HIF-2α in MDSCC, h) HIF-2α in PDSCC, B: Percentage distribution of the site of expression of HIF 1α and HIF 2α in OSCC and NOM. C: Percentage distribution of HIF 1α and HIF 2α stromal expression in OSCC and NOM. D: Percentage distribution of IRS scoring category of HIF-1α in OSCC as per grading. E: Percentage distribution of IRS scoring category of HIF-2α in OSCC as per grading. (C: Cytoplasmic, C +N: Cytoplasmic and nuclear, N: Nuclear, NOM: Normal oral mucosa, HIF: Hypoxia-inducible factor, WDSCC: Well-differentiated squamous cell carcinoma, MDSCC: Moderately differentiated squamous cell carcinoma, PDSCC: Poorly differentiated squamous cell carcinoma, OSCC: Oral squamous cell carcinoma)

to the activated status of HIF-α when it is translocated to the nucleus to dimerize with the β subunit. The HIF-α/β complex controls several pathways involved in metabolic reprogramming, immune evasion, angiogenesis, cell

Table 2. Comparison of *HIF-1α* and *HIF-2α* Expression in the Study Groups

Category	IRS HIF-1α (Mean±SD)	P* Value	IRS HIF-2α (Mean±SD)	P* Value	MLI HIF-1α (Mean±SD)	P Value	MLI HIF-2α (Mean±SD)	P* Value
OSCC (n=50)	4.58+1.75		3.50+1.31		43.70+18.86		47.48+10.19	
NOM (n=50)	1.08+0.488	<0.001	1.48+0.61	<0.001	3.14+2.81	<0.001	5.47+4.44	<0.001

(* Mann- Whitney U test, two-sided, P.value<0.05) (HIF, Hypoxia-inducible factor; IRS, Immune reactive score; MLI, Mean labeling index; NOM, Normal oral mucosa; OSCC, oral squamous cell carcinoma; SD, Standard deviation)

Table 3. Correlation between *HIF-1α, HIF- 2α*, and *α-SMA*

		IRS HIF-1α	IRS HIF-2α	α-SMA
IRS HIF 1α	Pearson Correlation	1	0.550**	0.611**
	Sig. (2-tailed)		<0.0001	<0.0001
IRS HIF 2α	Pearson Correlation	0.550**	1	0.531**
	Sig. (2-tailed)	<0.0001		<0.0001
α-SMA	Pearson Correlation	0.611**	0.531**	1
	Sig. (2-tailed)	<0.0001	<0.0001	

**, Correlation is significant at the 0.01 level (2-tailed). (Pearson correlation coefficient (r) value ranges from – 1 to +1); (CAF, Cancer-associated fibroblasts, α- SMA: Alpha smooth muscle actin, IRS: Immune reactive score)

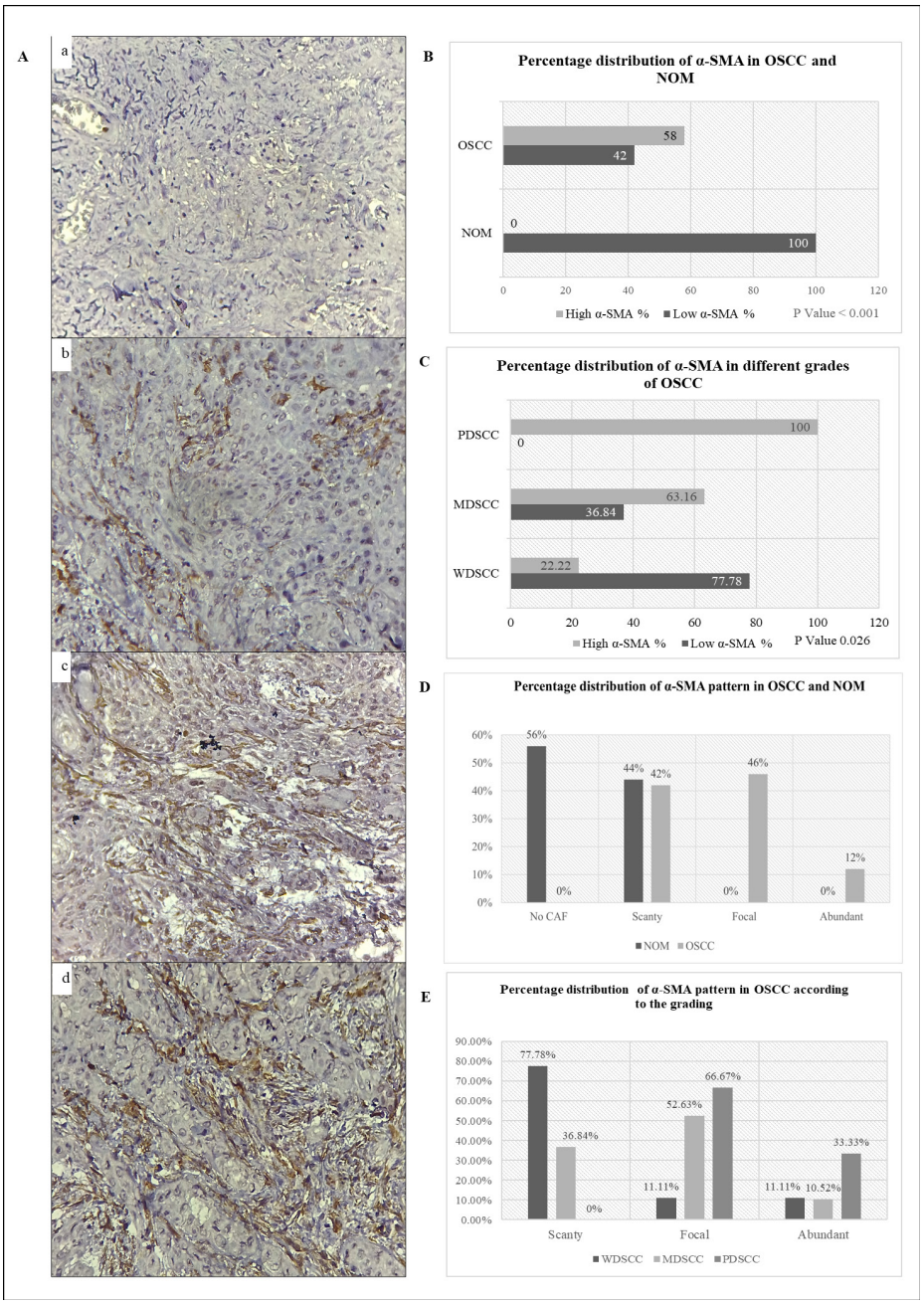
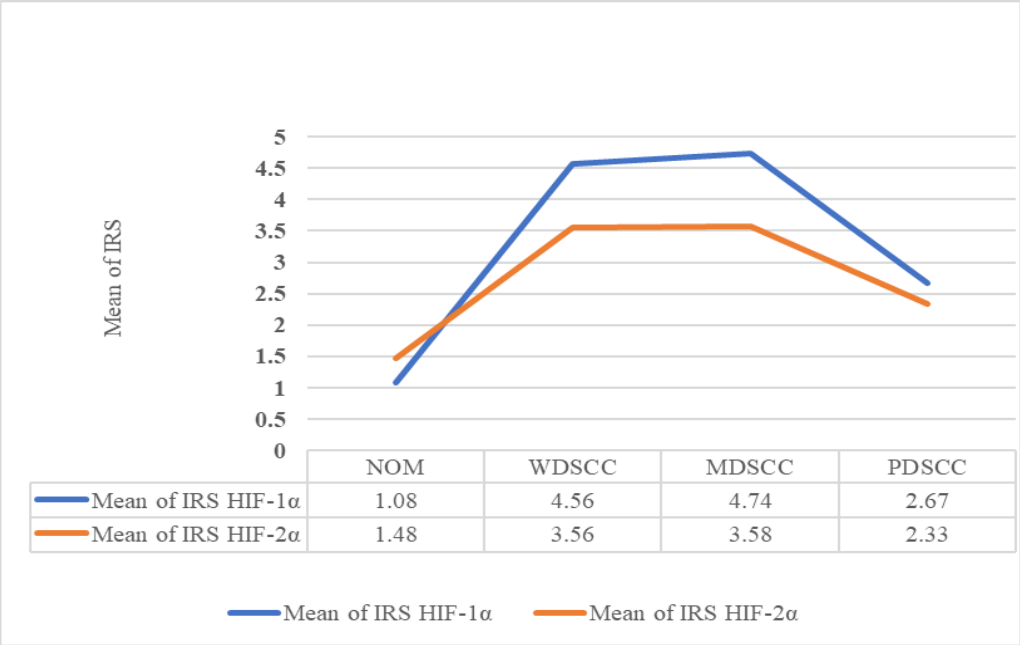


Figure 2. *α-SMA* Expression in OSCC and NOM. A: Photomicrographs showing immunohistological staining of α-SMA. a) α-SMA in NOM, 10×, b) Scanty α-SMA expression in OSCC 10×, c) Focal α-SMA expression in OSCC 10×, d) Abundant α-SMA expression surrounding the tumor islands in OSCC 10×, B: Percentage distribution of α-SMA in OSCC and NOM, C: Percentage distribution of α-SMA in different grades of OSCC, D: Percentage distribution of α-SMA patterns in OSCC and NOM, E: Percentage distribution of α-SMA patterns in different grades of OSCC. (CAF: Cancer-associated fibroblasts, α-SMA: Alpha smooth muscle actin, NOM: Normal oral mucosa, WDSCC: Well-differentiated squamous cell carcinoma, MDSCC: Moderately differentiated squamous cell carcinoma, PDSCC: Poorly differentiated squamous cell carcinoma, OSCC: Oral squamous cell carcinoma)



Graph 1. Comparison of *HIF 1α* and *HIF 2α* expression in NOM and different grades of OSCC. (HIF, Hypoxia-inducible factor; IRS, Immune reactive score; MLI, Mean labeling index; NOM, Normal oral mucosa; WDSCC, Well-differentiated squamous cell carcinoma; MDSCC, Moderately differentiated squamous cell carcinoma; PDSCC, Poorly differentiated squamous cell carcinoma)

proliferation, epithelial-mesenchymal transition, and tumor progression [20, 21]. Therefore, higher *HIF-1α* and *HIF-2α* expression and nuclear positivity could act as diagnostic indicators of malignancy.

This study showed higher *HIF-1α* and *HIF-2α* stromal expression in OSCC compared to normal tissue. Moreover, stromal immunostaining of *HIF-1α* increased with the histologic grade of OSCC. *HIF-1α* and *HIF-2α* were also expressed in the TME cells, such as endothelial cells, fibroblasts, and immune cells [22]. Joseph et al. also reported higher stromal positivity of *HIF-2α* in OSCC compared to normal tissues [14].

HIF-1α is the master regulator of hypoxia and is predominantly expressed in response to acute hypoxia. *HIF-2α* has similar domain arrangement and binding characteristics as *HIF-1α*, but it is elevated in response to chronic hypoxia [20]. Differences in the expression of *HIF-1α* and *HIF-2α* may be due to variations in oxygen levels required for activation, temporospatial arrangement, signaling pathways, and degradation patterns [6, 23]. E3 ligases, such as hypoxia-associated factor (HAF), mediate oxygen-independent degradation of *HIF-1α* in tissues but not of *HIF-2α*. Moreover, ubiquitin-independent degradation of HIFs may cause alterations from their expected levels of expression. Koh MY showed that *HIF-2α* demonstrates more sustained expression under hypoxic conditions compared to *HIF-1α* [23].

HIF expression is not uniform across tumors. Moreover, even similar tumors may exhibit differential expression of HIFs owing to mechanisms other than hypoxia [24]. *HIF-1α* can be upregulated by altered growth factors, activation of the PAM (PI-3K/AKT/mTOR) pathway, genetic up-regulation [24], and activation of oncogenes under normoxic conditions, such as Ras mutation or Src activation [23]. In many cases, HIF expression may be below the detection threshold of immunohistochemical analysis. Additionally, in some well-oxygenated tumors, HIF may not be induced at all [24].

In this study, WDSCC and MSDCC showed higher immunostaining of HIFs. Staining was more prominent in the keratinized/keratin pearl areas. However, no significant variations in HIFs expression were observed among different grades of OSCC, which is in line with the findings of previous studies [12, 14]. Fillies et al. in 2005 noted that *HIF-1α* expression was usually more prominent in the keratinized tumor parts [11]. Joseph et al. observed greater *HIF-2α* expression in WDSCC [14]. Mahapatra et al. in 2023 reported a reduction in the positivity of both markers as the tumor severity increased from well to poorly differentiated [10].

In this study, none of the demographic and clinicopathologic features showed an association with the degree of *HIF-1α* and *HIF-2α* positivity in OSCC. Similar studies have testified variable results regarding the association of demographic and clinicopathologic features with HIF expression [10, 11, 13, 19, 21, 25, 26].

CAFs are a heterogeneous group of cells that have pro- and anti-tumorigenic effects. They are sensitive to oxygen levels. α-SMA/smooth muscle aortic alpha-actin (ACTA2) is one of the most reliable markers for identifying CAFs [27]. In this study, *α-SMA* expression was significantly higher in OSCC compared to NOM, in consensus with Gandhi et al.'s findings in 2023 [28]. Gupta et al. in 2015 suggested that myofibroblasts were absent in NOM but present in OSCC due to the inductive effect of the genetically altered epithelium [29]. Our study showed an increase in CAF expression with the increasing grade of OSCC, with all PDSCC cases showing high *α-SMA*

expression. According to Guiquan et al.'s study in 2021, αSMA expression positively correlated with the nuclear expression of *HIF-1α* in head and neck cancer [30]. The current study showed a positive correlation between *HIF-1α*, *HIF-2α*, and *α-SMA* expressions in OSCC.

No significant associations between HIF expression and clinicopathological parameters, including histopathologic grades of OSCC, were observed in this study, which may be attributable to the relatively small number of WDSCC and PDSCC cases. HIF expression can be influenced by factors other than hypoxia, and those factors should be studied further.

In conclusion, this study highlights that OSCC expressed hypoxia markers significantly. The higher nuclear and stromal positivity of *HIF-1α* and *HIF-2α* in tumor tissues suggests the possible clinical utility of hypoxia markers. Additionally, this study underscores the influence of hypoxia in inducing *α-SMA*-positive CAFs in OSCC. As both *HIF-1α* and *HIF-2α* are significantly involved in OSCC, both can be considered separate therapeutic targets for oral cancer treatment. As the hypoxia–CAF axis can modify the behavior of OSCC, CAF-directed therapies can also be considered for oral cancer. As HIF can be upregulated by factors such as genetic alterations, these mechanisms should be further explored.

Author Contribution Statement

Concept, Planning, and Design: IM, VTB, MB, Research, Procedure: IM, LMC, APM, Statistical analysis: IM, SPS, VTB, Draft Manuscript: IM, SPS, VTB, Revision of Draft: IM, VTB, MB, SPS, APM, LMC, Manuscript Approval: All authors

Acknowledgements

Funding statement

The authors are thankful to the State Board for Medical Research (SBMR) for financial assistance to the research project.

Scientific body approval and Ethical Declaration

This study has been reviewed and approved by the Institutional Review Board and Institutional Ethics Committee of Govt Dental College Kottayam (letter no IEC/M/23/2022/28/DCK 18/05/2022)

Availability of data

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest

Nil.

References

1. Ferlay J, Ervik M, Lam F, Laversanne M, Colombet M, Mery L, et al. Global Cancer Observatory: Cancer Today [internet]. Lyon: France: International Agency for Research on Cancer; 2024. [updated 2024 Feb, accessed on December 9, 2024]. Available from: https://gco.iarc.who.int/today
2. Hu J, Li X, Yang L, Li H. Hypoxia, a key factor in the immune microenvironment. Biomed Pharmacother. 2022;151:113068. https://doi.org/10.1016/j.biopha.2022.113068.
3. Yang D, Liu J, Qian H, Zhuang Q. Cancer-associated fibroblasts: from basic science to anticancer therapy. Exp Mol Med. 2023;55(7):1322-32. https://doi.org/10.1038/s12276-023-01013-0.
4. Sahai E, Astsaturov I, Cukierman E, DeNardo DG, Egeblad M, Evans RM, et al. A framework for advancing our understanding of cancer-associated fibroblasts. Nat Rev Cancer. 2020;20(3):174-86. https://doi.org/10.1038/s41568-019-0238-1.
5. Greer SN, Metcalf JL, Wang Y, Ohh M. The updated biology of hypoxia-inducible factor. EMBO J. 2012;31(11):2448-60. https://doi.org/10.1038/emboj.2012.125.
6. Patel SA, Simon MC. Biology of hypoxia-inducible factor-2alpha in development and disease. Cell Death Differ. 2008;15(4):628-34. https://doi.org/10.1038/cdd.2008.17.
7. Fraga A, Ribeiro R, Coelho A, Vizcaíno JR, Coutinho H, Lopes JM, et al. Genetic polymorphisms in key hypoxia-regulated downstream molecules and phenotypic correlation in prostate cancer. BMC Urol. 2017;17(1):12. https://doi.org/10.1186/s12894-017-0201-y.
8. Gao ZJ, Wang Y, Yuan WD, Yuan JQ, Yuan K. *HIF-2α* not *HIF-1α* overexpression confers poor prognosis in non-small cell lung cancer. Tumour Biol. 2017;39(6):1010428317709637. https://doi.org/10.1177/1010428317709637.
9. Hu X, Lin J, Jiang M, He X, Wang K, Wang W, et al. *HIF-1α* promotes the metastasis of esophageal squamous cell carcinoma by targeting SP1. J Cancer. 2020;11(1):229-40. https://doi.org/10.7150/jca.35537.
10. Mahapatra N, Panda A, Dash K, Bhuyan L, Mishra P, Mohanty A. The study of expression of hypoxia-inducible factor-1 alpha (HIF-1 Alpha) and hypoxia-inducible factor-2 alpha (HIF-2 Alpha) in oral squamous cell carcinoma: An immunohistochemical study. Cureus. 2023;15(9):e45189. https://doi.org/10.7759/cureus.45189
11. Fillies T, Werkmeister R, van Diest PJ, Brandt B, Joos U, Buerger H. HIF1-alpha overexpression indicates a good prognosis in early stage squamous cell carcinomas of the oral floor. BMC Cancer. 2005;5:84. https://doi.org/10.1186/1471-2407-5-84.
12. Sumera S, Ali A, Yousafzai YM, Durrani Z, Alorini M, Aleem B, et al. Overexpression of hypoxia-inducible factor-1α and its relation with aggressiveness and grade of oral squamous cell carcinoma. Diagnostics (Basel). 2023;13(3):451. https://doi.org/10.3390/diagnostics13030451.
13. Eckert AW, Lautner MH, Schütze A, Taubert H, Schubert J, Bilkenroth U. Coexpression of hypoxia-inducible factor-1α and glucose transporter-1 is associated with poor prognosis in oral squamous cell carcinoma patients. Histopathology. 2011;58(7):1136-47. https://doi.org/10.1111/j.1365-2559.2011.03806.x.
14. Joseph I, Elizabeth J, Rao UK, Ranganathan K. Study of hypoxia-inducible factor-2α expression in the malignant transformation of oral submucous fibrosis. J Oral Maxillofac Pathol. 2020;24(1):33-9. https://doi.org/10.4103/jomfp.JOMFP_42_19.
15. Roy P, Datta J, Roy M, Mallick I, Mohandas M. Reporting of tumor budding in colorectal adenocarcinomas using ×40 objective: A practical approach for resource constrained set-ups. Indian J Cancer. 2017;54(4):640-5. https://doi.org/10.4103/ijc.IJC_642_17.
16. Wang C, Huang H, Huang Z, Wang A, Chen X, Huang L, et al. Tumor budding correlates with poor prognosis and

epithelial-mesenchymal transition in tongue squamous cell carcinoma. J Oral Pathol Med. 2011;40(7):545-51. https://doi.org/10.1111/j.1600-0714.2011.01041.x.
17. Fedchenko N, Reifenrath J. Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue - a review. Diagn Pathol. 2014;9:221. https://doi.org/10.1186/s13000-014-0221-9.
18. Ramos-Vega V, Venegas Rojas B, Donoso Torres W. Immunohistochemical analysis of cancer-associated fibroblasts and podoplanin in head and neck cancer. Med Oral Patol Oral Cir Bucal. 2020;25(2):e268-e276. https://doi.org/10.4317/medoral.23335.
19. Lim E, Kuo CC, Tu HF, Yang CC. The prognosis outcome of oral squamous cell carcinoma using *HIF-2α*. J Chin Med Assoc. 2017;80(10):651-6. https://doi.org/10.1016/j.jcma.2017.06.005.
20. Albadari N, Deng S, Li W. The transcriptional factors HIF-1 and HIF-2 and their novel inhibitors in cancer therapy. Expert Opin Drug Discov. 2019;14(7):667-82. https://doi.org/10.1080/17460441.2019.1613370.
21. Youssef HESM, Abo-Azma NEE, Megahe EME. Correlation of hypoxia-inducible factor-1 alpha (HIF-1a) and vascular endothelial growth factor (VEGF) expressions with clinico pathological features of oral squamous cell carcinoma (OSCC). Tanta Dent J. 2015;12:S1-S14. https://doi.org/10.1016/j.tdj.2015.05.010
22. Bigos KJ, Quiles CG, Lunj S, Smith DJ, Krause M, Troost EG, et al. Tumour response to hypoxia: understanding the hypoxic tumour microenvironment to improve treatment outcome in solid tumours. Front Oncol. 2024;14:1331-5. https://doi.org/10.3389/fonc.2024.1331355.
23. Koh MY, Powis G. HAF : the new player in oxygen-independent HIF-1alpha degradation. Cell Cycle. 2009;8(9):1359-66. https://doi.org/10.4161/cc.8.9.8303.
24. Talks KL, Turley H, Gatter KC, Maxwell PH, Pugh CW, Ratcliffe PJ, et al. The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages. Am J Pathol. 2000;157(2):411-21. https://doi.org/10.1016/s0002-9440(10)64554-3.
25. Patel NR, Jain L, Mahajan AM, Hiray PV, Shinde SS, Patel PA. An immunohistochemical study of HIF-1 Alpha in oral epithelial dysplasia and oral squamous cell carcinoma. Indian J Otolaryngol Head Neck Surg. 2019;71(4):435-41. https://doi.org/10.1007/s12070-019-01597-y.
26. Eckert AW, Schutze A, Lautner MH, Taubert H, Schubert J, Bilkenroth U. HIF-1alpha is a prognostic marker in oral squamous cell carcinomas. Int J Biol Markers. 2010;25(2):87-92. https://doi.org/10.1177/172460081002500205.
27. Kim I, Choi S, Yoo S, Lee M, Kim IS. Cancer-associated fibroblasts in the hypoxic tumor microenvironment. Cancers (Basel). 2022;14(14):3321. https://doi.org/10.3390/cancers14143321.
28. Gandhi V, Mahajan A, Kansal YP. An immunohistochemical analysis for evaluating the diagnostic role of myofibroblasts in oral squamous cell carcinoma using α-smooth muscle actin antibody. Adv Biomed Res. 2023;12:123. https://doi.org/10.4103/abr.abr_160_21.
29. Gupta K, Metgud R, Gupta J. Evaluation of stromal myofibroblasts in oral leukoplakia, oral submucous fibrosis, and oral squamous cell carcinoma--an immunohistochemical study. J Cancer Res Ther. 2015;11(4):893-8. https://doi.org/10.4103/0973-1482.147700.
30. Zhu G, Cao B, Liang X, Li L, Hao Y, Meng W, et al. Small extracellular vesicles containing miR-192/215 mediate hypoxia-induced cancer-associated fibroblast development in head and neck squamous cell carcinoma. Cancer Lett. 2021;506:11-22. https://doi.org/10.1016/j.canlet.2021.01.006.