Original Article

Clinicopathological Implications of HIF-1α and MCT-1 Co-Expression in Oral Squamous Cell Carcinoma

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KEY WORDS

Oral Squamous Cell Carcinoma;
Hypoxia-Inducible Factor-1α;
Immunohistochemistry;
Tumour Hypoxia;
Prognosis;

Received: 17 November 2025;
Revised: 9 February 2026;
Accepted: 21 June 2026;

ABSTRACT

Background: One of the hallmarks of tumour progression is metabolic reprogramming, which also promotes resistance to the host microenvironment. Despite recent advances in diagnosis, oral squamous cell carcinoma (OSCC) represents a constant threat to public health.

Purpose: To evaluate the expression of hypoxia-inducible factor-1α (HIF-1α) and monocarboxylate transporter-1 (MCT-1) and to correlate their expression with clinicopathological features of OSCC

Material and Method: A retrospective observational study was conducted in 60 cases of OSCC. Among them, 25 were non-metastatic, 25 metastatic, and 10 recurrent cases of OSCC were included, and other variants of OSCC were excluded. The cases were stained immunohistochemically with HIF-1α and MCT-1, and were subjected to statistical analysis using ANOVA and the Pearson correlation coefficient test.

Results: HIF-1α expression was predominantly observed in the nucleus and cytoplasm of tumour cells and showed higher expression in metastatic OSCC, tumour centre, and advanced TNM stages. MCT-1 expression was mainly localised to the tumour cell membrane and was significantly elevated in metastatic OSCC. A positive correlation was observed between HIF-1α and MCT-1 expression.

Conclusion: The findings indicate that increased expression of HIF-1α and MCT-1 is associated with aggressive tumour behaviour in OSCC. These markers may serve as potential prognostic indicators and therapeutic targets in OSCC.

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Cite this article as:

Introduction

Oral squamous cell carcinoma (OSCC) represents the most prevalent malignant neoplasm within the oral cavity, characterised by aggressive local invasion and a significant propensity for cervical lymph node metastasis, which remains a critical determinant of prognosis. Despite advancements in early detection and therapeutic modalities, the overall survival rate for OSCC patients has demonstrated only marginal improvement, particularly in advanced and metastatic stages [1]. The biological behaviour of OSCC is influenced by both genetic alterations and dynamic changes within the tumour mic-

roenvironment [2].

Hypoxia is a hallmark of solid tumours, including OSCC, resulting from rapid tumour proliferation and inadequate vascularisation. Tumour hypoxia triggers a cascade of adaptive cellular responses that promote angiogenesis, metabolic reprogramming, invasion, and resistance to therapy. These adaptive mechanisms are primarily regulated by hypoxia-inducible factor-1α (HIF-1α), a transcription factor central to the cellular response to reduced oxygen tension. Increased expression of HIF-1α has been associated with tumour aggressiveness, nodal metastasis, advanced TNM stage, and poor prognosis in OSCC [3].

A key consequence of hypoxia-driven adaptation is metabolic reprogramming, wherein tumour cells preferentially rely on aerobic glycolysis for energy production, leading to increased lactate accumulation within the tumour microenvironment [4-5]. Efficient regulation of intracellular pH and lactate transport is essential for tumour cell survival under these conditions. Monocarboxylate transporters (MCTs), particularly monocarboxylate transporter-1 (MCT-1), facilitate lactate efflux across the cell membrane and contribute to the maintenance of an acidic extracellular environment [5-6]. Overexpression of MCT-1 has been implicated in enhanced tumour proliferation, invasion, and metastatic potential in various malignancies, including head and neck squamous cell carcinomas [6].

Emerging evidence suggests a close functional relationship between hypoxia-regulated signalling pathways and metabolic transport mechanisms within the tumour microenvironment. HIF-1α has been shown to influence the expression of metabolic enzymes and transporters, thereby reinforcing a hypoxia-adapted and aggressive tumour phenotype [7]. While several studies have evaluated the individual expression of HIF-1α or MCT-1 in OSCC, limited data are available on their combined expression and clinicopathological relevance, particularly concerning metastatic and recurrent disease patterns [3, 6-7].

Therefore, the present study aimed to evaluate the immunohistochemical (IHC) expression of HIF-1α and MCT-1 in OSCC and to correlate their expression with clinicopathological parameters, including tumour site, histopathological grading, and metastatic status. Understanding the coordinated expression of these markers may provide insights into tumour metabolism-driven progression and identify potential prognostic indicators and therapeutic targets in OSCC.

Materials and Method

A retrospective institutional observational study was conducted on histopathologically confirmed cases of OSCC reported between January 2019 and December 2021. Clinical staging and patient information were retrieved from the archives of our dental college. The study received approval from the institutional ethical committee (VMSDC/IEC/185). A total of 60 histopathologically confirmed cases of OSCC were selected from the archives. For each case, two representative areas were collected: one from the center of the tumour, which is the central part of the tumour mass, and one from the invasive front of the tumour, which is the advancing edge adjacent to connective tissue. Among these, 25 cases were non-metastatic, 25 were metastatic, and 10 were recurrent cases of OSCC. The study included OSCC with regional lymph node metastasis and primary tumours located in any area of the oral cavity. Other variants of OSCC, as well as specimens obtained from patients who had undergone radiotherapy and chemotherapy, were excluded from this study.

From the formalin-fixed paraffin-embedded blocks, sections were stained for hematoxylin and eosin (H&E) stain and IHC analysis for all 60 cases by two observers. Broder's grading was done for H&E-stained sections [8]. For IHC, the sections were evaluated for HIF-1α and MCT-1 expression. Klein's scoring criteria [9] was used to score the IHC positive cells at 40× magnification; the percentage of all the positively stained cells in the respective section was seen. Score 1- <30% of positive cells, score 2- 30-60% of positive cells, and score 3- >60% of positive cells. Intensity of stains was scored 1- weak, 2- mild, 3- severe. The final score was graded as follows: 1-2 as mild expression, 2-4 as moderate expression, and 4-6 as severe expression.

The data obtained was subjected to statistical analysis, SPSS version 20.0, using the Chi-Square test, ANOVA for comparison between study groups, and the Pearson correlation coefficient test for correlation between the IHC-stained markers. A p Value of <0.05 was considered statistically significant.

Results

Immunoexpression of HIF-1α was detected in both the

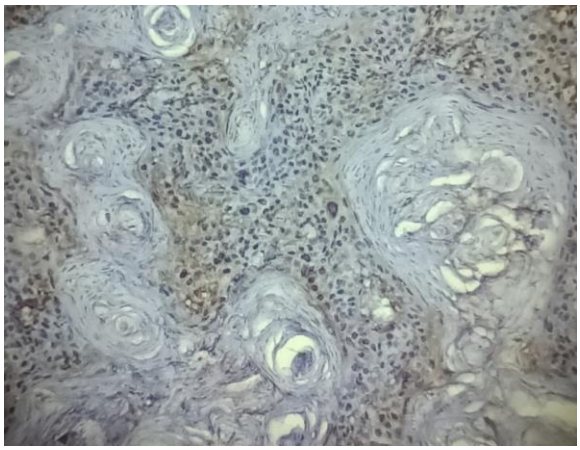

Figure 1: Immunohistochemical expression of hypoxia-inducible factor-1α (HIF-1α) in oral squamous cell carcinoma (OSCC)

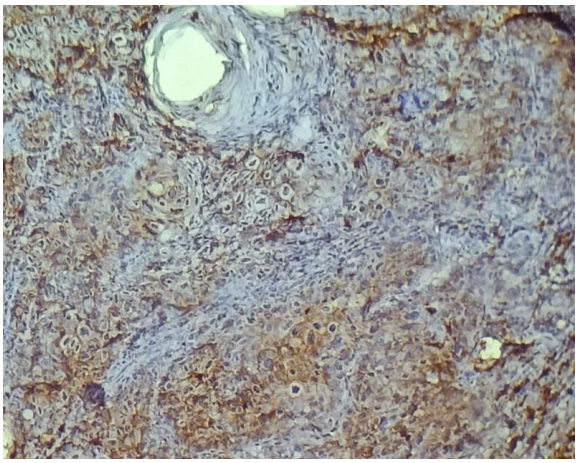

Figure 2: Immunohistochemical expression of monocarboxylate transporter-1 (MCT-1) in oral squamous cell carcinoma (OSCC)

nucleus and cytoplasm of tumour cells (Figure 1). In non-metastatic OSCC cases, score 1 expression was present in 64% of tumour cells, score 2 in 16%, and score 3 in 20%, with staining intensity predominantly mild (92%) and moderate in 8% of cases. In metastatic OSCC, score 1 expression was observed in 56% of tumour cells, score 2 in 44%, and score 3 in 28%, with staining intensity being mild in 52%, moderate in 32%, and severe in 16% of cases. In recurrent OSCC, score 1 expression was noted in 60% and score 2 in 40% of tumour cells, with mild staining intensity in 70% of cases and absence of staining in 30% of cases. The combined immunoreactivity score indicated that 56% of OSCC cases exhibited moderate HIF-1α expression. Metastatic OSCC cases demonstrated significantly higher HIF-1α expression compared to non-metastatic and recurrent OSCC cases, with this difference being statistically significant (p= 0.001).

The immunoexpression of MCT-1 was predominantly localised along the cell membrane of tumour cells (Figure 2). In non-metastatic OSCC, score 1 expression was observed in 56% of tumour cells, score 2 in 28%, and score 3 in 16%, with staining intensity classified as mild in 48%, moderate in 36%, and severe in 16% of cases. In contrast, metastatic OSCC exhibited a predominance of score 3 expression, observed in 84% of tumour cells, while score 1 and score 2 expressions were noted in 12% and 4% of cases, respectively. The staining intensity in metastatic OSCC was mild in 28%, moderate in 32%, and severe in 40% of cases. In recurrent OSCC, score 1 expression was present in 60% of tumour cells, score 2 in 30%, and score 3 in 10%, with staining intensity being mild in 80%, moderate in 10%, and severe in 10% of cases. Overall, 42% of OSCC cases exhibited severe MCT-1 expression. Notably, metastatic OSCC demonstrated significantly higher MCT-1 expression compared to non-metastatic and recurrent OSCC cases, with this difference being statistically significant (p= 0.028).

The clinicopathological findings of OSCC cases are detailed in Table 1. Of the 60 cases analysed, 53% were male, and 47% were female. A statistically significant correlation was identified between HIF-1α expression and age, with elevated expression levels observed in patients over 45 years of age (p= 0.048). Furthermore, a significant association was noted between HIF-1α expression and tumour site, with increased expression in tumours located in the buccal mucosa (38%), followed by the alveolar mucosa (32%) (p= 0.033). Additionally, HIF-1α expression was significantly correlated with advanced TNM stage, with higher expression levels in Stage III tumours (p= 0.028). No statistically significant association was found between MCT-1 expression and clinical parameters.

Histopathological evaluation indicated that 45% of OSCC cases were moderately differentiated squamous cell carcinoma, followed by well-differentiated (43%) and poorly differentiated (12%) carcinomas. Analysis of tumour regions revealed that HIF-1α expression was significantly higher in the tumour center compared to the invasive front (p= 0.022). Comparative analysis among non-metastatic, metastatic, and recurrent OSCC demonstrated that metastatic OSCC exhibited higher expression levels of both HIF-1α and MCT-1 (p= 0.001

Table 1: Clinicopathological findings in correlation with hypoxia-inducible factor-1α (HIF-1α) and monocarboxylate transporter-1 (MCT-1) expression in oral squamous cell carcinoma (OSCC)

Clinical & Histopathological Parameters	Total (N)	%	HIF-1α Chi-square	*p* Value	MCT-1 Chi-square	*p* Value
Age						
< 45 years	28	23	3.921	0.048*	0.515	0.473
> 45 years	92	77				
Gender						
Male	64	53	1.491	0.222	1.480	0.224
Female	56	47				
Location						
Tongue	26	22	12.118	0.033*	4.734	0.449
Floor of mouth	4	3				
Buccal mucosa	46	38				
Alveolus	38	32				
Lip	2	2				
Palate	4	3				
Histopathological grading						
Well differentiated	52	43	0.972	0.615	2.667	0.264
Moderately differentiated	54	45				
Poorly differentiated	14	12				
Tumour region						
Center	60	50	5.217	0.022*	2.157	0.142
Periphery	60	50				
TNM staging						
Stage I	22	18	7.160	0.028*	6.333	0.096
Stage II	28	23				
Stage III	40	33				
Stage IV	30	25				
Tumour status						
Non-metastatic	50	42	15.318	0.001**	7.160	0.028*
Metastatic	50	42				
Recurrent	20	17				

Footnote: Chi-square test was used to assess the association between HIF-1α and MCT-1 expression and clinicopathological parameters. Comparisons were made between marker-positive and marker-negative cases within each clinicopathological category.
* $p < 0.05$ – statistically significant
** $p < 0.01$ – highly significant
Abbreviations: Oral squamous cell carcinoma (OSCC); hypoxia-inducible factor-1α (HIF-1α); monocarboxylate transporter-1 (MCT-1); tumour necrosis metastasis (TNM)

and p= 0.028, respectively).

Two observers independently performed IHC evaluation, and good inter-observer agreement was observed. Pearson's correlation analysis revealed a positive correlation between HIF-1α and MCT-1 expression in non-metastatic OSCC cases (p= 0.006).

Discussion

OSCC exhibits an altered metabolism when compared to the normal host tissue. Apart from genetic and epigenetic changes in the tumour, metabolic adaptation of tumour cells within the microenvironment contributes to tumour proliferation and invasion [10]. In tumour cells, aerobic glycolysis takes place even in the presence of sufficient oxygen levels, which leads to increased lactic acid concentration around the tumour cells, which in turn activates HIF-1α [11]. In normal host tissue, MCT-1 found in the blood-brain barrier, red blood cells and skeletal muscles, is responsible for regulating substrate concentration and pH [10].

In the current study sample, 57% of the cases were male, while 47% were female. Similar findings have been documented in prior research, where OSCC (OSCC) was observed to be more prevalent among males than females within their respective cohorts [12-13]. Regarding age distribution, OSCC was more frequently identified in individuals over 45 years of age in the present cohort, aligning with the observations reported by Youssef *et al*. [14]. In this study, the buccal mucosa emerged as the most commonly affected site, followed by the alveolar mucosa and tongue. Conversely, previous studies have identified the tongue, buccal mucosa, and floor of the mouth as prevalent sites of involvement [7, 12-14]. A significant proportion of cases in the present series were classified as advanced TNM stage (Stage III), consistent with findings reported by Costa *et al*. [16] and Lin *et al*. [17] Histopathological analysis revealed that the majority of cases were moderately differentiated squamous cell carcinomas, followed by well-differentiated and poorly differentiated tumours, mirroring observations reported by Yasin *et al*. [15].

IHC analysis revealed that HIF-1α expression was more pronounced in the central region of the tumour compared to the periphery. Additionally, moderate expression of HIF-1α was noted, with an increased expression observed in metastatic OSCC. Sumera *et al.* [18] observed that moderate to marked expression of HIF-1α in OSCC was noted, with no association with Broder's grading system and TNM staging. Bharti *et al.* [19] found a marked association between HIF-1α expression and the tumour and nodal stages of OSCC. According to Patel *et al*. [20], there is a notable progressive increase in the expression of HIF-1α as oral epithelial dysplasia advances to OSCC, which is expressed with higher TNM stages MCT-1 expression did not demonstrate any correlation with clinical findings. However, MCT-1 was expressed in metastatic OSCC. Dang *et al.* [21] investigated that MCT-1 inhibition impairs cell proliferation,

suggesting its role in glycogen-dependent proliferation, and it shows increased levels in metastatic tumour tissue. Leu *et al.* [22] investigated the protein expression of MCT-1 and MCT 4 in head and neck cancers and reported that MCT-1-positive cases showed worsened progression-free survival with a hazard ratio of 3.1 and proposed that MCT-1 was a promising biomarker in head and neck squamous cell carcinoma compared to MCT 4.

Alterations in cell adhesion and the epithelial- mesenchymal transition significantly contribute to tumour invasion and metastasis in OSCC. Chandolia *et al*. [23] reported that elevated N-cadherin expression in OSCC is correlated with aggressive tumour behaviour and a poorer prognosis. Lymphangiogenesis and angiogenesis are critical components of the OSCC tumour microenvironment. Agarwal *et al*. [24] demonstrated that lymphatic and vascular markers, such as D2-40 and CD34, play a role in tumour progression and metastatic potential in OSCC.

In this study, HIF-1α demonstrated a positive correlation with MCT-1, exhibiting significant expression. Rademakers *et al*. [10] examined the staining pattern and co-expression of HIF-1α with MCT-1 and 4, GLUT-1, CAIX, and LDH-5, finding that HIF-1α exhibited strong co-expression with the monocarboxylate transporter protein. Yamaguchi *et al*. [25] investigated cell lines derived from lung adenocarcinoma and assessed the energy metabolism of MCT-1 and MCT4 in normoxia and hypoxia. HIF-1α knockdown leads to decreased levels of MCT4 under hypoxia, and hypothesized that MCT4 can be a potential cancer target. Khammanivong *et al.* [26] reported that OSCC cases with elevated MCT-1 and MCT-4 levels showed a poor prognosis.

This is in accordance with the hypothesis that hypoxia leads to increased levels of lactate mediated by MCT, which in turn activates HIF-1α, promoting angiogenesis, cancer stem cell formation, progression, and consequently, controls the behaviour of OSCC. Still, a greater number of OSCC cases with different hypoxia and lactate transporter-related markers is needed to rule out the exact behaviour of the OSCC.

Conclusion

Based on the above literature and the results from the current study, HIF-1α plays an important role in metabolic and functional changes in cancer cells. Acidic microenvironment in a reduced oxygen state worsens the prognosis of OSCC by suppressing the immunity, which leads to therapeutic resistance of tumour cells. Also, a positive correlation between HIF-1α and MCT-1 denotes that they may represent a potential therapeutic target for OSCC treatment modality.

Acknowledgement

We sincerely acknowledge the Research Committee of our institution for providing internal funding support for this study.

Conflicts of Interest

Nil

References

[1] Bugshan A, Farooq I. Oral squamous cell carcinoma: Metastasis, potentially associated malignant disorders, aetiology, and recent advancements in diagnosis. F1000Res. 2020; 9: 229.

[2] Majumdar B, Patil S, Sarode SC, Sarode GS, Rao RS. Clinico-pathological prognosticators in oral squamous cell carcinoma: an update. Transl Res Oral Oncol. 2017; 2: 1–10.

[3] Zhou J, Huang S, Wang L, Yuan X, Dong Q, Zhang D, *et al.* Clinical and prognostic significance of HIF-1α overexpression in oral squamous cell carcinoma: a meta-analysis. World J Surg Oncol. 2017; 15: 104.

[4] Brizel DM, Schroeder T, Scher RL, Walenta S, Clough RW, Dewhirst MW, *et al*. Elevated tumour lactate concentrations predict an increased risk of metastases in head-and-neck cancer. Int J Radiat Oncol Biol Phys. 2001; 51: 349–353.

[5] Walenta S, Schroeder T, Mueller-Klieser W. Metabolic mapping with bioluminescence: basic and clinical relevance. Biomol Eng. 2002; 18: 249–262.

[6] Simoes-Sousa S, Granja S, Pinheiro C, Fernandes D, Longatto-Filho A, Laus AC, *et al*. Prognostic significance of monocarboxylate transporter expression in oral cavity tumours. Cell Cycle. 2016; 15: 1865–1873.

[7] van Kuijk SJ, Yaromina A, Houben R, Niemans R, Lambin P, Dubois LJ. Prognostic Significance of Carbonic Anhydrase IX Expression in Cancer Patients: A Meta-Analysis. Front Oncol. 2016; 6: 69.

[8] Bhargava A, Saigal S, Chalishazar M. Histopathological

grading systems in oral squamous cell carcinoma: A review. J Int Oral Health. 2010; 2: 1-10.

[9] 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.

[10] Rademakers SE, Lok J, van der Kogel AJ, Bussink J, Kaanders JH. Metabolic markers in relation to hypoxia: staining patterns and colocalization of pimonidazole, HIF-1α, CAIX, LDH-5, GLUT-1, MCT-1 and MCT4. BMC Cancer. 2011; 11: 167.

[11] Sonveaux P, Vegran F, Schroeder T, Wergin MC, Verrax J, Rabbani ZN, *et al*. Targeting lactate-fueled respiration selectively kills hypoxic tumour cells in mice. J Clin Invest. 2008; 118: 3930–3942.

[12] Ribeiro M, Teixeira SR, Azevedo MN, Fraga AC Jr, Gontijo AP, Vêncio EF. Expression of hypoxia-induced factor-1 alpha in early-stage and metastatic oral squamous cell carcinoma. Tumour Biol. 2017; 39: 1010428317705740.

[13] Rahadiani N, Habiburrahman M, Handjari DR, Stephanie M, Krisnuhoni E. Clinicopathological characteristics predicting advanced stage and surgical margin invasion of oral squamous cell carcinoma: a single-center study on 10 years of cancer registry data. Oncol Lett. 2022; 24: 364.

[14] Youssef H, Aboazma N, Megahed E. Correlation of hypoxia-inducible factor-1 alpha (HIF-1α) and vascular endothelial growth factor (VEGF) expressions with clinicopathological features of oral squamous cell carcinoma (OSCC). Tanta Dent J. 2015; 12(Suppl 1): S1–S14.

[15] Yasin MM, Abbas Z, Hafeez A. Correlation of histopathological patterns of OSCC patients with tumour site and habits. BMC Oral Health. 2022; 22: 305.

[16] Costa AL, Araújo Júnior RF, Ramos CC. Correlation between TNM classification and malignancy histological feature of oral squamous cell carcinoma. Braz J Otorhinolaryngol. 2005; 71: 181–187.

[17] Lin NC, Hsu JT, Tsai KY. Survival and clinicopathological characteristics of different histological grades of oral cavity squamous cell carcinoma: a single-center retrospective study. PLoS One. 2020; 15: e0238103.

[18] 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: 451.

[19] Bharti A, Urs AB, Kumar P. Significance of HIF-1α expression and LOXL-2 localization in progression of oral squamous cell carcinoma. Asian Pac J Cancer Prev. 2021; 22: 341–347.

[20] 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: 435–441.

[21] Dang CV, Le A, Gao P. MYC-induced cancer cell energy metabolism and therapeutic opportunities. Clin Cancer Res. 2009; 15: 6479–6483.

[22] Leu M, Kitz J, Pilavakis Y, Hakroush S, Wolff HA, Canis M, *et al*. Monocarboxylate transporter-1 (MCT-1) protein expression in head and neck cancer affects clinical outcome. Sci Rep. 2021; 11: 4578.

[23] Chandolia B, Rajliwal JP, Bajpai M, Arora M. Prognostic Potential of N-Cadherin in Oral Squamous Cell Carcinoma via Immunohistochemical Methods. J Coll Physicians Surg Pak. 2017; 27: 475-478.

[24] Agarwal D, Pardhe N, Bajpai M, Gupta S, Mathur N, Vanaki SS, et al. Characterization, Localization and Patterning of Lymphatics and Blood Vessels in Oral Squamous Cell Carcinoma: A Comparative Study Using D2-40 and CD-34 IHC Marker. J Clin Diagn Res. 2014; 8: ZC86-ZC89.

[25] Yamaguchi A, Mukai Y, Sakuma T, Narumi K, Furugen A, Yamada Y, *et al*. Monocarboxylate transporter 4 involvement in energy metabolism and drug sensitivity in hypoxia. Sci Rep. 2023; 13: 1501.

[26] Khammanivong A, Saha J, Spartz AK, Sorenson BS, Bush AG, Korpela DM, *et al*. A novel MCT-1 and MCT4 dual inhibitor reduces mitochondrial metabolism and inhibits tumour growth of feline oral squamous cell carcinoma. Vet Comp Oncol. 2020; 18: 324–341.