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Role Of Glut - 1 In Oral Squamous Cell Carcinoma With Histopathological Correlation

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ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity and is associated with significant morbidity and mortality. Despite advances in treatment, prognosis remains poor due to late diagnosis and aggressive tumor behavior. Altered glucose metabolism is a hallmark of cancer, and Glucose Transporter-1 (GLUT-1) plays a crucial role in facilitating increased glucose uptake in malignant cells.

OBJECTIVES: To assess the histopathological features of oral squamous cell carcinoma and to evaluate the immunohistochemical expression of GLUT-1 in OSCC compared with non-neoplastic oral epithelium. Additionally, the study aimed to correlate GLUT-1 expression with clinicopathological prognostic parameters including tumor size, histological grade, TNM stage, lymphovascular invasion, and perineural invasion.

Materials and methods: This prospective comparative study was conducted on 32 surgically resected OSCC specimens and 32 non-neoplastic oral squamous epithelium specimens received for routine histopathological examination. All tissues were processed and stained with hematoxylin and eosin for histopathological evaluation. Tumors were graded and staged according to the AJCC (8th edition, 2017) staging system. Immunohistochemical staining for GLUT-1 was performed on paraffin-embedded sections. GLUT-1 expression was assessed based on membranous staining, percentage of positive cells, and staining intensity. Statistical analysis was performed using SPSS version 22, with a p value <0.05 considered statistically significant.

RESULTS: All OSCC cases (100%) showed membranous GLUT-1 positivity. Strong GLUT-1 expression (3+) was observed in 62.5% of OSCC cases, while moderate expression was seen in 31.3% and low expression in 6.3%. None of the non-neoplastic cases showed strong GLUT-1 expression. Increased GLUT-1 expression was predominantly observed in tumors with advanced TNM stage (Stage III and IV), larger tumor size, and in cases with lymphovascular and perineural invasion. A highly significant difference in GLUT-1 expression was noted between OSCC and non-neoplastic tissues ($p < 0.001$).

Conclusion: GLUT-1 expression was significantly increased in OSCC compared to non-neoplastic oral epithelium and showed a strong association with adverse histopathological prognostic factors. GLUT-1 may serve as a useful prognostic biomarker for identifying aggressive OSCC and high-risk patients.

Keywords: *GLUT-1 expression; Immunohistochemistry; Oral squamous cell carcinoma; Prognostic biomarker; Tumor aggressiveness*

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1. INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common malignant neoplasm of the oral cavity and constitutes a major public health problem, particularly in developing countries where tobacco chewing, smoking, alcohol consumption, and betel nut use are highly prevalent. Despite advances in diagnostic and therapeutic strategies, the overall five-year survival rate of OSCC has remained relatively stagnant over the past several decades. This poor prognosis is largely attributed to late diagnosis, aggressive local invasion, early lymph node metastasis, and resistance to conventional treatment modalities

such as radiotherapy and chemotherapy [1]. Therefore, identification of reliable molecular biomarkers that reflect tumor biology and correlate with histopathological features is essential for improved prognostication and treatment planning.

Cancer cells exhibit profound metabolic alterations to sustain rapid proliferation and survival in a hostile tumor microenvironment. One of the hallmark metabolic changes in malignancy is increased glucose uptake and utilization through aerobic glycolysis, commonly known as the Warburg effect [2]. This metabolic reprogramming enables tumor cells to generate energy and biosynthetic intermediates even under hypoxic conditions. Glucose transporters (GLUTs), a family of facilitative transmembrane proteins, play a crucial role in regulating glucose entry into cells. Among these, glucose transporter-1 (GLUT-1) is the most widely studied and is known to be overexpressed in many solid tumors [3].

GLUT-1 expression is normally limited to tissues with high glucose demand, such as erythrocytes, endothelial cells of the blood–brain barrier, and placental tissue. However, in malignant conditions, GLUT-1 is frequently upregulated to meet the increased metabolic requirements of rapidly proliferating tumor cells [4]. Hypoxia, a common feature of solid tumors including OSCC, is a key stimulus for GLUT-1 overexpression. Hypoxia-inducible factor-1 alpha (HIF-1α) activates transcription of GLUT-1, thereby enhancing glucose uptake and promoting tumor cell survival under low oxygen tension [5]. This adaptive response contributes not only to tumor growth but also to treatment resistance and aggressive behavior. In OSCC, increased GLUT-1 expression has been reported to correlate with tumor progression, depth of invasion, nodal metastasis, and poor clinical outcome [6]. Immunohistochemical evaluation of GLUT-1 allows visualization of its expression pattern within tumor tissues, providing valuable insights into metabolic heterogeneity and tumor aggressiveness. Studies have demonstrated that well-differentiated OSCCs tend to show lower or peripheral GLUT-1 expression, whereas moderately and poorly differentiated tumors exhibit strong and diffuse membranous and cytoplasmic positivity [7]. This suggests a close association between GLUT-1 expression and histopathological grading of OSCC.

Histopathological grading remains a cornerstone in the assessment of OSCC, as it reflects the degree of tumor differentiation and predicts biological behavior. However, conventional histological parameters alone may not fully capture the molecular alterations driving tumor progression. Incorporation of metabolic markers such as GLUT-1 into routine histopathological evaluation may enhance prognostic accuracy [8]. Furthermore, the association of GLUT-1 expression with hypoxia and angiogenesis highlights its potential role in tumor invasion and metastasis.From a therapeutic perspective, GLUT-1 has emerged as a promising target for cancer treatment. Inhibition of glucose transport may selectively impair tumor metabolism while sparing normal tissues with lower glucose dependency [9]. In OSCC, targeting GLUT-1 could potentially enhance the efficacy of existing treatment modalities and overcome hypoxia-induced therapy resistance. Additionally, GLUT-1 expression may serve as a predictive biomarker for response to radiotherapy and chemotherapy.

OSCC is a metabolically active malignancy characterized by altered glucose metabolism and hypoxic adaptation. GLUT-1 plays a pivotal role in facilitating increased glucose uptake in tumor cells and is closely associated with histopathological grade, tumor aggressiveness, and prognosis. Evaluating the role of GLUT-1 in OSCC with histopathological correlation can provide deeper insights into tumor biology, aid in risk stratification, and open new avenues for targeted therapy. Hence, the present study aims to assess GLUT-1 expression in OSCC and correlate it with histopathological parameters to establish its significance as a diagnostic and prognostic biomarker [10].

2. METHODOLOGY

a. Study Design

The present study was designed as a **prospective comparative study** aimed at evaluating the expression of Glucose Transporter-1 (GLUT-1) in oral squamous cell carcinoma (OSCC) and correlating it with histopathological parameters. The study compared GLUT-1 expression in malignant oral epithelial tissues with that in non-neoplastic oral mucosal tissues serving as controls. A standardized histopathological and immunohistochemical approach was adopted to ensure uniformity and reproducibility of observations.

b. Study Setting

The study was conducted in the **Department of Pathology** of a tertiary care teaching hospital. Radical resection specimens of OSCC received from the Department of surgical oncology were included. All specimens were received in **10% neutral buffered formalin** and processed in the histopathology laboratory following standard operating procedures.

Study Duration

The study was carried out over a period of **18 months**, which included specimen collection, histopathological evaluation, immunohistochemical processing, interpretation of slides, and statistical analysis of the data.

Participants

The study population comprised patients diagnosed with oral squamous cell carcinoma who underwent surgical resection during the study period, along with non-neoplastic oral tissue specimens used as controls.

Inclusion Criteria (Cases):

- Histopathologically confirmed cases of oral squamous cell carcinoma
- Patients who underwent radical surgical resection
- Adequately preserved specimens received in 10% formalin
- Availability of complete clinicopathological data

Inclusion Criteria (Controls):

- Non-neoplastic oral mucosal tissues obtained from benign oral lesions or margins free of tumor

Exclusion Criteria:

- Incisional biopsies or inadequately fixed specimens
- Recurrent or previously treated cases of OSCC
- Specimens with extensive necrosis or autolysis
- Patients who received preoperative chemotherapy or radiotherapy

Study Sampling

A **consecutive sampling method** was employed. All eligible OSCC cases received during the study period that satisfied the inclusion criteria were included until the required sample size was achieved. Control tissues were selected from non-neoplastic cases processed during the same period.

Study Sample Size

The total sample size consisted of **64 cases**, which included:

- **32 cases of oral squamous cell carcinoma**
- **32 non-neoplastic control cases**

The sample size was selected based on feasibility and availability of cases during the study duration.

Study Groups

The study population was divided into the following groups:

- **Group I (Cases):** Histopathologically confirmed OSCC specimens
- **Group II (Controls):** Non-neoplastic oral mucosal tissues

Within the OSCC group, further subgrouping was performed based on histopathological grade and tumor stage for correlation analysis.

Study Parameters

The following parameters were evaluated:

- Patient demographic details
- Tumor site and size
- Histopathological type and grade of OSCC
- Lymphovascular invasion
- Lymph node metastasis
- Tumor staging according to AJCC (8th edition, 2017)
- GLUT-1 expression pattern and intensity on immunohistochemistry

c. Study Procedure

All surgical specimens were received in 10% formalin and subjected to **standard gross examination**. Detailed grossing was performed according to established protocols. Multiple tissue sections were taken from the tumor proper, surgical margins, and all identified lymph nodes. The tissues were processed routinely, embedded in paraffin wax, and sectioned at 4–5 µm thickness. The sections were stained with **hematoxylin and eosin (H&E)** and examined under light microscopy to assess histopathological features.Tumors were graded based on the degree of differentiation and staged according to the **American Joint Committee on Cancer (AJCC) 8th edition staging system (2017)**.

d. Study Data Collection

Histopathological findings were recorded using a structured proforma. Data regarding tumor grade, lymphovascular invasion, perineural invasion, lymph node status, and pathological stage were documented. Immunohistochemical findings for GLUT-1 expression were recorded separately and later correlated with histopathological parameters.

e. Processing for Immunohistochemistry and Data Analysis

Immunohistochemical detection of GLUT-1 was performed on **4 µm thick paraffin sections** mounted on poly-L-lysine

coated slides. Antigen retrieval was carried out using citrate buffer in a microwave oven. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide. Sections were incubated with primary mouse monoclonal antibodies against GLUT-1, followed by rabbit anti-mouse secondary antibodies. Enzyme labeling was done using streptavidin–horseradish peroxidase, chromogen development with diaminobenzidine (DAB), and counterstaining with hematoxylin.

Positive and negative controls were run with each batch. GLUT-1 expression was assessed based on **membranous staining**. Random microscopic fields were selected, and **300 tumor cells were counted** per case. The percentage of positive cells was graded as:

- 0: <10%
- 1+: 10–25%
- 2+: 26–50%
- 3+: >50%

Staining intensity was graded as 0 (negative), 1+ (mild), 2+ (moderate), and 3+ (intense).
Data were entered into Microsoft Excel and analyzed using **SPSS version 22**. Categorical variables were expressed as frequencies and percentages. The **Chi-square test** or **Fisher's exact test** was used for qualitative data. A *p* value of <0.05 was considered statistically significant.

f. Ethical Considerations

Ethical clearance was obtained from the **Institutional Ethics Committee** prior to commencement of the study. Patient confidentiality was strictly maintained, and all specimens were analyzed solely for academic and research purposes. No additional intervention was performed on patients as part of this study.

3. RESULT

Table 1 shows the correlation between GLUT-1 expression and perineural invasion (PNI). In tumors with perineural invasion, no cases demonstrated low GLUT-1 expression (1+). The majority of cases (80.0%) showed strong GLUT-1 positivity (3+), while 20.0% exhibited moderate expression (2+). This indicates a strong association between intense GLUT-1 expression and perineural infiltration.In contrast, tumors without perineural invasion displayed a broader distribution of GLUT-1 scores. Strong expression (3+) was observed in 59.3% of cases, moderate expression (2+) in 33.3%, and low expression (1+) in 7.4%. The predominance of strong GLUT-1 expression in PNI-positive cases suggests that GLUT-1 overexpression may contribute to aggressive tumor behavior and neural spread, which is a known adverse prognostic factor in OSCC.

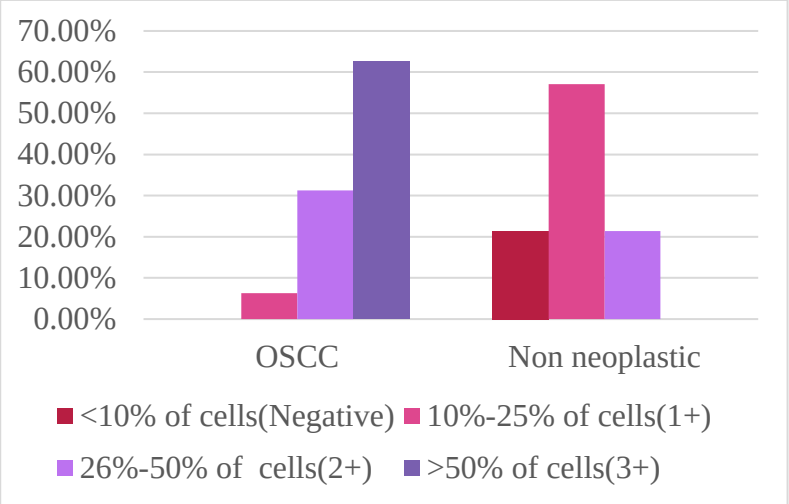
Table 1: - Distribution of subjects according to GLUT-1 SCORING and perineural invasion

	1+		2+		3+	
	N	%	N	%	N	%
Present	0	0.0%	1	20.0%	4	80.0%
Absent	2	7.4%	9	33.3%	16	59.3%

Table 2 compares GLUT-1 expression between OSCC and non-neoplastic oral tissues, highlighting a clear difference in expression patterns. In OSCC cases, none were negative for GLUT-1 expression. A majority of cases (62.5%) showed strong expression (3+), followed by 31.3% showing moderate expression (2+). Only a small proportion (6.3%) exhibited low expression (1+). This demonstrates a marked upregulation of GLUT-1 in malignant tissues.In contrast, non-neoplastic tissues predominantly showed low or absent GLUT-1 expression. Most cases (57.1%) exhibited low positivity (1+), while 21.4% were completely negative and another 21.4% showed moderate expression (2+). Importantly, none of the non-neoplastic cases demonstrated strong GLUT-1 expression (3+). This stark contrast underscores the role of GLUT-1 as a metabolic marker of malignancy, distinguishing neoplastic from normal oral epithelium.

Table 2:- Comparison of GLUT -1 SCORE in OSCC and Non-neoplastic cases

GLUT SCORE	OSCC		Non neoplastic	
	N	%	N	%
<10 % of cells(Negative)	0	0.0%	6	21.4%
10%-25% of cells (1+)	2	6.3%	16	57.1%
26%-50% of cells (2+)	10	31.3%	6	21.4%
>50% of cells (3+)	20	62.5%	0	0.0%



Graph 1:- Graph showing Comparison of GLUT -1 SCORE in OSCC and Non-neoplastic cases

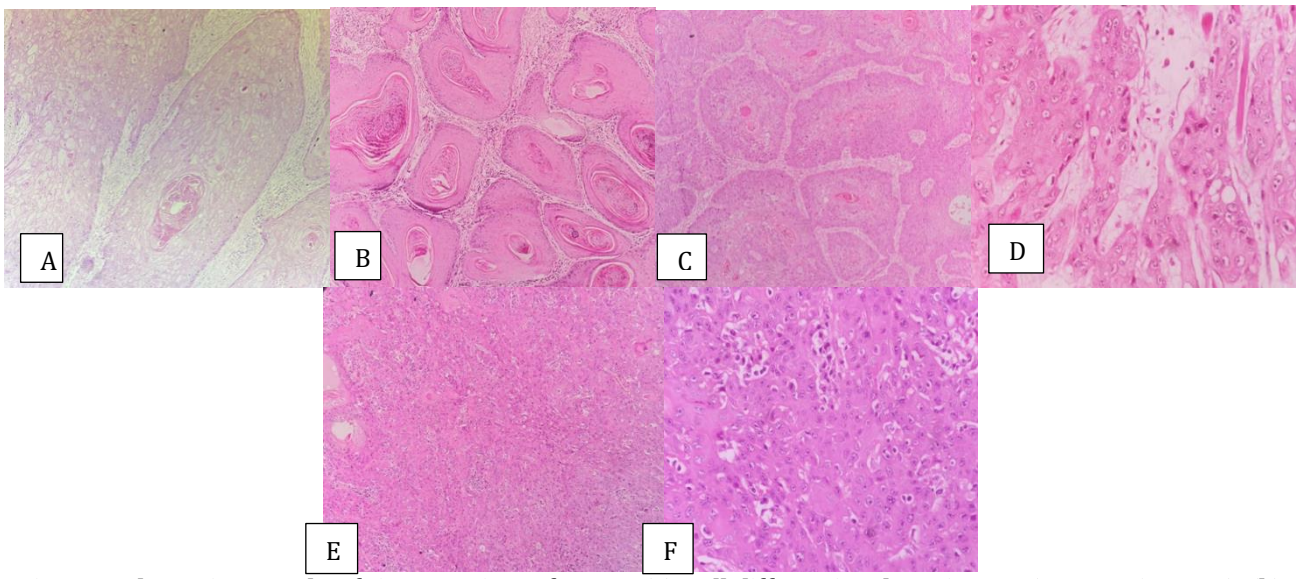


Figure1: Photomicrographs of tissue sections of OSCC (a)Well differentiated carcinoma, (H&E stain, x100). (b) Well differentiated carcinoma with numerous keratin pearls,(H&E stain, x400). (c)Moderately differentiated carcinoma,(H&E stain, x100). (d) Moderately differentiated carcinoma,(H&E stain, x400). (e) Poorly differentiated carcinoma,(H&E stain, x100). (f) Poorly differentiated carcinoma,(H&E stain, x400).

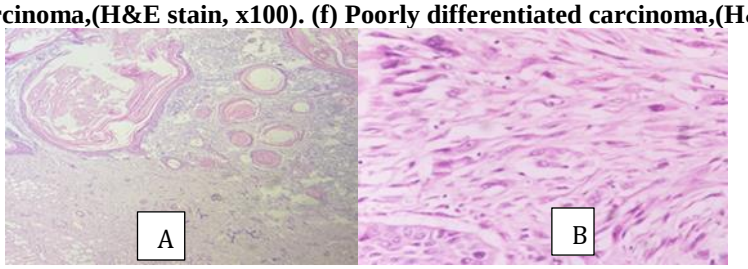


Figure 2:(a)Photomicrograph shows lymphnode metastasis with keratin pearls formation(H&E Stain,x100).(b)photomicrograph shows poorly differentiated spindle cell type OSCC(H&E Stain,x100).

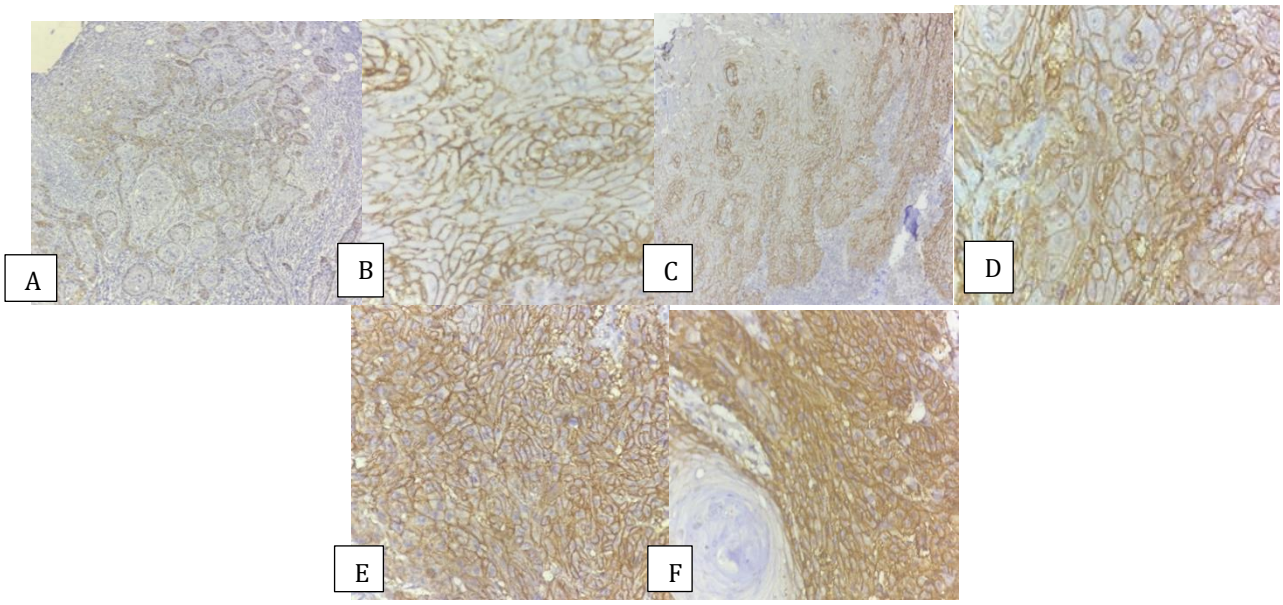


Figure 3: Photomicrographs shows immunoexpression of GLUT-1 in OSCC (a) Positive membrane staining of GLUT-1 with intensity 1+(IHC stain,x100). (b) Positive membrane staining of GLUT-1 with intensity 1+(IHC stain, x400). (c) Positive membrane staining of GLUT-1 with intensity 2+(IHC stain, x100). (d) Positive membrane staining of GLUT-1 with intensity 2+(IHC x400). (e) Positive membrane staining of GLUT-1 with intensity 3+(IHC stain, x100). (f) Positive membrane staining of GLUT-1 with intensity 3+(IHC stain, x400).

4. DISCUSSION

The present prospective comparative study evaluated GLUT-1 expression in oral squamous cell carcinoma (OSCC) and correlated it with histopathological parameters such as TNM stage, lymphovascular invasion, perineural invasion, and comparison with non-neoplastic oral tissues. The findings of this study demonstrated a consistent pattern of increasing GLUT-1 expression with advancing tumor aggressiveness, supporting the role of altered glucose metabolism in OSCC progression. In the current study, GLUT-1 expression was detected in all OSCC cases, with 62.5% showing strong positivity (3+), 31.3% moderate positivity (2+), and only 6.3% low positivity (1+), while none of the OSCC cases were negative. In contrast, non-neoplastic oral tissues predominantly exhibited absent or low GLUT-1 expression, with 21.4% negative, 57.1% showing low expression, and none demonstrating strong positivity. This clear difference between malignant and non-malignant tissues highlights GLUT-1 as a marker of malignant transformation and increased metabolic demand in OSCC, a finding that is in agreement with previously published literature.

The progressive increase in GLUT-1 expression with tumor stage observed in the present study further emphasizes its association with disease advancement. Early-stage tumors (TNM stage I and II) showed predominantly moderate expression, with 66.7% demonstrating a 2+ score and 33.3% showing 1+ expression, and notably no cases exhibiting strong positivity. In contrast, advanced-stage tumors (stage III and IV) showed a marked predominance of high GLUT-1 expression, with 69% of cases exhibiting strong positivity (3+), 27.5% moderate positivity, and only 3.4% low positivity. These findings strongly suggest that GLUT-1 overexpression parallels tumor progression and increasing metabolic requirements. Similar observations were reported by Harshani et al., [11] demonstrated significantly higher GLUT-1 expression in advanced clinical stages of OSCC, with a highly significant association between GLUT-1 expression and clinical staging ($p = 0.0004$). Their study, which included 30 OSCC cases, showed that higher immunohistochemical staining scores were associated with increased clinical stage, supporting the findings of the present study.

The association between GLUT-1 expression and histopathological aggressiveness was further reinforced by its correlation with lymphovascular invasion. In the present study, tumors with lymphovascular invasion demonstrated predominantly strong GLUT-1 expression in 77.8% of cases, with the remaining 22.2% showing moderate expression, and none showing low expression. In comparison, tumors without lymphovascular invasion still showed a predominance of strong expression (56.5%), but with a higher proportion of moderate (34.8%) and low (8.7%) expression. This trend suggests that higher GLUT-1 expression is more frequently associated with vascular invasion, indicating enhanced invasive and metastatic potential. These findings are biologically plausible, as increased glucose uptake facilitates tumor cell survival, migration, and invasion within hypoxic tumor microenvironments. Although Harshani et al. [11] primarily focused on clinical stage and histological grade, their observation of increasing GLUT-1 expression with aggressive disease indirectly supports the association observed in the present study.

Perineural invasion, a well-recognized adverse prognostic factor in OSCC, also showed a strong relationship with GLUT-1 expression in the present study. Among cases with perineural invasion, 80.0% demonstrated strong GLUT-1 positivity, while 20.0% showed moderate expression, and none exhibited low expression. In contrast, tumors without perineural invasion showed a relatively broader distribution, with 59.3% strong, 33.3% moderate, and 7.4% low expression. This predominance of strong GLUT-1 expression in perineural invasion–positive tumors suggests that enhanced metabolic activity may facilitate tumor spread along neural pathways, contributing to aggressive local behavior and poorer outcomes. Although direct evaluation of perineural invasion was not the primary focus of earlier studies, the overall association between high GLUT-1 expression and aggressive histopathological features aligns with the findings reported by Angadi et al., [12] observed increasing GLUT-1 expression with increasing histologic grade of OSCC ($p < 0.001$).

The results of the present study are also comparable with the observations of Ganvir et al., [13] evaluated GLUT-1 expression in relation to depth of invasion (DOI) in 90 OSCC patients across progressive histological grades. Their study demonstrated a highly significant correlation between GLUT-1 overexpression at the invasion front and increasing DOI, particularly in tumors with higher metastatic potential and recurrence risk. Although DOI was not directly assessed in the present study, the strong association of GLUT-1 expression with advanced TNM stage, lymphovascular invasion, and perineural invasion indirectly reflects increased invasive potential, thereby supporting the conclusions drawn by Ganvir et al. that GLUT-1 may serve as a marker of tumor aggressiveness and poor prognosis.

Further support for the findings of the present study comes from the work of Attur et al., [14] compared GLUT-1 expression in normal oral mucosa, potentially malignant disorders (PMDs), and OSCC. They demonstrated a progressive increase in GLUT-1 expression from normal tissues to PMDs and to increasing grades of OSCC, with statistically significant differences between groups. In their study, GLUT-1 expression was detected in all grades of OSCC, and higher expression was associated with poorer differentiation. The absence of strong GLUT-1 expression in non-neoplastic tissues and its predominance in OSCC cases in the present study closely mirrors their findings, reinforcing the role of GLUT-1 as a prognostic and predictive marker in oral carcinogenesis.

Ayala et al. [15] further highlighted the prognostic relevance of glucose transporters in OSCC by evaluating GLUT-1 and GLUT-3 expression in 142 cases. They reported GLUT-1 overexpression in 50.3% of OSCC cases with membranous staining and demonstrated that both GLUT-1 and GLUT-3 were indicators of poor prognosis due to enhanced glycolytic metabolism in aggressive tumors. While their study emphasized survival outcomes and metabolic aggressiveness, the present study complements these findings by demonstrating the association of GLUT-1 overexpression with adverse histopathological parameters, thereby reinforcing its prognostic significance.

The findings of the present study are in strong concordance with previous literature, collectively indicating that GLUT-1 expression increases with tumor stage, histopathological aggressiveness, and invasive behavior in OSCC. The consistent observation of strong GLUT-1 expression in advanced tumors and its relative absence in non-neoplastic tissues underscores its role as a metabolic marker of malignancy. Incorporation of GLUT-1 immunohistochemical assessment into routine histopathological evaluation may therefore enhance prognostic stratification and assist in identifying patients with aggressive disease who may benefit from closer surveillance and more intensive therapeutic strategies.

5. CONCLUSION

The present study emphasizes the significant role of glucose transporter-1 (GLUT-1) in the progression and biological behavior of oral squamous cell carcinoma (OSCC). Immunohistochemical analysis demonstrated 100% membranous GLUT-1 positivity in OSCC cases, with a majority showing strong expression and high staining intensity, whereas non-neoplastic oral tissues exhibited absent to low expression. This clear distinction highlights the role of altered glucose metabolism in oral carcinogenesis.A progressive increase in GLUT-1 expression was observed with advancing tumor aggressiveness. Higher expression was associated with larger tumor size, advanced TNM stage (Stage III and IV), poorer histological differentiation, and the presence of lymphovascular and perineural invasion. Although associations with lymphovascular and perineural invasion were not statistically significant, the consistent trend toward higher GLUT-1 expression in these adverse prognostic groups suggests biological relevance.Overall, the findings support GLUT-1 as a valuable metabolic and prognostic biomarker in OSCC. Incorporation of GLUT-1 immunohistochemistry into routine histopathological evaluation may aid in prognostic stratification and identification of high-risk patients. Further large-scale studies are required to validate its prognostic significance and therapeutic potential.

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