Original Research

Evaluation of glucose transporter-1 expression in oral epithelial dysplasia and oral squamous cell carcinoma: An immunohistochemical study

Preethi Patlolla, N D V N Shyam, G Kiran Kumar, Vaishali Narayen, Paremala Konda, Priyanka Mudududla

Department of Oral Pathology, Government Dental College and Hospital, Hyderabad, Telangana, India

Abstract

Introduction: Oral squamous cell carcinoma (OSCC) is the most common malignancy of oral cavity and is commonly preceded by oral potentially malignant disorders. Glucose transporter-1 (GLUT-1) protein expression is upregulated in malignant cells that show increased glucose uptake. Alterations in GLUT-1 expression have been reported in several potentially malignant and malignant lesions.

Aims and Objectives: The aims and objectives of this study were to analyze and assess the role of GLUT-1 immunomarker in oral epithelial dysplasia (OED) and OSCC, to demonstrate and analyze the presence, location and intensity of GLUT-1 immunomarker in low-risk and high-risk OEDs and in different grades of OSCC and to correlate the expression of GLUT-1 immunomarker between normal oral mucosa (NOM), OED and different grades of OSCC.

Materials and Methodology: A total of ninety paraffin-embedded tissue blocks, 15 each of NOM; low-risk and high-risk OED and well, moderately and poorly differentiated OSCC were stained with the immunomarker GLUT-1.

Results and Observation: GLUT-1 immunoexpression was statistically significant in terms of number of positive cells, staining intensity, IRS score and level of staining within the epithelium and also within the cell between NOM, OED and OSCC.

Conclusion: Increased GLUT-1 expression has a consistent role in the malignant transformation of OED and aggressiveness of OSCC.

Keywords: Glucose transporter 1, immunohistochemistry, oral epithelial dysplasia, oral leukoplakia, oral squamous cell carcinoma

Address for correspondence: Dr. Preethi Patlolla, Room No. 331, Department of Oral Pathology, Government Dental College and Hospital, Afzal Gunj, Hyderabad - 500 012, Telangana, India.
E-mail: drpreethirddy1989@gmail.com

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INTRODUCTION

Oral cancer is the most prevalent cancer and predominant cause of morbidity and mortality. Among all other cancers, it occupies sixth position worldwide and shows epidemiologic variations between different geographic regions. About 300,373 new cases and 145,353 cancer deaths have been reported in the world. In India, it ranks third position and constitutes >30% of all cancers

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reported. Tobacco use either as smokeless or in smoking form and alcohol consumption are frequently associated with oral cancer.[1]

Oral squamous cell carcinomas (OSCC) account for >90% of all oral cancers. It can arise either *de novo* or from oral potentially malignant disorders that include oral leukoplakia, erythroplakia, oral lichen planus and oral submucous fibrosis that histologically represent the oral epithelial dysplasia (OED).[2,3]

Most of the OSCC cases are diagnosed in advanced stages. This can be due to lack of awareness among patients as most of the cases are painless in initial stages. There is a need to develop newer immunohistochemical markers to identify OSCC cases in initial stages so that treatment can be initiated to improve the survival rate of this dreadful disease.[1-3]

Oral carcinogenesis is a complex process that causes alterations in various genes; these genetic alterations produce altered proteins. In recent years, there is overexpression of altered proteins related to cell metabolism that has a role in the development of OSCC and also in the progression of OED to OSCC.[4]

Glucose homeostasis within the body is predominantly maintained by the glucose transporter (GLUT) protein family comprising 14 isoforms. Increased expression of certain members of GLUT protein family has been reported in various cancers such as lung, pancreas, prostate and esophagus, suggesting that the tumor cells use glycolytic pathway for their long-term maintenance and can proliferate very rapidly even in low oxygen tension environment for their survival.[4,5]

GLUT-1 glucose transporter is a transmembrane glycoprotein that is involved in Na+-independent transport of glucose into cells. Studies revealed alterations in GLUT-1 expression in several potentially malignant and malignant lesions.[5] Thus, the present study is carried out to detect the alterations in the mechanisms of glucose transport by means of GLUT-1 immunoexpression and to evaluate these changes that occur in the OED and also in the malignant transformation of OED to OSCC.

MATERIALS AND METHODOLOGY

Sample selection

The present retrospective study was carried out in the Department of Oral and Maxillofacial Pathology, Government Dental College and Hospital, Hyderabad, after the approval from the institutional ethical committee affiliated to the Ethics Committee, Osmania Medical College, Hyderabad, with Regd. No. ECR/300/Inst/AP/2013/RR-16.

A total of ninety samples of formalin-fixed, paraffin-embedded tissue blocks were retrieved from the archives of the Department of Oral and Maxillofacial Pathology. Group I including 15 normal oral mucosa (NOM) samples that were considered controls were obtained from mucosa during disimpaction of third molars. The Group II (n = 30) category comprised 15 cases each of low-risk (Group IIa) and high-risk (Group IIb) OED. The Group III category included 45 cases of OSCC (n = 45), with 15 cases each of well-differentiated (WDOSCC), moderately differentiated (MDOSCC) and poorly differentiated OSCC (PDOSCC) (Group IIIa, IIIb and IIIc, respectively).

Serial sections of 3-μm thickness were taken from selected tissue blocks for the analysis of GLUT-1 immunomarker and placed onto silane-coated slides. Following deparaffinization by heating on a slide warmer for 1 h at 60°C and treatment with xylene, the sections were rehydrated in decreasing grades of isopropyl alcohol and brought to water. The tissue sections were kept in EZ-retriever system for antigen retrieval containing retrieval buffer and treated at 95°C for five cycles: 5 min for the first cycle and 3 min each for the remaining four cycles. The sections were then brought to room temperature and then rinsed in distilled water followed by washing in wash buffer. Further, the slides were treated with polyExcel hydrogen peroxide (10 min) to block endogenous peroxidase activity and were washed in wash buffer three times for 3 min each.

Then, the tissue sections were incubated with prediluted primary antibody against GLUT-1 (Rabbit Monoclonal, PathnSitu Biotechnologies, Pleasanton, California, USA.) at room temperature for 30 min and were washed with wash buffer. Later, the slides were treated with polyExcel target binder (10 min at room temperature) and then washed gently with wash buffer. The tissue sections were then incubated with secondary antibody, i.e., polyExcel polyhorseradish peroxidase, at room temperature for 10 min. The slides were then washed gently with wash buffer, and the tissue sections were completely covered with freshly prepared substrate chromogen solution (polyExcel Stunn DAB) at room temperature for 5 min. The slides were then washed gently with distilled water for 5 min. The sections were then placed in Harri's hematoxylin for 2 min and then washed gently under running tap water for bluing. Then, the tissue sections were dehydrated through

grades of isopropyl alcohol, i.e., 70%, 95% alcohol and absolute alcohol. The sections were cleared in xylene bath and mounted using DPX.

Assessment of GLUT-1-positive cells was performed using a compound binocular light microscope at ×10, ×20 and ×40 magnifications. For each case, five fields were selected randomly.

The percentage of positive cells was scored as follows:

- 0: no positive cells
- 1: 10% positive cells
- 2: 10%–50% positive cells
- 3: 51%–80% positive cells and
- 4: More than or equal to 80%.

The staining intensity was graded as follows: 1 – mild, 2 – moderate and 3 – intense.

The product of two scores (i.e., percentage of positive cells and staining intensity) gives the IRS value, which ranges from 0 to 12.[6]

Statistical analysis

The collected data were statistically analyzed using Statistical Package for Social Sciences version 20.0 (SPSS, Inc. Chicago, IL, USA). The observed data were analyzed by Pearson's Chi-square test, one-way analysis of variance and *post hoc* tests. Confidence intervals were set at 95%, and $P < 0.05$ was interpreted as statistically significant.

RESULTS

The mean percentage of GLUT-1-positive cells in NOM (Group I), low-risk OED (Group IIa), high-risk OED (Group IIb), WDOSCC (Group IIIa), MDOSCC (Group IIIb) and PDOSCC (Group IIIc) was 31.35, 44.84, 55.66, 67.81, 75.18 and 84.75, respectively [Figure 1 and Table 1].

The intensity of staining of GLUT-1 was calculated. In NOM, out of the 15 cases, 12 were mild and 3 were moderate. Out of the 15 cases of low-risk OEDs, 9 were mild, 5 were moderate and 1 was intensely stained. In high-risk OEDs, two were mild, seven were moderate and six were intense. Out of 15 WDOSCC, 10 were mild, 3 were moderate and 2 cases were intense; in MDOSCC, 2 were mild, 11 were moderate and 2 cases were intense and in PDOSCC, 1 was mild, 8 were moderate and 6 cases were intense.

IRS classification was compared among groups using Chi-square test. There was a statistically significant difference with *P* value (0.001) in IRS classification from NOM to low-risk OED to high-risk OED to WDOSCC to MDOSCC to PDOSCC [Table 2].

Location of GLUT-1 immunoexpression within the epithelium was compared in NOM and OED (low and high risk). Out of 15 cases of NOM, 13 cases showed expression in basal and suprabasal cells and 2 cases up to mid-spinous layer. In low-risk OED, five cases showed expression in basal and suprabasal layers, followed by nine cases showing expression till mid-spinous layers and in one case till superficial spinous layers. In high-risk OED, in majority of the cases (12 out of 15), there was GLUT-1 expression till superficial spinous layers, with two cases to basal and suprabasal and one case only up to mid-spinous

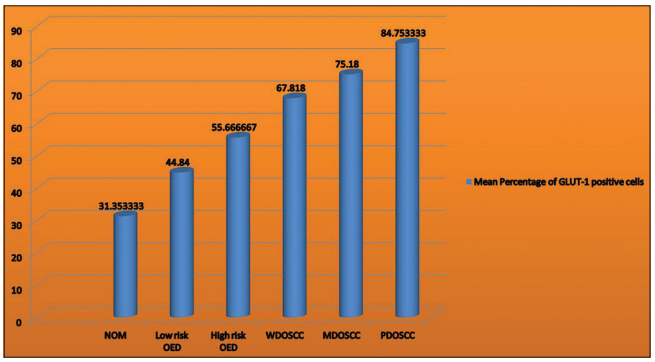

Figure 1: Percentage of glucose transporter-1-positive cells among Groups I, IIa, IIb, IIIa, IIIb and IIIc

Table 1: Percentage of glucose transporter-1-positive cells among Groups I, IIa, IIb, IIIa, IIIb and IIIc

Group	*n*	Mean	SD	SE	95% CI for mean		*F* statistic	*P*
					Lower bound	Upper bound		
NOM	15	31.353333	3.7425863	0.9663316	29.280758	33.425909	179.164	0.0001 (highly significant)
Low-risk OED	15	44.840000	4.2123628	1.0876274	42.507271	47.172729		
High-risk OED	15	55.666667	2.8452132	0.7346309	54.091040	57.242293		
WDOSCC	15	67.818000	7.5071111	1.9383278	63.660700	71.975300		
MDOSCC	15	75.180000	9.2603764	2.3910189	70.051774	80.308226		
PDOSCC	15	84.753333	4.0388942	1.0428380	82.516668	86.989998		
Total	90	59.935222	19.0735252	2.0105261	55.940350	63.930095		

One-way ANOVA test, $P<0.05$ significant. NOM: Normal oral mucosa, OED: Oral epithelial dysplasia, OSCC: Oral squamous cell carcinoma, WDOSCC: Well-differentiated OSCC, MDOSCC: Moderately differentiated OSCC, PDOSCC: Poorly differentiated OSCC, CI: Confidence interval, SD: Standard deviation, SE: Standard error

layers. A statistically significant correlation with "*P*" value of 0.001 of GLUT-1 immunoexpression within the epithelium from NOM to OED was observed [Figure 2].

The location of GLUT-1 immunoexpression within the invading islands of tumor cells was compared in different grades of OSCC. With increase in the grade of OSCC, the staining pattern changed from peripheral cells to combined peripheral and central cells in the islands with a statistically significant correlation with "*P*" = 0.001 [Table 3].

The location of GLUT-1 expression within the cell was also compared among all groups. There was a statistically significant difference with "*P*" value (0.001) in the location of GLUT-1 from membrane to combined membrane and cytoplasm from NOM to OED to WDOSCC to MDOSCC to PDOSCC [Figure 3].

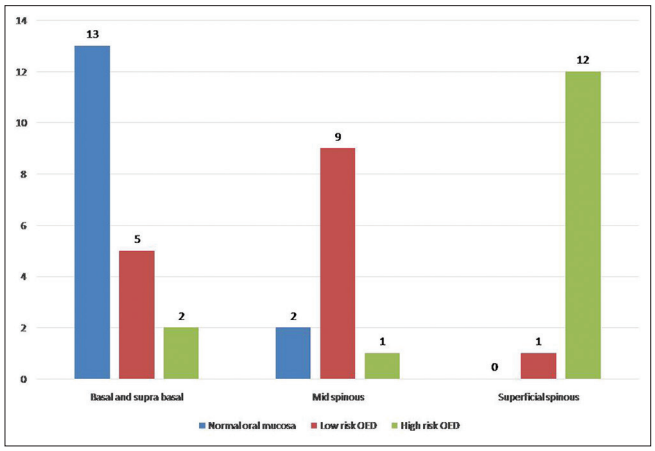

Figure 2: Location of glucose transporter-1 staining within the epithelium among Groups I, IIa and IIb

DISCUSSION

Most of the epithelial malignancies are characterized by multistep progression from OED to invasive OSCC. Most of the cancer cells reprogram their metabolism to promote growth, survival, proliferation and long-term maintenance. The general features of this altered metabolism are increased glucose uptake and fermentation of glucose to lactic acid even in the presence of oxygen and fully functioning mitochondria termed as Warburg effect or aerobic glycolysis which was initially described by a German scientist Otto Warburg (1920).[7]

In aerobic glycolysis, one molecule of glucose generates less amount of Adenosine triphosphate compared to that obtained by mitochondrial oxidative phosphorylation (OXPHOS). The glucose metabolic rate through aerobic glycolysis is higher so that the formation of lactate from glucose occurs 10–100 times quicker when compared to complete oxidation of glucose in mitochondria through OXPHOS pathway.[7]

An actively dividing cell (normal or transformed) needs to double its DNA content and also other components, including membranes, proteins and organelles. This requires increased uptake of nutrients, particularly glucose that produces the energy needed for the biosynthesis of these components and amino acids that provide the building blocks used for protein synthesis. Halting the breakdown of glucose at pyruvate or lactate allows these carbons to be shifted to anabolic pathways such as lipid and nucleotide production.[8]

GLUT-1 is also known as solute carrier family 2, facilitated GLUT member 1 (SLC2A1). It is a uniporter protein

Table 2: IRS classification among Groups I, IIa, IIb, IIIa, IIIb and IIIc

Group	IRS classification			Total	χ^2	*P*
	Positive weak	Positive mild	Positive strong			
NOM	12	3	0	15	44.009	0.0001 (highly significant)
Low-risk OED	9	6	0	15		
High-risk OED	2	7	6	15		
WDOSCC	10	3	2	15		
MDOSCC	2	11	2	15		
PDOSCC	0	9	6	15		
Total	35	39	16	90		

Chi-square test, $P<0.05$ significant. NOM: Normal oral mucosa, OED: Oral epithelial dysplasia, OSCC: Oral squamous cell carcinoma, WDOSCC: Well-differentiated OSCC, MDOSCC: Moderately differentiated OSCC, PDOSCC: Poorly differentiated OSCC, IRS (Immunoreactive Score)

Table 3: Association of location of glucose transporter-1 within cells of tumor island between Groups IIIa, IIIb and IIIc

Group	GLUT-1 location in tumor island		Total	χ^2	*P*
	Peripheral	Peripheral and central			
WDOSCC	14	1	15	28.889	0.001 (highly significant significant)
MDOSCC	4	11	15		
PDOSCC	0	15	15		
Total	18	27	45		

Chi square test, $P<0.05$ significant. GLUT-1: Glucose transporter-1, OSCC: Oral squamous cell carcinoma, WDOSCC: Well-differentiated OSCC, MDOSCC: Moderately differentiated OSCC, PDOSCC: Poorly differentiated OSCC

localized to the short arm of chromosome 1 (1p34.2). It is the prime transporter for basal glucose uptake in many cell types. It is typically expressed in erythrocytes, endothelial cells of the blood–brain barrier and placental cells, where there is increased use of glucose. Changes in GLUT-1 expression and rates of glucose transport are affected by growth rates, oxygen supply and malignant transformation.[5] Literature revealed that several tumor markers such as matrix metalloproteinases, cadherins, mucins, interleukins, human papillomavirus-16, estimated glomerular filtration rate and p53 have been used to identify and also to determine prognosis in OED and different grades of OSCC.

Earlier studies showed increased expression of GLUT-1 as a significant initial event for the development of carcinomas. GLUT-1 marker immunoexpression has been studied in various cancers such as prostate and lung. Very few studies have been conducted to evaluate the role of GLUT-1 immunomarker expression in OED and OSCC.[4,5]

In the present study, the mean percentage of GLUT-1 immunopositive cells increased from NOM (31.35%) to low-risk OED (44.84%) to high-risk OED (55.66%) [Figures 4-6].

Similar findings have been reported in a study conducted by Angadi and Angadi to evaluate GLUT-1 immunoexpression in different grades of OED and also by Mendez *et al*. in cervical dysplasias. As there is increase in the grade of OED, the number of positive cells increases, reflecting the increased proliferative capacity of the high-grade lesions, suggesting that oncogene-triggered mechanisms might be directly involved in the upregulation of GLUT-1.[9,10]

In our study, with increasing grades of OSCC from WDOSCC (Group IIIa) to MDOSCC (Group IIIb) to PDOSCC (Group IIIc), there is gradual increase in the percentage of positive cells, and there is a correlation between GLUT-1 immunopositive expression and the grades of OSCC [Figures 7-9].

These findings are in agreement with those of Angadi and Angadi and Harshani *et al*. where the percentage of positivity increased with the grade of OSCCs from WDOSCC to MDOSCC to PDOSCC. In contrast, a study done by Tian *et al*. found no correlation between staining pattern and grade of differentiation in OSCC.[9,11,12]

The increase in GLUT-1 positivity in increasing grades of OSCC can be due to the regulation of glucose influx into cancer cells by GLUT-1, assisting in energy preservation, especially in weakly perfused or hypoxic

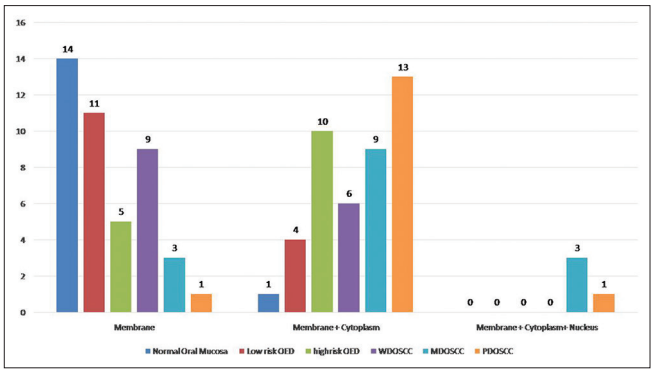

Figure 3: Location of glucose transporter-1 within cell among Groups I, IIa, IIb, IIIa, IIIb and IIIc

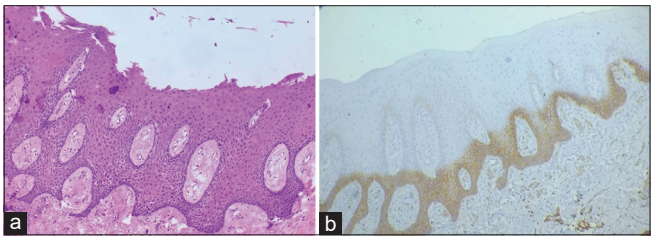

Figure 4: (a) Photomicrograph showing normal oral mucosa (H and E, ×10). (b) Photomicrograph showing mild expression of glucose transporter-1 in basal and parabasal layers of normal oral mucosa (IHC, ×10)

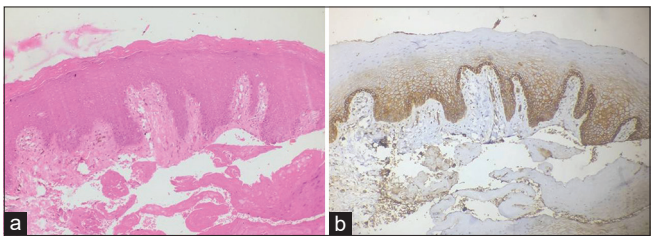

Figure 5: (a) Photomicrograph showing low-risk oral epithelial dysplasia (H&E, ×4). (b) Photomicrograph showing moderate expression of glucose transporter-1 extending up to the mid-spinous layers in low-risk oral epithelial dysplasia (IHC, ×4)

regions characterized by an inadequate supply of glucose.[9]

In our study, the intensity of staining of GLUT-1 increased from low-risk to high-risk OEDs. These findings are in accordance with the results of Angadi and Angadi and may be linked to the glycogen content of cells being high in nondysplastic areas of epithelium or absent in areas of dysplasia.[9] With increase in the grade of OED from low risk to high risk, there is increased expression of GLUT-1 that is significantly associated with reduced glycogen levels.

In the present study, majority of the cases of WDOSCC and MDOSCC showed moderate staining and in PDOSCC, most of the cases showed moderate and intense staining. These findings are similar to that of the results obtained by

Angadi and Angadi and Ohba *et al*., wherein they observed that there is a progressive shift in the intensity of staining from mild to intense as the grade of OSCC increased from WDOSCC to PDOSCC.[9,13]

The intense expression of GLUT-1 in PDOSCC may be due to less/poor differentiation of tumor cells and scanty glycogen content. Glycogen storage is said to be inversely correlated with the GLUT-1 expression.

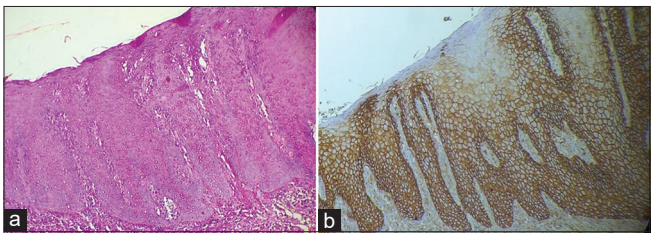

Figure 6: (a) Photomicrograph showing high-risk oral epithelial dysplasia (H&E, ×10). (b) Photomicrograph showing intense expression of glucose transporter-1 extending up to the superficial spinous layers in high-risk oral epithelial dysplasia (IHC, ×10)

The IRS classification was calculated by multiplying the percentage of GLUT-1 immunopositive cells and its staining intensity. The possible reason for the upregulation of GLUT-1 with increasing grades of OEDs and OSCCs is that GLUT-1 may play a role in tumor cell survival by providing sufficient energy to support their high growth rate and metabolic rate in an environment that is generally less than ideal from a physiologic standpoint or not natural.

The extent of GLUT-1 expression in different layers within the epithelium was observed. In NOM, GLUT-1 staining was detectable predominantly in basal and suprabasal layers. In majority of cases of low-risk and high-risk OEDs, the expression of GLUT-1 was noticed till suprabasal layers and superficial spinous layers, respectively [Figures 4-6].

Our findings coincide with those of the study done by Burstein *et al*., who observed the increased expression of GLUT-1 from basal layers to superficial layers as the

Figure 7: (a) Photomicrograph showing well-differentiated oral squamous cell carcinoma (H&E, ×4). (b) Photomicrograph showing moderate expression of glucose transporter-1 in well-differentiated oral squamous cell carcinoma (IHC, ×4). (c) Photomicrograph showing peripheral cells of tumor island in well-differentiated oral squamous cell carcinoma showing membranous expression of glucose transporter-1 (IHC, ×40)

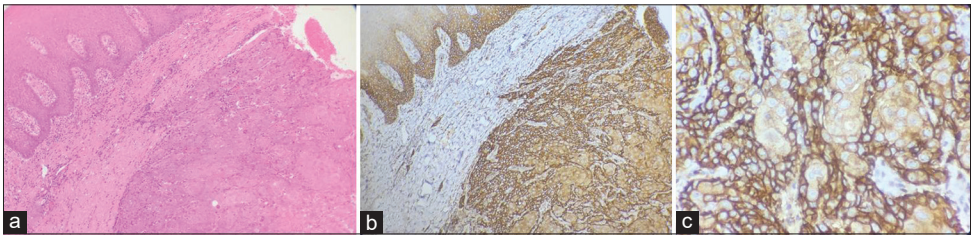

Figure 8: (a) Photomicrograph showing moderately differentiated oral squamous cell carcinoma (H&E, ×4) (b) Photomicrograph showing moderate expression of glucose transporter-1 in tumor islands of moderately differentiated oral squamous cell carcinoma (IHC, ×4). (c) Photomicrograph showing peripheral cells of tumor island in moderately differentiated oral squamous cell carcinoma showing intense glucose transporter-1 expression (IHC, ×40)

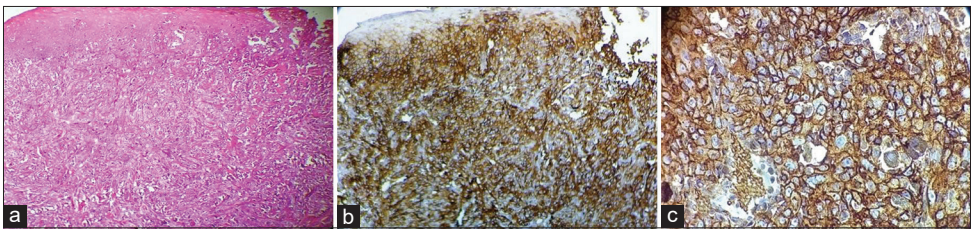

Figure 9: (a) Photomicrograph showing poorly differentiated oral squamous cell carcinoma (H&E, ×4). (b) Photomicrograph showing intense expression of glucose transporter-1 in all cells in poorly differentiated oral squamous cell carcinoma (IHC, ×4). (c) Photomicrograph showing intense expression of glucose transporter-1 in peripheral and central cells of tumor island in poorly differentiated oral squamous cell carcinoma with membranous and cytoplasmic staining (IHC, ×40)

severity of the dysplasia increases and suggested that the enhancement of GLUT-1 is an early alteration in the progression of squamous cell carcinoma.[14]

In our study, the location of GLUT-1 immunostaining within the invading islands in different grades of OSCC was investigated. In WDOSCC, GLUT-1 expression was predominantly evident at the periphery of tumor islands and absent in the central keratin pearls known as prostromal pattern. In keratin pearls, there is increased accumulation of glycogen which is inversely correlated with GLUT-1 expression, suggesting that differentiated and mature cells present in keratinized regions lack GLUT-1 expression. The presence of glycogen is related to cellular maturation of squamous epithelium and disappears when there is loss of differentiation during neoplastic transformation. In PDOSCC, it has been suggested that hypoxia-driven GLUT-1 stimulation creates an antistromal staining pattern in areas devoid of squamous differentiation and keratinization [Figures 7-9].

In the present study, the location of GLUT-1 expression within the cell was evaluated and compared among different groups. As the grade of OED increased from low risk to high risk, there is a shift in the location of GLUT-1 from membrane to combined membrane and cytoplasm staining, and there is a significant correlation between the location of GLUT-1 within the cell and grade of dysplasia.

As there is an increase in the grade of OSCC from WDOSCC to PDOSCC, the location of GLUT-1 showed a progressive switch from membrane to cytoplasmic staining and then to a combination of both, and there is a significant correlation between the location of GLUT-1 and histological grade of OSCC. This could be due to the co-localization of GLUT-1 with the Golgi that leads to combined membrane and cytoplasmic staining.[15]

In our study, the immunoexpression of GLUT-1 was statistically significant in terms of number of positive cells, staining intensity, IRS score and level of staining within the epithelium and also within the cell between all the studied groups.

However, the limitations associated with our study include a small sample size. Thus, studies with a larger sample size are required to precisely predict the role of GLUT-1 immunoexpression in different grades of OED and OSCC.

CONCLUSION

Our study demonstrates that GLUT-1 has a role in the pathogenesis of OED and OSCC. Its level of expression and activity may be associated with the malignant transformation of OED and aggressiveness of OSCC. The increased GLUT-1 expression associated with the degree of dysplasia reflects the expanding glycolytic response to hypoxia and the high energy requirement of proliferating tumor cells. GLUT-1 expression in proliferating cells may be related to the aggressiveness of the tumor and their response to various individual treatment strategies.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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