Expression of Glut-1 in Oral Epithelial Dysplasia and OSCC: An Immunohistochemical Study

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ABSTRACT

Introduction: Glucose transporters are membrane proteins active in the transport of hexoses like glucose and fructose across plasma membranes. GLUT-1 is the key target gene for hypoxia inducible factor (HIF) which facilitates glucose influx into cells under various conditions that have greater metabolic requirements like cell division, malignant transformation and nutrient depletion. Study aimed to determine the expression of Glut-1 in normal oral mucosa, oral epithelial dysplasia and in different histological grades of oral squamous cell carcinoma so as to evaluate the role of Glut-1 as a prognostic marker.

Material and methods: A retrospective study was conducted on 25 formalin-fixed paraffin- embedded tissue blocks, comprising of 5 cases of normal oral mucosa, 5 cases of oral epithelial dysplasia, 5 cases of well-differentiated OSCC, 5 cases of moderately-differentiated OSCC and 5 cases of poorly-differentiated OSCC which are histopathologically diagnosed using Hematoxylin and Eosin (H&E).Immunohistochemical staining was done for Glut-1 expression which was determined on the basis of location, intensity and area of stained cells.

Results:The expression of GLUT-1 increased significantly from normal oral mucosa to oral epithelial dysplasia and to different grades of oral squamous cell carcinoma. The intracellular location of GLUT-1 expression changed significantly from membranous to cytoplasmic location then to a combination of both membranous and cytoplasmic positivity during the transition from normal oral mucosa to oral epithelial dysplasia and to different grades of oral squamous cell carcinoma.

Conclusion: The expression of GLUT-1 wasincreasedfrom oral epithelial dysplasia to varying grades of oral squamous cell carcinoma. The enhanced expression of this protein determined increased glycolytic activity of the tumor cells due to high energy requirement with an increase in the grade of the tumor. Glut-1 can be used as a prognostic marker in OSCC.

Keywords: Oral Epithelial Dysplasia, Oral Squamous Cell Carcinoma, Prognostic Marker, Glucose Transporter

INTRODUCTION

Oral cancer is the sixth most common cancer worldwide, with epidemiologic variations between different geographic areas. In India, it ranks the third most common malignancy, which accounts for more than 30% of all cancers stated in the country.[1,2] Squamous cell carcinoma (SCC) is the most common clinical entity accounting for more than 90% of all oral malignancies and occurs with a growing incidence rate in young and middle-age population.[3] Oral squamous cell carcinomas generally follow clinically detectable potentially malignant disorders including oral leukoplakia, erythroplakia, and oral submucous fibrosis, which histologically represent the process of oral epithelial dysplasia (OED).[4]

OED is the histological indicator of potentially malignant disorders diagnosed on the basis of architectural as well as cytological changes, for which the rate of progression to oral squamous cell carcinoma (OSCC) may ranges from 6% to 36%.[4,5]

OSCC occurs as a result of tobacco exposure, immunodeficiency, multiple mutations, genetic and epigenetic changes. Oral squamous cell carcinoma (OSCC) is a locally aggressive neoplasm with rapid progression and significantly reduced oxygen concentration.[6]

Tumour hypoxia is a well-known phenomenon which is caused by an imbalance between oxygen supply and consumption within the tumour microenvironment. Under sustained hypoxic conditions, some tumour cells can survive, and they adapt themselvesthrough hypoxia induced cellular changes, that can lead to more aggressive phenotype, hence results in invasion and metastasis.[7,8] Malignant cells show an increased glucose uptake which helps to nourish the cancer cells throughout their expansive activity. The up-regulation of glucose transport across the plasma membrane is intervened by facilitative glucose transporter (GLUT) proteins but GLUT-1 is also known as facilitated glucose transporter member 1. GLUT-1 was the first facilitative sugar transporter discovered and is competent to transport primarily glucose.[9] GLUT-1 is normally expressed in the membranes of red blood cells, brain capillary endothelium, perineurium of peripheral nerves, salivary gland ducts, and the germinal centers of lymph nodes.[10] GLUT-1 positivity in malignant cells signifies increased proliferative activity, energy requirements and aggressive behaviour.[11] The aim of the present study was to determine the immunohistochemical

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expression of the glucose transporter, GLUT-1 in oral epithelial dysplasia and oral squamous cell carcinoma so as to elucidate the biological behavior of malignant neoplasias.

MATERIAL AND METHODS

A retrospective study was carried out on 25 formalin-fixed paraffin- embedded tissue blocks comprising of 5 cases of normal oral mucosa, 5 cases of oral epithelial dysplasia, 5 cases of well-differentiated OSCC, 5 cases of moderately-differentiated OSCC and 5 cases of poorly-differentiated OSCC, histopathologically diagnosed (using H&E) and retrieved from archives of the Department of Oral Pathology IDST College, Modinagar, and from a private laboratory in Jaipur.From each paraffin embedded tissue block, 3-4 micron thin sections were obtained by using semiautomatic rotary microtome and the sections were lifted on to the pre-coated (3- Aminopropyl triethoxy silane) slides for immunohistochemical (IHC) staining using primary anti-glucose transporter, GLUT-1 rabbit polyclonal antibody(Biogenex supersensitive detection system, USA). After staining and mounting, all the stained slides were examined by two observers under the microscope to eliminate any subjective bias. GLUT-1 expression in the endothelial cells and erythrocytes was taken as positive internal control. Glut-1 expression was determined by IHC on the basis of location, intensity and area of stained cells.

Evaluation of GLUT-1 immunoreactivity: GLUT-1 immunostaining was evaluated by a semi-quantitative scoring system as:

a) Percentage of GLUT-1 immunoreactivity; b) Intensity of staining and c) Location of immunoreactivity

In each section, five high power light microscopic fields were randomly selected. Two observers individually noted the percentage of GLUT-1 positivity in each field and the region of staining was scored as follows:

Score 0 - no staining of cells in any microscopic field

Score 1+ - less than 25% of tissue stained positive

Score 2+ - between 25% and 50% of tissue stained positive

Score 3+ - between 50% and 75% of tissue stained positive

Score 4+ - more than 75% of tissue stained positive

The intensity scores were recorded by comparing it with the positive control slides. The intensity of VEGF reaction was scored as follows:

Score 0 – No staining was evident

Score 1- Mild staining when intensity was less than the positive control

Score 2- Moderate staining when intensity was equal to the positive control

Score 3- Intense staining when intensity was greater than the positive control

The intracellular location of GLUT-1 staining was evaluated on the basis of presence or absence of immunoreactivity in cell membrane and cytoplasm. The location of GLUT-1 staining was scored as follows:

Score 0- No immunoreactivity

Score 1- Cell membrane immunoreactivity

Score 2- Cytoplasm immunoreactivity

Score 3- Combination of cell membrane and cytoplasm immunoreactivity

STATISTICAL ANALYSIS

Data on immunohistochemical expression of GLUT-1 in all the tissue sections was obtained and statistically analysed with the help of Statistical Package for Social Sciences (SPSS) software version 21.0 using frequency, percentage, Pearson Chi-square test, Pearson correlation coefficient test and Cronbach's alpha reliability test. A probability value of <0.05 was considered to be statistically significant.

RESULTS

In all the study groups included in the present study, majority of the patients were males (85%) and rest were females (15%). A good interobserver reliability was found on applying Cronbach's alpha reliability test to the observations obtained from all two observers for the assessment of percentage of GLUT-1 immunoreactivity, intensity of GLUT-1 staining and intracellular location of GLUT-1 immunoreactivity (Table 1).

Evaluation of GLUT-1 immunostaining: GLUT-1 expression was easily apparent as brown staining and occurred in both cell membrane and cytoplasm of epithelial cells. GLUT-1 positive expression was observed in all the cases of oral squamous cell carcinoma and oral epithelial dysplasia than in normal oral mucosa.GLUT-1 immunoexpression in normal oral mucosa was membranous in basal layer which progressively diminished in suprabasal layers whereas in oral epithelial dysplasia, GLUT-1 immunoexpression was determined in the basal and suprabasal layers of epithelium as well as diminished reactivity in the most superficial layers in some cases.GLUT-1 expression was occurred predominantly at tumour periphery and it was much weaker in the centre of tumour areas (Figure 1).

Percentage of GLUT-1 immunoreactivity

In normal oral mucosa, 40% (02) and 60% (03) of the cases showed score 0 and score 1+ respectively whereas 20% (01), 20% (01), 40% (02) and 20% (01) of the cases of oral epithelial dysplasia revealed score 0, score 1+, score 2+ and score 3+ respectively. In well-differentiated oral squamous cell carcinoma, 20% (01), 40% (02) and 40% (02) of the cases showed score 1+, score 2+ and score 3+ respectively whereas 40% (02), 40% (02) and 20% (01) of cases of moderately-differentiated oral squamous cell carcinoma revealed score 2+, score 3+ and score 4+ respectively. The score 2+, score 3+ and score 4+ of GLUT-1 percentage was found to be 20% (01), 40% (02) and 40% (02)of the cases of poorly-differentiated oral squamous cell carcinoma respectively. The p value was found to be statistically significant (Table 2).

Intensity of GLUT-1 immunostaining

In normal oral mucosa, 40% (02), 40% (02) and 20% (01)of the cases showed score 0, score 1 and score 2 respectively whereas 40% (02), 40% (02) and 20% (01) of the cases of

Reliability Analysis	Cronbach's α Test		
	GLUT-1 percentage	GLUT-1 intensity	GLUT-1 location
Observer 1 & Observer 2	0.984	0.964	0.976

Table-1: Interobserver reliability analysis for GLUT-1 immunostaining

Group	GLUT-1 percentage				
	Score 0	Score 1+	Score 2+	Score 3+	Score 4+
NOM	02 (40.0%)	03 (60.0%)	00 (00.0%)	00 (00.0%)	00 (00.0%)
OED	01 (20.0%)	01 (20.0%)	02 (40.0%)	01 (20.0%)	00 (00.0%)
WDSCC	00 (00.0%)	01 (20.0%)	02 (40.0%)	02 (40.0%)	00 (00.0%)
MDSCC	00 (00.0%)	00 (00.0%)	02 (40.0%)	02 (40.0%)	01 (20.0%)
PDSCC	00 (00.0%)	00 (00.0%)	01 (20.0%)	02 (40.0%)	02 (40.0%)
Chi-square test	28.822				
Degree of freedom (df)	8				
p - value	0.001				
NOM- Normal oral mucosa, OED- Oral epithelial dysplasia, WDSCC- Well-differentiated Oral squamous cell carcinoma, MDSCC-Moderately-differentiated Oral squamous cell carcinoma, PDSCC- Well-differentiated Oral squamous cell carcinoma					

Table-2: Percentage of GLUT-1 immunoreactivity in normal oral mucosa, oral epithelial dysplasia and in different grades of oral squamous cell carcinoma

Group	GLUT-1 Intensity			
	Score 0	Score 1	Score 2	Score 3
NOM	02 (40.0%)	02 (40.0%)	01 (20.0%)	00 (00.0%)
OED	00 (00.0%)	02 (40.0%)	02 (40.0%)	01 (20.0%)
WDSCC	00 (00.0%)	01 (20.0%)	03 (60.0%)	01 (20.0%)
MDSCC	00 (00.0%)	00 (00.0%)	02 (40.0%)	03 (60.0%)
PDSCC	00 (00.0%)	00 (00.0%)	01 (20.0%)	04 (80.0%)
Chi-square test	30.180			
Degree of freedom (df)	6			
p - value	0.0001			
NOM- Normal oral mucosa, OED- Oral epithelial dysplasia, WDSCC- Well-differentiated Oral squamous cell carcinoma, MDSCC-Moderately-differentiated Oral squamous cell carcinoma, PDSCC- Well-differentiated Oral squamous cell carcinoma				

Table-3: Intensity of GLUT-1 immunoreactivity in normal oral mucosa, oral epithelial dysplasia and in different grades of oral squamous cell carcinoma

| Group	GLUT-1 location			
	Score 0	Score 1	Score 2	Score 3
NOM	02 (40.0%)	03 (60.0%)	00 (00.0%)	00 (00.0%)
OED	00 (00.0%)	02 (40.0%)	02 (40.0%)	01 (20.0%)
WDSCC	00 (00.0%)	02 (40.0%)	02(40.0%)	01 (20.0%)
MDSCC	00 (00.0%)	01 (20.0%)	02 (40.0%)	02 (40.0%)
PDSCC	00 (00.0%)	00 (00.0%)	02 (40.0%)	03 (60.0%)
Chi-square test	28.040			
Degree of freedom (df)	7			
p - value	0.001			
NOM- Normal oral mucosa, OED- Oral epithelial dysplasia, WDSCC- Well-differentiated Oral squamous cell carcinoma, MDSCC-Moderately-differentiated Oral squamous cell carcinoma, PDSCC- Well-differentiated Oral squamous cell carcinoma				

Table-4: Intracellular location of GLUT-1 immunoreactivity in normal oral mucosa, oral epithelial dysplasia and in different grades of oral squamous cell carcinoma

oral epithelial dysplasia revealed score 1, score 2 and score 3 respectively. In well-differentiated oral squamous cell carcinoma, 20% (01), 60% (03) and 20% (01) of the cases showed score 1, score 2 and score 3 respectively whereas 40% (02) and 60% (03) of cases of moderately-differentiated oral squamous cell carcinoma revealed score 2 and score 3 respectively. The score 2 and score 3of GLUT-1 intensity was found to be 20% (01) and 80% (04) respectively. The p value was found to be statistically significant (Table 3).

Intracellular location of GLUT-1 immunoreactivity
In normal oral mucosa, 40% (02) and 60% (03)of the cases showed score 0 and score 1 respectively whereas 40% (02), 40% (02) and 20% (01) of the cases of oral epithelial dysplasia revealed score 1, score 2 and score 3 respectively.
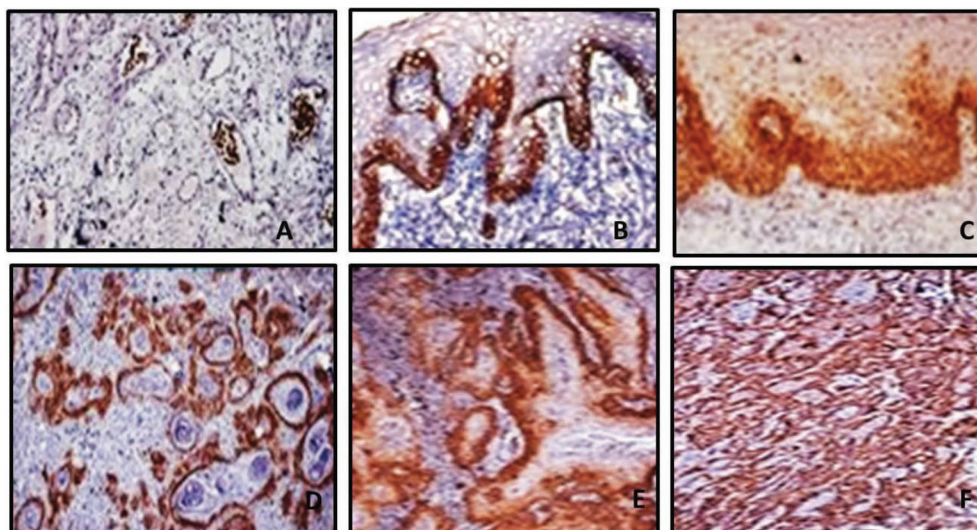
Figure-1: Photomicrograph showing GLUT-1 expression in A) Positive control, B) Normal oral mucosa, C) Oral epithelial dysplasia, D) Well-differentiated oral squamous cell carcinoma, E) Moderately-differentiated oral squamous cell carcinoma and F) Poorly- differentiated oral squamous cell carcinoma (100X)

In well-differentiated oral squamous cell carcinoma, 40% (02), 40% (02) and 20% (01) of the cases showed score 1, score 2 and score 3 respectively whereas 20% (01), 40% (02) and 40% (02) of cases of moderately-differentiated oral squamous cell carcinoma revealed score 1, score 2 and score 3 respectively. The score 2 and score 3 of GLUT-1 intensity was found to be 40% (02) and 60% (03) respectively. The p value was found to be statistically significant (Table 4).

DISCUSSION

Malignant neoplastic cells illustrate an altered metabolism that is characterized by the increased absorption and consumption of glucose.[12,13] This phenomenon can be described by numerous mechanisms, including the adaptation of cells to the hypoxic tumor microenvironment and the maintenance of tumor cell viability, that be determined by the ability of these cells to perform anaerobic glycolysis.[12] Hypoxia is an important characteristic of all solid tumors and associated with a poor prognosis.

Glucose transporters are membrane proteins active in the transport of hexoses like glucose and fructose across plasma membranes are divided into two families such as Facilitative glucose transporters (Glut family) and Na^+ coupled glucose transporters (SGLT family). Glut-1 is also called as erythrocyte, brain or Hep G2-type glucose transporter. GLUT-1 is the key target gene for hypoxia inducible factor (HIF) which facilitates glucose influx into cells under various conditions that have greater metabolic requirements like cell division, malignant transformation and nutrient depletion.[14]

The present study was done to determine the expression of Glut-1 in normal oral mucosa, oral epithelial dysplasia and in different histological grades of oral squamous cell carcinoma in order to evaluate the role of Glut-1 as a prognostic marker. In the present study, GLUT-1 immunoexpression in normal oral mucosa was membranous in basal layer which progressively diminished in suprabasal layers whereas in oral epithelial dysplasia, GLUT-1 immunoexpression was determined in the basal and suprabasal layers of epithelium as well as diminished reactivity in the most superficial layers in some cases. GLUT-1 expression was occurred predominantly at tumour periphery and it was much weaker in the centre of tumour areas. These findings were in accordance with the study done by Angadi et al, Rudlowski et al and Kim et al.[15-17] The occurrence of glycogen is linked to cellular maturation of squamous epithelium and disappears with loss of differentiation during neoplastic transformation. In well differentiated tumours, greater accumulation of glycogen in keratin pearls has inversely correlated with GLUT-1 immunoexpression, suggesting that differentiation and mature cells present in keratinized regions lack GLUT-1 expression. In poorly differentiated tumours, it has been proposed that hypoxia driven GLUT-1 stimulation creates an antistromal staining pattern in area devoid of squamous differentiation/keratinization.[16,17]

In the present study, percentage and intensity of GLUT-1 increased significantly from normal oral mucosa to oral epithelial dysplasia and to different grades of oral squamous cell carcinoma. These results were similar to the study carried out by Angadi et al.[15] The intracellular location of GLUT-1 expression in our study changed significantly from membranous to cytoplasmic location then to a combination of both membranous and cytoplasmic positivity during the transition from normal oral mucosa to oral epithelial dysplasia and to different grades of oral squamous cell carcinoma. These findings were in agreement with the study done by Angadi et al[15] and Vasconcelos et al[18] but Harshaniet al[19] evaluated that GLUT-1 expression was predominantly membranous in all grades of OSCC.Anti-GLUT-1 antibody typically identifies membrane bound proteins on epithelial cells. GLUT-1 induction ensuing hypoxia includes a sequence of changes to its intrinsic activity, kinetics and expression. Initially, there is "unmasking" of the protein which increases its affinity for glucose. Further stimulation results in translocation of existing glucose transporters

from cytoplasmic vesicles to the plasma membrane, and an eventual increase in the synthesis of GLUT-1 mRNA.[20] Ariely et alexaminedthat cytoplasmic and membranous expression of GLUT-1 in tumors was associatedwith the duration and extent of hypoxia present in different areas. They proposed that co-localization of GLUT-1 with the Golgi leads to combined membrane and cytoplasmic expression.[20]

CONCLUSION

The expression of GLUT-1 wasincreasedfrom oral epithelial dysplasia to varying grades of oral squamous cell carcinoma. The enhanced expression of this protein determined increased glycolytic activity of the tumor cells due to high energy requirement with an increase in the grade of the tumor. Glut-1 can be used as a prognostic marker in OSCC. Further studies on larger samples need to be conducted in order to clarify the role of GLUT-1 in oral epithelial dysplasia and oral carcinogenesis from a clinical, biological and molecular point of view.

REFERENCES

1. Shah JP and Gil Z. Current Concepts in Management of Oral Cancer-Surgery. Oral Oncol 2009; 45: 394-401.
2. Sankaranarayanan R, Ramadas K, Thomas G, et al. Effect of screening on oral cancer mortality in Kerala, India: a cluster-randomised controlled trial. Lancet 2005; 365: 1927–1933.
3. Neville BW, Day TA. Oral cancer and precancerous lesions. CA Cancer J Clin 2002; 52:195–215.
4. Tilakaratne WM, Sherriff M, Morgan PR, Odell EW. Grading oral epithelial dysplasia: analysis of individual features. J Oral Pathol Med 2011; 40:533-540.
5. Napier SS, Speight PM. Natural history of potentially malignant oral lesions and conditions: an overview of the literature. J Oral Pathol Med 2008; 37: 1-10.
6. Ryu MH, Park HM, Chung J et al. Hypoxia-inducible factor- 1alpha mediates oral squamous cell carcinoma invasion via upregulation of alpha5 integrin and fibronectin. Biochem Biophys Res Commun 2010; 393:11–15.
7. Hockel M and Vaupel P. Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. J Natl Cancer Inst 2001; 93:266–276.
8. Poon E, Harris AL and Ashcroft M. Targeting the hypoxiainducible factor (HIF) pathway in cancer. Expert Rev Mol Med 2009; 11: 1–23.
9. Calvo MB, Figueroa A, Pulido EG, et al. Potential role of sugar transporters in cancer and their relationship with anticancer therapy. Int J Endocrinol 2010; 2010: 205357.
10. Kato Y, Tsuta K, Seki K, et al. Immunohistochemical detection of GLUT-1 can discriminate between reactive mesothelium and malignant mesothelioma. Mod Pathol 2007; 20: 215-20.
11. Birnbaum MJ, Haspel HC and Rosen OM. Cloning and characterization of a cDNA encoding the rat brain glucose-transporter protein. Proc Natl Acad Sci USA 1986; 83:5784–5788.
12. Pérez-Sayáns M, Suárez-Peñaranda JM, Pilar GD, et al. Hypoxia-inducible factors in OSCC. Cancer Lett 2011;313: 1-8.
13. Ortega AD, Sánchez-Aragó M, Giner-Sánchez D, et al. Glucose avidity of carcinomas. Cancer Lett 2009;276: 125-35.
14. Kunkel M, Reichert TE, Benz P, et al. Overexpression of Glut- 1 and increased glucose metabolism in tumors are associated with a poor prognosis in patients with oral squamous cell carcinoma. Cancer 2003;97:1015–1024.
15. Angadi VC, Angadi PV. GLUT-1 immunoexpression in oral epithelial dysplasia, oral squamous cell carcinoma, and verrucous carcinoma. J Oral Sci 2015; 57:115–122.
16. Rudlowski C, Becker AJ, Schroder W, et al. GLUT1 messenger RNA and protein induction relates to the malignant transformation of cervical cancer. Am J Clin Pathol 2003; 120:691–698.
17. Kim CH, Kim MY. Correlation between glucose transporter type-1 expression and 18F-FDG uptake on PET in oral cancer. J Korean Assoc Oral Maxillofac Surg 2012; 38:212–220.
18. Vasconcelos MG, Vasconcelos RG, Pereira de Oliveira DH, et al. Distribution of hypoxia-inducible factor-1a and glucose transporter-1 in human tongue cancers. J Oral Maxillofac Surg 2015; 73:1753–1760. 28.
19. Harshani JM, Yeluri S, Guttikonda VR, et al. Glut-1 as a prognostic biomarker in oral squamous cell carcinoma. J Oral Maxillofac Pathol 2014; 18:372–378.
20. Airley R, Loncaster J, Davidson S, Bromley M, Roberts S, Patterson A et al. Glucose transporter Glut-1 expression correlates with tumor hypoxia and predictsmetastasis-free survival in advanced carcinoma of the cervix.Clin Cancer Res 2001; 7: 928-934.