Original Article

Study of expression of GLUT-1 in oral potentially malignant disorders and oral squamous cell carcinoma: An immuno-histochemical analysis

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Abstract

Background: Glucose is the chief source of energy for cells, and glucose transporter 1 (GLUT-1) is one of the most common glucose transporters in humans. Tumour cells are known to express hypoxia-related protein, and these may allow tumour cells to survive under a sustained hypoxic environment. Surviving cells develop a more aggressive phenotype, which results in poor prognosis.

Aims and Objectives: Expression and comparison of GLUT-1 in normal tissues, potentially malignant disorders (PMDs), and oral squamous cell carcinoma (OSCC) and comparison of expression in different grades of OSCC.

Material and Methods: A total of 57 cases (10 normal, 17 PMD, and 30 cases of OSCC) were stained immuno-histochemically with GLUT-1. The expression was scored as 0, 1, 2, 3, and 4 for negative, mild, moderate, severe, and intense staining, respectively.

Results: GLUT-1 expression was detected in all grades of OSCC. A significant correlation was found on comparing normal and OSCC, normal and PMDs, and PMD and OSCC. The expression of GLUT-1 was significant when compared with different histopathological grades of OSCC.

Conclusions: Expression of GLUT-1 increased from normal to PMDs to increasing grades of OSCC and hence can be used as a prognostic predictive marker for OSCC.

Keywords: GLUT-1, biomarker, hypoxia, prognosis

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INTRODUCTION

Transport of glucose across the plasma membrane is the most imperative cellular nutrient conveyance, which permits the passage down its chemical gradient.[1] Glucose controls transcription, enzymatic activity, hormone secretion, and the activity of neurons. These functions typically are secondary to glucose uptake, which is controlled primarily by the glucose transporter family.[2]

Hypoxia in tumours is chiefly a patho-physiologic consequence of structurally and functionally disturbed micro-circulation and the decline of diffusion conditions.

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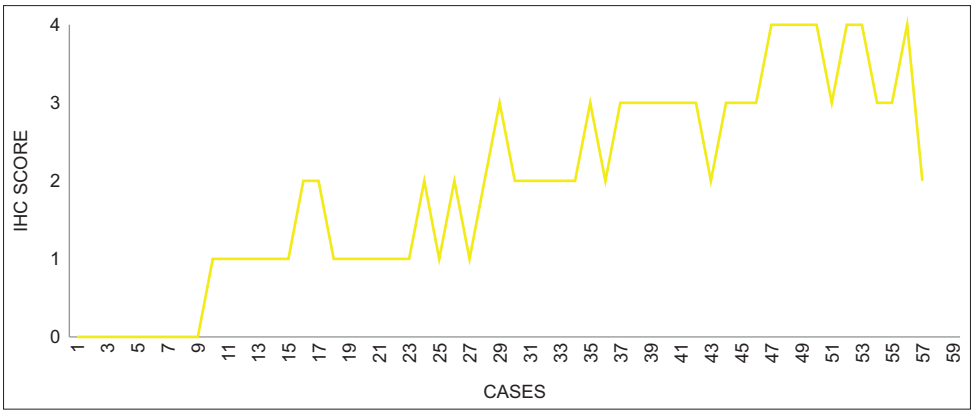

Graph 1: IHC score and expression of GLUT-1 in normal, PMDs, and OSCCs. Normal tissue, 1 to 10 cases; PMDs, 11 to 27 cases; OSCC, 28 to 57 cases

Under persistent hypoxia, tumour cells may die due to insufficient oxygen supply; however, some tumour cells can subsist and they acclimatise themselves into these circumstances via hypoxia-induced cellular modifications (genomic instability through point mutations, gene amplification, and chromosomal re-arrangements).[3] These hypoxia-induced cellular adaptations can direct to a supplementary unreceptive phenotype that will upshot in invasion and metastasis.[4]

The glucose transporter 1 (GLUT-1) isoform is also called as the erythrocyte, brain, or hepatic G2-type glucose transporter, encompassing 3–5% of the total red blood cell membrane. It arbitrates cellular glucose acceptance and thus facilitates anaerobic glycolysis.[1,5] The activity of GLUT-1 is controlled by oncogenes and growth factors and is also subjective to growth rate, oxygen supply, and malignant transformation. Over-expression of GLUT-1 could facilitate growth and proliferation of tumour cells by supporting the high metabolic ingestion in a hypoxic tumour micro-environment. Hypoxia-induced factor (HIF1) regulated protein GLUT-1 has been seconded as an endogeneous/intrinsic marker of hypoxia.[5]

The influence of GLUT-1 on prognosis and its use as a biomarker mark tumour hypoxia and the adaptive upregulation of anaerobic glycolysis. This eventually encourages tumour cell survival, signifying that GLUT-1 may be mirrored to be a negative biomarker of prognosis in patients with oral squamous cell carcinoma (OSCC).[6]

Management of OSCC is steered by complete resection of the primary lesion, but long-term survival is still poor because of a high rate of loco-regional recurrence and new malignant conversions. To precisely categorise high-risk patients and to foresee clinical outcomes, reliable and novel prognostic markers are imperatively desired. In view of this, the present study was undertaken to evaluate the prognostic significance of GLUT-1 in potentially malignant lesion and OSCC.

MATERIALS AND METHOD

Approval was taken from institutional ethical committee on 14th March 2014.

The present study included 57 cases, with 17 pre-diagnosed potentially malignant disorders (PMDs), 30 OSCC, and 10 normal cases as control. PMDs and OSCC were selected from archives of the department. Ten normal tissues were procured from the patients undergoing surgery in the institute who had no history of tobacco in any form.

The selected cases were grouped as group I, normal tissue; group II, PMDs (9 leukoplakia, 4 oral sub-mucous fibrosis, and 4 oral lichen planus); group III, 10 well-differentiated OSCC (WDSCC); group IV, 10 moderately differentiated OSCC (MDSCC); and group V, 10 poorly differentiated OSCC (PDSCC).

4 μ thick sections were prepared for routine haematoxylin and eosin (H&E) staining and evaluated for PMDs and grades of OSCC [Figure 1] (Broders grading system of tumours). The second set of 4 μ thick sections was taken on Poly L Lysil (PLL)-coated slides for immuno-staining with GLUT-1 protein (Bio-Genex Super Sensitive polymer-HRP IHC detection system). Immuno-staining was done by using primary monoclonal mouse anti-human GLUT-1 antibody (Clone SPM498). The pyogenic granuloma section was taken as a positive control with each batch of staining.

IHC procedure

4 μm sections on PLL-coated slides were de-paraffinised and rehydrated using decreasing grades of alcohol and distilled water. Antigen retrieval was carried out by placing

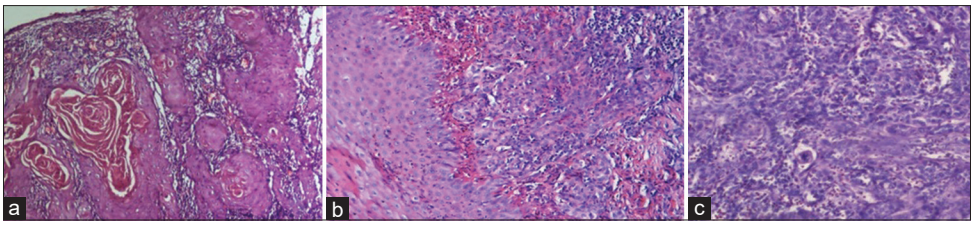

Figure 1: Photomicrograph showing H&E-stained section with (a) WDSCC, (b) MDSCC, and (c) PDSCC (x100 magnification)

the hydrated slides in a pressure cooker with tris ethylene diamine tetra-acetic acid buffer for 5 minutes, with 15 psi pressure at 105°C. Endogenous peroxidase was blocked by treating the slides with 3% hydrogen peroxide for 10 minutes, followed by buffer wash.

The sections were covered with primary monoclonal mouse anti-human GLUT-1 antibody for 1 h and a super enhancer for 20 minutes, followed by incubation with SS label (polymer horseradish peroxidase) for 30 minutes. Each step was followed by buffer wash. This was followed by freshly prepared substrate DAB chromogen solution for 10 minutes, washed and counter-stained with Harris's hematoxylin for 10 minutes, dehydrated, and mounted with coverslip using DPX.

OBSERVATION AND RESULT

The presence of brown colour at the site of target antigen was indicative of positive immunoreactivity. Five high-power (X400) fields were selected having the maximum number of positive cells for anti-GLUT-1 antibody, and 300 cells were counted for a positive or negative staining.

Percentage of staining –>No. of IHC positive cells (GLUT-1) ×100/No. of cells counted.

Grading of GLUT-1positivity

The intensity of GLUT-1 positivity was estimated based on the following criteria: score 0, <10% positive tumour cells (negative); score 1, 10–25% positive tumour cells (mild); score 2, 25–50% positive tumour cells (moderate); score 3, 50–75% positive tumour cells (severe); score 4, >75% positive tumour cells (intense). The results were analysed for significance using Chi-square test.

The positive cells showed membrane staining and/or cytoplasmic staining. RBCs in pyogenic granuloma (positive control) showed positive staining [Figure 2]. RBCs within the tissue served as an internal control. The normal tissue showed the basal cell positive [Figure 3], and PMDs showed basal and supra basal positivity [Figure 4]. Invading islands in WDSCC showed GLUT-1-positive cells in the periphery but keratin in the centre as negative [Figure 5]. A progressive

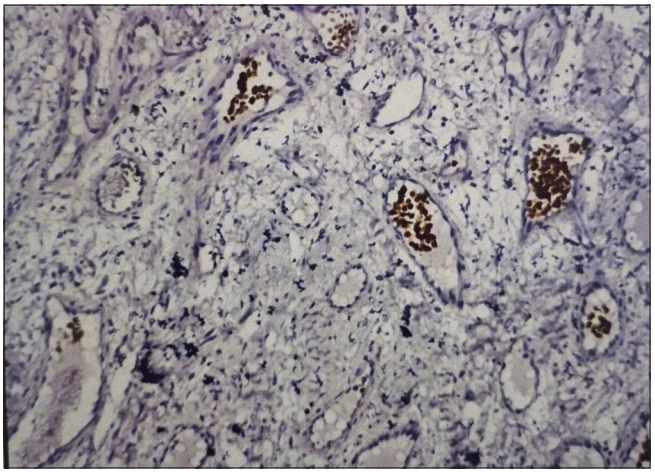

Figure 2: Photomicrograph of pyogenic granuloma as positive control for GLUT-1 (x100 magnification)

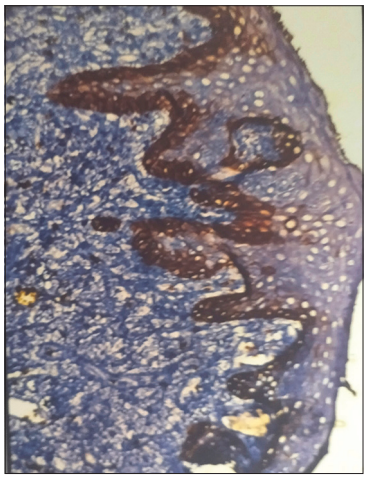

Figure 3: Photomicrograph showing GLUT-1 expression in normal tissue (x100 magnification)

increase in the intensity of staining for GLUT-1 was noted in MDSCC [Figure 6] and PDSCC, which showed even central cells of the island positive [Figure 7].

Nine cases in the normal tissue had score 0, 13 of PMD had score 1, and 14 of OSCC were scored 3 [Table 1]. Normal mucosa had score 0 and score 1, while PMD showed score 1 and score 2. Comparison by Chi-square test showed a value of 23.018 df = 2 P = 0.0003, which showed no significant difference of GLUT-1 expression

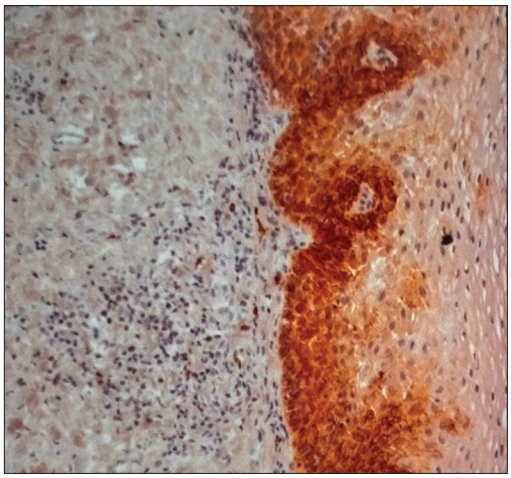

Figure 4: Photomicrograph showing GLUT-1 expression in PMD (x100 magnification)

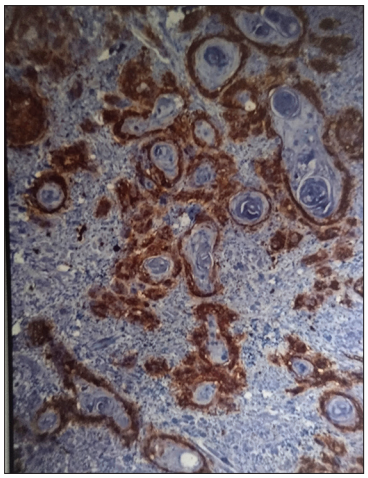

Figure 5: Photomicrograph showing GLUT-1 expression in WDSCC (x100 magnification)

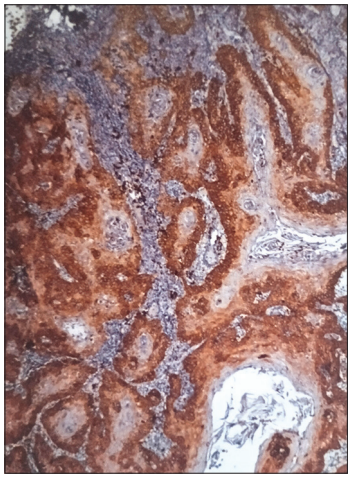

Figure 6: Photomicrograph showing GLUT -1 expression in MDSCC (x100 magnification)

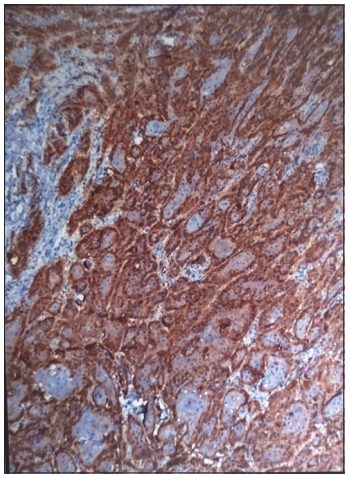

Figure 7: Photomicrograph showing GLUT-1 expression in PDSCC (x40 magnification)

Table 1: Expression of GLUT-1 in normal tissue, PMDs, and OSCC

IHC Score	Normal	PMD	OSCC
Score 0	9	0	0
Score 1	1	13	0
Score 2	0	4	9
Score 3	0	0	14
Score 4	0	0	7
Total	10	17	30

Table 2: Comparison of expression of GLUT-1 between normal tissue and PMDs

IHC Score	Normal %	PMD %
Score 0	90	0
Score 1	10	76.5
Score 2	0	23.5
Score 3	0	0
Score 4	0	0
Total	100	100

Chi-square=23.018, df=2, *P*=0.0003

between normal mucosa and PMD [Table 2]. OSCC showed scores 2, 3, and 4. Comparing with normal mucosa by Chi-square test showed a value of 40.0 with df = 4 and $P = 0.0001$. This showed that the cells of OSCC showed significantly higher positivity to GLUT-1 as compared to normal tissues [Table 3].

OSCC showed significantly higher expression for GLUT-1 as compared to PMD with $P = 0.0001$ [Table 4], which signifies that OSCC cells sustain hypoxia conditions better than PMDs. Comparison of different grades of OSCC revealed that the majority of cases of WDSCC had score 2, MDSCC had score 3, and PDSCC had score 4. Chi-square analysis showed that PDSCC had significantly higher expression for GLUT-1 as compared to better differentiated cases [Table 5]. Graph 1 shows IHC score and expression of GLUT 1 in normal, PMDs, and OSCCs.

DISCUSSION

Solid tumours include zones of poor perfusion, low pH,

Table 3: Comparison of expression of GLUT-1 between normal tissue and OSCCs

IHC Score	Normal %	OSCC %
Score 0	90	0
Score 1	10	0
Score 2	0	30
Score 3	0	46.7
Score 4	0	23.3
Total	100	100

Chi-square=40.0, df=4, *P*=0.0001

Table 4: Comparison of expression of GLUT-1 between PMDs and OSCCs

IHC Score	PMD %	OSCC %
Score 0	0	0
Score 1	76.5	0
Score 2	23.5	30
Score 3	0	46.7
Score 4	0	23.3
Total	100	100

Chi-square=35.005, df=3, *P*=0.0001

Table 5: Comparison of expression of GLUT-1 in different grades of OSCCs

IHC Score	WDSCC (%)	MDSCC (%)	PDSCC (%)
Score 0	0	0	0
Score 1	0	0	0
Score 2	80	10	0
Score 3	20	90	30
Score 4	0	0	70

Chi-square=32.810, df=4, *P*=0.0004

severe hypoxia, and nutrient exhaustion, and hence, these regions exhibit higher requirement of oxygen, equated to surrounding tissues. A neoplastic cell has plentiful properties, which brand it self-sustaining, and increased metabolic activity as an adaptation in the path. Energy for the same is provided by amplified utilisation of glucose. This increased demand is reinforced by transport of glucose into the neoplastic cells by a specific group of transport molecules labelled as GLUTs.

Glucose transporters are membrane proteins that convey hexose sugar across the plasma membrane and are classified as facilitative glucose transporters (GLUT family) and Na+ coupled glucose transporters (SGLT family). The GLUT family is composed of 13 components, among which GLUT-1 is the first to be cloned and most studied.[7] Normal cells like erythrocytes, germinal cells from the testis and lymph nodes, renal tubules, perineurium, endothelial cells in blood-brain barrier vessels, and salivary gland ducts express GLUT-1.[8] This signifies that normal cells with high metabolic activity express GLUT-1, and hence, it is considered as an indicator of high metabolic activity. The protein assists increased glucose transport during mitosis, differentiation, low perfusion, nutrient depletion, and carcinogenesis.[9]

The present analysis revealed that the normal tissue expressed positive staining in basal cells and a weak suprabasal staining confined to two layers only [Table 1]. Reisser C *et al*.[10] noted immunostaining in normal mucosa, confined to the basal compartment and the first suprabasal cell layer. Similar to our result, they noted higher staining for GLUT-1 in the dysplastic potentially malignant epithelium [Table 2].

OSCC cases in the present study showed a significantly increased immuno-reactivity as compared to normal mucosa with $P = 0.0001$ [Table 3]. A progressive increase in immunoreactivity for GLUT-1 was noted from PMD to OSCC with $P = 0.0001$ [Table 4], suggesting OSCC cells endure hypoxic conditions better than PMD. In the same path, Reisser C *et al*.[10] observed weak expression of GLUT-1 in normal mucosa and increasing expression in pre-neoplastic and head and neck SCC. On the contrary, Pariera KMA *et al*.[11] noted a greater expression of GLUT-1 in the dysplastic lesions than in the carcinoma and proposed that GLUT-1 is an essential protein in the initial stages of carcinogenesis.

Over-expression of GLUT enables a survival advantage for cells to sustain a high metabolic rate and fast growth in an atmosphere that is often critical to normal cells. Expression of GLUT-1 transporter protein is induced by certain oncogenes such as ras and src and regulated by growth factors (platelet-derived growth factor and epidermal growth factor), hormones, and metabolic signals.[6] Malignant cells over-express GLUT-1 molecules, which elucidates their increased glucose consumption to afford energy for proliferation and progression.[3]

GLUTs are stockpiled in dedicated endosomes in the cytoplasm and on stimulation transfigure as cell membrane protein and unmasking enhances its magnetism for glucose. Additional stimulation leads to an eventual increase in the synthesis of GLUT-1 mRNA.[3,8,9] This explains the localisation of GLUT-1 in the cytoplasm or cell membrane or both, and this may be associated with degree of hypoxia. The present study noted membrane and/or cytoplasm staining in immuno-positive cells.

A prostromal staining pattern with peripheral immune staining in a well-differentiated island and negative in the central keratin pearls was noted. The presence of glycogen is coupled to maturation and disappears with de-differentiation. Hence, glycogen in central keratin pearls is inversely correlated with GLUT-1 immune expression. In undifferentiated tumors, hypoxia stimulation creates

an antistromal pattern in areas devoid of squamous differentiation/keratinisation.[6,8,12]

GLUT-1 over-expression is a manifestation of tumour hypoxia and the adaptive upregulation of anaerobic glycolysis that may ultimately promote tumour cell survival.[6] High glycolytic activity produces high levels of lactate and Hþ ions that get transported outside the cell where they directly promote tumour aggressiveness through invasion and metastasis.[13]

Comparison between different grades of OSCC revealed a significantly increasing degree of immunoreactivity of GLUT-1 (a *P*-value of 0.0004) with a decreasing grade of differentiation [Table 5], which was in conjunction of the observation of Harshani *et al*.,[6] Angadi *et al*.,[12] and others. Usman *et al*.,[14] Tian M *et al*.,[15] Airley *et al*.,[16] Kunkel *et al*.,[17] and Choi *et al*.[4] found no correlation between GLUT-1 staining pattern and histopathological grades of OSCC.[5] On the contrary, a statistically significant relationship was seen with advanced clinical stages[14] and with metastatic lesions.[18]

Ganvir *et al*. noted principal countenance of GLUT-1 at the tumour margin than the centre. Tumour cells require additional nourishment in deep invasion areas with hypoxia in contrast to wide and shallow invasion areas in central parts of tumour. They postulated that GLUT-1 expression at invasion front was supplementary to progression and aggressiveness and also predicts prognosis.[13] GLUT-1 over-expression was associated with shorter survival and hence is suggested to be used as a predictive or negative biomarker of prognosis in OSCC.

The result of the present study showed that expression of GLUT-1 progressively increased in PMDs and OSCC. PDSCC showed a higher immunoscore compared to differentiated lesions, indicating the expression of GLUT-1 increased from normal to PMDs to WDSCC to MDSCC to PDSCC. The expression of GLUT-1 can be used as a prognostic negative biomarker.

GLUT-1 was found to have non-significant correlation to gender, age, use of alcohol, or regional reappearance but was seen to be prejudiced by rate of proliferation, oxygen supply, malignant conversion, progressive tumour stages, histological grade, clinical stage, lymph node involvement, tobacco use, and distant metastasis.[7] Yu M *et al*., in their analysis, found that GLUT-1 over-expression was associated with high aggressive and invasive potential of the lesion. Invasion and metastasis lead to nutrient starvation reflecting lack of energy; upregulation of GLUT-1 supports the metabolic consumption helping in homing of tumour cells: a potential mechanism for poor prognosis.[19] GLUT-1 over-expression is also hypothesised to be attributable with radio- and chemo-resistance caused by hypoxia.[17]

Kunkel *et al*. associated strong versus low expression of GLUT-1 and noted 2.65 times increased risk of tumour-related death in patients with strong expression. Similarly, moderate versus strong expression of GLUT-1 was studied, which showed 4.9 times increased risk of tumour-related death.[5,20]

CONCLUSION

GLUT-1 is registered as an autogenous marker of tumour hypoxia and proposes indication on tumour behaviour and prognosis. If used at the early stages during diagnosis, GLUT-1 can act as a marker for screening of high-risk OSCC. Interruption of glucose uptake by inhibiting/blocking GLUT proteins could revise the metabolism of malignant cells, which could be approached by blocking the signaling components using selective GLUT inhibitors or by inhibiting anaerobic glycolysis, which alkalises the tumour microenvironment, leading to enhanced sensitivity of chemotherapeutic agents on tumour cells.

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Conflicts of interest
There are no conflicts of interest.

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