Original Article

Can Increased Metabolic Status be a Grading Tool for Oral Squamous Cell Carcinoma? A Glucose Transporter 1 Immunoexpression Study

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Abstract

Background: Glucose transporter-1 (GLUT-1) is a GLUT protein whose expression is upregulated in malignant cells where enhanced uptake of glucose is observed. **Aim:** The aim of this study is to evaluate the expression of GLUT-1 protein in oral squamous cell carcinoma (OSCC) tissue sections using immunohistochemistry and to describe the relationship between increased metabolic status and the grades of OSCC. **Materials and Methods:** This is cross-sectional study with 76 formalin-fixed paraffin-embedded tissue blocks of OSCC, obtained from the archives of the department. All the cases were scored using Bryne's grading system by three oral pathologists independently. The tissue sections were then stained using immunohistochemistry with anti-GLUT-1 rabbit monoclonal antibody. **Results:** Staining intensity and localization of positively stained slides were evaluated. Overall, a significant correlation between Bryne's histopathological grading system for OSCC and GLUT-1 immunohistochemical expression was observed. Thus, high GLUT-1 expressions are observed with increasing grades of OSCC. **Conclusion:** This study shows that a significant positive correlation exists between GLUT-1 immunoexpression and histological grading of OSCC. Thus, GLUT-1 expression can be used as a diagnostic adjunct and prognostic marker for OSCC patients.

Keywords: *Glucose transporter 1, grading, immunoexpression, metabolic status, oral squamous cell carcinoma*

Introduction

Oral squamous cell carcinoma (OSCC) is a common malignant tumor which is estimated to be the third most common cancer of all cancers reported. The 5-year survival rate of OSCC is reported to be about 40%–50%.[1] This tumor has been shown to have rapid progression and significantly reduced oxygen concentration.[2] The rapidly progressive tumors can survive even hypoxic conditions due to hypoxia-related cellular adaptations which results in altered phenotype of the tumor and renders the tumor more aggressive with increased potential for invasion and metastasis. Warburg[3] reported that malignant cells show increased glucose uptake and enhanced glycolytic metabolism of carbohydrates, even in the presence of oxygen.

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Glucose transporter-1 (GLUT-1) is a GLUT protein and is composed of 14 members of the mammalian facilitative GLUT family.[4] It is the most dominant member of the family. GLUT-1 immunopositivity in malignant cells of OSCC indicates increased proliferative activity, increased energy demands and aggressive nature of the tumor. It has been found to be overexpressed in various malignant tumors such as nonsmall cell lung cancer, colorectal cancer, breast cancer, and OSCC.[5] Furthermore, its overexpression has been reported to be related to poor clinical prognosis in carcinoma patients.

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Due to increased incidence and poor clinical outcome in spite of the latest treatment modalities, there is an urgent need to identify high-risk patients and to find out new reliable and novel prognostic markers for better clinical outcomes.

Therefore, the aim of the present study was to evaluate the expression of GLUT-1 protein in OSCC tissue sections using immunohistochemistry and to find out whether increased metabolic status in such cases can be used as a grading tool for OSCC.

Materials and Methods

The present cross-sectional study was conducted in the Department of Oral and Maxillofacial Pathology of Kalinga Institute of Dental Sciences, Bhubaneswar, Odisha, India. Before the commencement of the study, required ethical clearance was taken from the Institutional Ethical Committee, Ref. No-KIMS/KIIT/IEC/27/2017.

Sample size

This study was conducted in 4 months between April and July 2018. The total number of samples collected at the Department of Oral Pathology Kalinga Institute of Dental Sciences, Bhubaneswar, Odisha, India during this time was 95. With a 95% confidence level and a confidence interval of 5%, the sample size needed was 76. Hence, a total of 76 formalin-fixed paraffin-embedded (FFPE) tissue blocks of OSCC were obtained from the archives of the department. Histopathologically, diagnosed cases of OSCC were scored using Bryne's grading system[6] by three oral pathologists independently and an average scoring was recorded for each case.

Immunohistochemical staining

Histological sections of 3-μm thickness were cut from the original FFPE blocks and mounted on poly-L-lysin coated positively charged slides before immunohistochemical staining. Antigen retrieval was done by pressure cooker technique using Tris-EDTA buffer at pH 9.0 after deparaffinization and rehydration. The sections were then subjected to peroxide block for 15 min to block endogenous peroxidize activity. The sections were then stained using a primary anti- GLUT GLUT-1 rabbit monoclonal antibody (PathNSitu Biotechnologies Pvt. Ltd., California, USA). The incubation time was 45 min after which the sections were treated by Poly Excel HRP/DAB detection system (PathNSitu Biotechnologies Pvt. Ltd., California, USA). Human cervical carcinoma sections and erythrocyte membranes served, respectively, as external and internal positive controls.

Immunohistochemical evaluation

The immunohistochemical evaluation was done by three observers who were blinded to their respective histopathologic grades. Samples were considered positive for the expression of GLUT-1 if cells showed brown staining on the cell membrane, cytoplasm, or nucleus. Scoring of GLUT-1 expression was evaluated by three independent observers. Five representative areas of the epithelium at the invasive front were evaluated with an Olympus CX-21 microscope under ×400. Following scores were used to categorize the specimen: 0 (0% of positive cells), 1 (1%–25% of positive cells), 2 (25%–50% of positive cells), and 3 (>50% of positive cells). The intensity of staining was scored 0 (no expression), 1 (mild expression, i.e., less than the intensity expressed by the internal control, endothelial cells); 2 (intense expression, i.e., if similar to that of endothelial cells expression). The location of staining was scored 0 for no staining, 1 for expression in membrane only, 2 for cytoplasmic positivity, and 3 for expression both in membrane and cytoplasm.

Statistical analysis

All the evaluations were scored and tabulated and sent for statistical analysis. Data analysis was performed using statistical software SPSS version 18.0 (Chicago, SPSS Inc). Mean values with standard deviations were calculated for all groups. Chi-square test was applied for analyzing the percentage of positivity, intensity of staining, and localization of staining. $P < 0.005$ was considered as statistically significant. The analysis of variance test was used to compare between overall GLUT 1 expression and different groups of carcinomas. Independent sample *t*-test and *post hoc* test were used to assess the significance of pairwise differences between the various study groups. A value of $P < 0.05$ was taken to be statistically significant. Pearson correlation was applied to assess the correlation between the Grades of carcinoma and overall GLUT 1 expression.

Results

In the present study, a total of 76 OSCC diagnosed cases comprising 53 males and 23 females with the mean age of 63.75 among males and 63.52 of females were included in the study. The data pertaining to age and gender of the patients is stated in Table 1. The inter-rater reliability coefficient for the three observers

Table 1: Distribution of age and gender in the study group

	n	Mean age±SD	*P*
Gender (*n*=76)			
Male	53	63.75±6.47	0.340
Female	23	63.52±5.34	

SD=Standard deviation

Table 2: Relationship between grades of oral squamous cell carcinoma with glucose transporter 1 expression

	Percentage of positivity cells			Glut 1 staining intensity		Location of Glut 1		
	<25%	25%-50%	>50%	Mild (%)	Intense (%)	Membranous (%)	Cytoplasmic (%)	Both (%)
Grade I (*n*=43)	35 (81.1)	8 (18.9)	-	33 (76.7)	10 (23.3)	35 (81.4)	8 (19.6)	-
Grade II (*n*=23)	7 (30.4)	14 (60.9)	2 (8.7)	16 (69.6)	7 (30.4)	7 (30.4)	14 (60.9)	2 (8.7)
Grade III (*n*=10)	-	-	10 (100)	-	10 (100)	-	2 (20)	8 (80)
P	0.00			0.01		0.00		

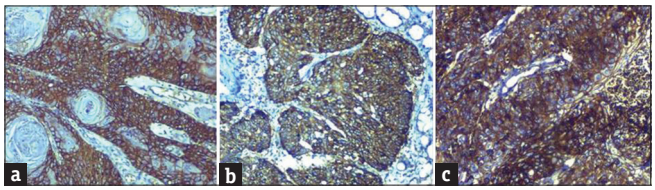

Figure 1: Expression of glucose transporter-1 in (a) Grade I, (b) Grade II, and (c) Grade III oral squamous cell carcinoma

was 0.796 (P = 0.452). On the basis of average Bryne's histopathological grading system[6] evaluated by the observers, 43 (56.57%), 23 (30.26%), and 8 (13.15%) of the cases belonged to Grade 1, II, and III, respectively.

Our findings of the immunohistochemical staining with GLUT-1 antibody are summarized in Figure 1 and Table 2. We observed 100% positivity of the sample for GLUT-1 antibody. The distribution of GLUT 1 positive cells in the tumor nests of Grade I OSCC tissues was restricted to the outer layer with no expression in the center with keratin pearls. In cases of Grade II OSCC, the expression of GLUT-1 in the tumor nests was found to be more diffuse in the inner layers with membrane positivity in the center and cytoplasmic in the peripheral areas. In Grade III squamous cell carcinoma, the expression in both cell membrane and cytoplasm was seen in most of the tumor cells. Nearly 81.1% of Grade I cases showed <25% positivity, whereas none of them showed >50% positivity. More than 50% positivity of tumor cells was observed to be the highest (100%) in Grade III cases. Approximately 23.3% of Grade I cases showed intense expression, 19.6% showed cytoplasmic staining, and none showed both membranous and cytoplasmic staining. All the Grade III cases and 69.6% of Grade II showed of the intense expression. Both Grade II and Grade III cases showed combined expression of both cytoplasm and membrane. When comparison of the location of GLUT-1 expression was done, it was observed that membranous expression was predominant in Grade I with 81.4%, the cytoplasmic expression being predominant in Grade II (60.9%) and finally, both the patterns combined are predominant in Grade III with 80%.

Overall, the correlation between Bryne's histopathological grading system for OSCC and GLUT-1 immunohistochemical expression proved to be statistically significant with a positive correlation coefficient of 0.885 (P = 0.00). Thus, high GLUT-1 expressions are observed with increasing grades of OSCC which is depicted in Figure 2.

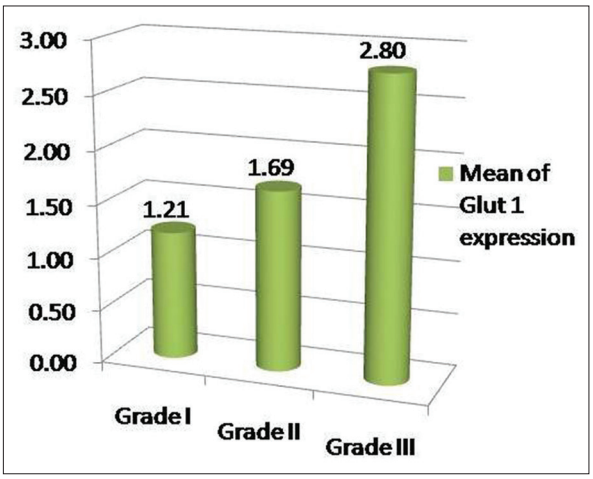

Figure 2: Comparison of Grades of oral squamous cell carcinoma with mean glucose transporter-1 expression

Discussion

GLUT-1, a GLUT protein has an important role in cellular growth.[3] It helps in glucose influx within the cells under stressful conditions which require higher metabolic requirements like during malignant transformation and continuous cell divisions. Because of its overexpression the tumor cells survive, as they are being supported by adequate energy which in turn helps the cell sustain its high metabolic growth rate.[7] Thus on basis of this important role of GLUT-1, it was hypothesized that it has an important relationship with malignancy. Numerous studies in the literature support this hypothesis and its association with malignancy.[8-10] This study was done to evaluate the expression of GLUT-1 in histopathological grades of OSCC.

Apart from histological grading, the tumor-node-metastasis classification is also used to determine tumor extent, treatment modality, and likely prognosis. Hence, no single gold standard exists as yet for prediction of tumor prognosis. Hence, on the basis of increased metabolic activity and increased expression

of GLUT-1 in malignancies, our study aimed to find whether increased metabolic status of the tumor can be useful as a grading tool for OSCC.

In the present study, a total sample of 76 cases were included with a mean age of 63.63 ± 5.9 years. Gender difference though present, with the male population being 69.7%, no statistically significant difference was observed. A higher predominance OSCC in male was in accordance with studies reported by Ayala *et al*.[11] and Malhotra *et al*.[12] In contrast, Harshani *et al*.[13] reported a higher female predominance.

In this study, all the 76 cases of OSCC showed GLUT 1 positivity. The higher percentage of positive cells was observed with increase in histopathological grade of carcinoma reflecting the higher proliferative activity of Grade III when compared to that of Grade I and Grade II. This was statistically significant with value of $P = 0.00$ [Table 2]. This was in consonance with studies by Angadi *et al*.[14] and Azad *et al*.[15] Similar inference was also drawn by Mendez *et al*.[16] and Rudlowski *et al*.[17] in cervical cancer.

In the present study, we observed that the intensity of staining progressively increased from Grade I to Grade III which was statistically significant. ($P = 0.01$). All 100% cases of Grade III cases showing intense staining which was in consonance with Harshani *et al*.[13] Angadi *et al*.[14] in their study on OSCC reported that 80% of well-differentiated OSCC showed mild staining which in consonance with our result where approximately 77% of Grade I OSCC showed mild staining intensity. There was an absence of GLUT 1 expression in the central keratinized area of keratin pearls suggesting the presence of differentiated mature cells in these areas [Figure 1]. The higher intensity of GLUT 1 staining indicates the severity of the disease. These results were also in accordance with several other studies.[15,16,18,19]

While evaluating the localization of GLUT-1 immunostaining, we observed that membranous pattern was observed in 81.4% of Grade I and 30.4% of Grade II cases while no membranous expression was seen in Grade III cases. Similarly, cytoplasmic expression was observed in 21.4% of Group I, 60.9% of Group II and 20.0% of Group III cases. Finally, both combined membranous and cytoplasmic expressions were observed in 8.7% of Grade II and 80% of Grade III cases with no such expression seen in Group I cases [Table 2]. Thus, our results show that as histopathological grading increases, membranous pattern of expression shifts to cytoplasmic and later to both together. Our results were further supported by studies done by Angadi *et al*.,[14] Azad *et al*.,[15] Ayala *et al*.,[11] and Vasconcelos *et al*.[20] However, contrary to this, no correlation with immunoexpression pattern of GLUT-1 was observed by Choi *et al*.[21] and predominantly membranous expression of GLUT 1 was found in all grades of OSCC by Harshani *et al*.[13] and Angadi *et al*.[14] This was explained by Azad *et al*.[15] in their study that anti-GLUT-1 antibody recognizes membrane-bound proteins on epithelial cells. During hypoxic conditions, the unmasking of GLUT protein occurs in the cell membrane to increase movement of glucose into the cell. Furthermore, this stimulation leads to translocation of GLUT from cytoplasmic vesicle to plasma membrane, thus suggesting that all the above-mentioned changes can lead to combined membranous and cytoplasmic expression because of co-localization of GLUT-1 within the Golgi bodies. In the present study, we did not observe any nuclear staining pattern which though has been reported earlier in a few cases of OSCC.[11,13]

When correlation was assessed between the overall GLUT 1 score and the grades of carcinoma, a positive correlation was found with Pearson correlation coefficient being 0.885 and $P = 0.00$. Thus, GLUT 1 may prove to be a useful adjunct to histopathological grading. Eckert *et al*.[9] from the results of their study suggested that GLUT-1 expression is an independent marker for routine assessment of OSCC. Various studies have proved that GLUT 1can be a potential prognostic marker. Kunkel *et al*.[8] and Ayala *et al*.[11] in their study on OSCC cases opined that GLUT-1 could be used as a negative indicator of prognosis. However, Choi *et al*.,[21] Kim and Kim[22] Tian *et al*.[23] in their study on expression of Glut-1 in OSCC reported no significant correlation between the immunostaining pattern of GLUT-1 and tumor differentiation.

The clinical significance of this study can be explained on the basis that OSCC is one of the most common malignant tumors of oral cavity these days with increasing incidence. Although there are treatment modalities such as surgical resection, radiotherapy, and chemotherapy, the long-term survival of OSCC patients is still poor. Therefore, pretreatment modalities such as the characterization of tumor hypoxia and detection of prognostic markers may be useful. With their application, a surgeon can establish a risk-adapted treatment strategy, and hence that best treatment can be provided to the patient at the earliest.

The present study has its own drawbacks, first the small sample size and secondly including more clinical parameters to the study with extended variables like the effect of alcohol and tobacco usage, to better understand the relationship between progression of tumor and

GLUT-1 as a prognostic marker. As we review the literature, mixed results are available from various authors, but still, more such studies should be conducted to validate the results.

Conclusion

GLUT 1 has been expressed in multitude of malignancies and also suggested to be considered as a negative predictor of prognosis. The over-expression of this marker depicts the aggressive behavior of the tumor. The present study witnessed a shift in the expression pattern from the membrane to the cytoplasm to both as we progress from Grade I to Grade III. This definitely is pointing toward the potential of this biomarker to establish the grade of OSCC and to predict the prognosis. However, more studies on this aspect will definitely aid in achieving the goal.

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Conflicts of interest

There are no conflicts of interest.

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