International Journal of Dentistry and Oral Science (IJDOS)
ISSN: 2377-8075

Studying The Expression of Glucose Transporter Type 1 in the Histological Grades of Oral Squamous Cell Carcinoma

Research Article

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Abstract

Introduction: One of the widespread features of human malignant tumors, including oral carcinoma, is the overexpression of glucose transporters, particularly Glut-1.
Objectives: This study aimed at investigating the prognostic value of Glucose transporter 1 expression in various histological grades of the oral squamous cell carcinoma (OSCC).
Materials and Methods: 45 Paraffin blocks, belonged to 45 patients who were diagnosed with OSCC, were selected for immune stain GLUT-1 test at the Pathology Department, Damascus University. Tumor grading scale by WHO 2010 was chosen in this study. The immuno-reactive score was calculated for GLUT-1 by multiplying proportion and intensity score of stain. The obtained data were processed and analyzed by SPSS 21. Kruskal-Wallis test was used to measure correlation between GLUT-1 staining and grade of tumor. $P<0.005$ was taken as significant.
Results: The expression of Glut-1 was observed in all grades of OSCC. In grade III, it was noticed that this expression is higher in comparison to grades I, and II. There was a moderate negative correlation between the age and the tumor grade, while gender was not significantly relevant to the tumor grade. The Glut-1 expression had no significant relation to age and gender.
Conclusions: As histological grades of OSCC increased, there were higher immune-histochemical staining scores. Thus, it can be relied on Glut-1 as a marker for the histological grades of OSCC, and the relevant overexpression can signify the malignant behavior of tumor.

Keywords: Glucose Transporter Type 1; Oral Squamous Cell Carcinoma; Prognosis.

Introduction

Oral cancer has the sixth highest mortality rate compared to other cancers [1], and oral squamous cell carcinoma (OSCC) is the fifth most common cancer worldwide [2], and the second most spread cause of morbidity and mortality [3].

Oral squamous cell carcinoma (OSCC) can be defined as an abnormal neoplasm that is accompanied by fast progression and insufficient oxygen levels in the affected cellular areas [4]. In a state of constant oxygen depletion, some malignant cells are able to survive by cellular changes resulting from hypoxia, which cause further aggressive phenotype, and leads to metastasis and invasion [5].

The primary source of energy for cells is represented by glucose, and it is generally broken into water and carbon dioxide to produce ATP in the presence of oxygen. This process is called oxidative phosphorylation. In the absence of oxygen, different conditions lead glucose to be converted to lactate, with the assistance of anaerobic glycolysis [3, 6]. Malignant cells consume glucose through glycolytic pathway, which enhances a condition of hypoxia.

GLUTs are a group of 14 proteins that assist the transport of glucose through the membrane, with no reliance on energy [7]. (GLUT-1) is one of the aforementioned proteins.

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Received: January 16, 2021
Accepted: January 29, 2021
Published: February 12, 2021

Citation: Shahed Tello, Ahmad Al Manadili. Studying The Expression of Glucose Transporter Type 1 in the Histological Grades of Oral Squamous Cell Carcinoma. *Int J Dentistry Oral Sci.* 2021;8(2):1555-1559. **doi:** http://dx.doi.org/10.19070/2377-8075-21000309

The overexpression of GLUT-1 was observed in a variety of cancers, such as stomach, colon, pancreas, lung, endometrium, breast, and particularly in SCC [8].

There is a link between the abnormal expression of GLUT-1 found in malignancies, and the invasion and metastasis of tumors, such as oral cancers [9]. The hypoxic microenvironment for the tumor is connected with this abnormal expression of GLUT-1. Multiple studies have found that restricting the expression deviations of GLUT-1 can enhance the efficacy of curing malignant tumors [10, 11]. Thus, suppressing GLUT-1 can be a new treatment strategy for head and neck cancer.

Previous studies conducted in Pakistan have not explored the overexpression of GLUT-1 in relation to oral SCC grades. Plus, some studies have clarified cytoplasmic, and others showed nuclear positivity of GLUT-1.

Objectives

The present study aimed to correlate the relation of immunehistochemical overexpression of GLUT-1 in the different histopathological grades of OSCC.

Materials and Methods

Ethical approval was gained from the Research and Ethical Committee of the Faculty of Dentistry at Damascus University, Syria. In addition to that, a written informed consent was obtained from all study participants (approval ref.: 1364/2018).

Study design: A descriptive cross-sectional study was conducted at the Department of Pathology, in cooperation with Maxillofacial Department at Damascus University. 45 samples related to patients who had been diagnosed with oral squamous cell carcinoma were used in this study. The age range of the patients was 40–86 years.

Study Procedures: All 45 samples used in this study can be classified as follows: 16 tissue biopsy samples were taken from the lip, 12 samples from the tongue, 10 samples from the buccal mucosa, and 7 samples from the hard palate. Paraffin blocks of diagnosed cases of OSCC were chosen from February 2017 to June 2018, and the relevant clinical and demographic details were gained. Histopathological grading by Bryne's Invasive Tumor Front Grading system (1989 and 1992) was conducted on H & E stained sections.

For immunohistochemistry, 3-micrometer-thick sections were cut from paraffin-embedded material, incubated with primary antibody GLUT-1 (1:100 dilutions) at 37 ºC for 1 hour. PBS buffer was applied for washing, and secondary antibody was used for ½ hour at 37 ºC. Slides were then counterstained with hematoxylin, dehydrated, mounted, and evaluated by two histopathologists. GLUT-1 staining was estimated on the basis of presence or absence of immune-stains in cell membrane-cytoplasm-nucleus at supra-basal level. Positive control was the staining of basal layer of epithelium.

Statistical Analysis: Data analysis was performed using SPSS version 25. Descriptive statistical analysis was carried out. Moreover, Kruskal-Wallis test was used to relate the expression of GLUT-1 with Bryne's grades. Dunnett's test (1967) was done with Bonferroni correction for multiple comparisons. Spearman correlation coefficient was used to study the relationship between tumor grade and age, while the Rank Biserial correlation test was used to study the relationship between tumor grade and gender. Level of significance and confidence interval were set at 5 and 95%, respectively. P value less than 0.05 was considered statistically significant.

Results

Differentiation of oral squamous cell carcinoma showed well differentiation in 15 (33.3%) cases, moderate in 15 (33.3%) cases, and poor in 15 (33.3%) cases. GLUT-1 was positive in 45 (100%). Intensity of GLUT-1 positivity was weak positive in 16 (35.5%) cases, mild positive in 17 (37.8%) cases, and strong positive in 12 (26.7%) cases.

-OSCC grade (n=15):

All cancerous cells showed positive expression for GLUT-1 in all 15 samples; 26.7% was mild, and 73.3% was weak (Figure 1).

-OSCC grade (n=15):

All cancerous cells showed positive expression for GLUT-1 in all 15 samples; 33.3% was weak, 6.7%was strong, and 60.0% was mild (Figure 2).

-OSCC grade (n=15):

All cancerous cells showed positive expression for GLUT-1 in all 15 samples; 26.7% was mild, and 7.3% was strong (Figure 3).

-OSCC grade:

The absence of GLUT-1 expression in keratin pearls (Figure 4).

Figure 1. Weak positivity for Glucose transporter-1 (GLUT-1) in all cancerous cells, grade 1 oral squamous cell carcinoma (OSCC) magnification ×400.

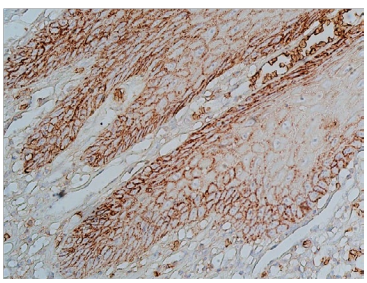


Figure 2. Mild positivity for Glucose transporter-1 (GLUT-1) in all cancerous cells, grade 2 oral squamous cell carcinoma (OSCC) magnification ×400.

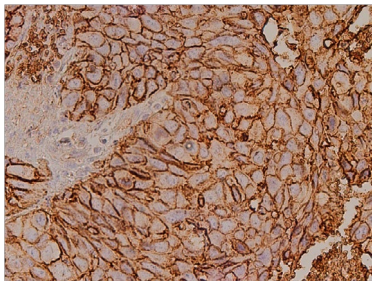


Figure 3. Strong positivity for Glucose transporter-1 (GLUT-1) in all cancerous cells, grade 3 oral squamous cell carcinoma (OSCC) magnification ×400.

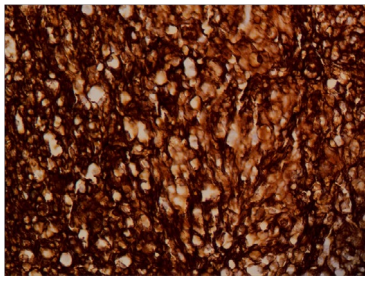
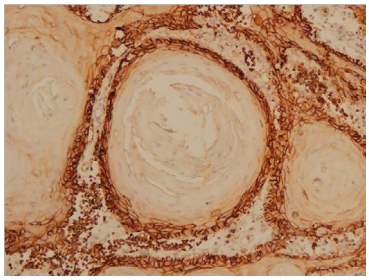


Figure 4. The absence of GLUT-1 expression in keratin pearls in OSCC grade 1 magnification ×400.



Discussion

Glut-1 is a high-affinity glucose transporter protein, and its function is significant in cellular growth [12]. Glut-1 supports the increase of glucose levels in the cells in difficult conditions that may necessitate stronger metabolic requirements, such as malignant transformation and constant cell divisions. Glut-1 overexpression maintains the cancer cells alive, as they get support through sufficient energy, that helps the cells keep the high increase of metabolic rate [13]. Accordingly, it was hypothesized that GLUT-1 has a significant role in preserving the malignant activity. Many studies from the literature promote this hypothesis, and the relation it has with malignancy [14-16]. The present study was performed to evaluate the expression of GLUT-1 in OSCC histopathological grades.

In the present study, a total sample of 45 cases were included with a mean age of 63.91 ± 13.44 years. Despite gender variation, with the male population being 66.7%, no statistically significant difference was observed. A higher dominance of OSCC among male patients goes in line with studies by Ayala et al., [17] and Malhotra et al [18]. On the other hand, Harshani et al [19] reported a higher dominance among females.

A study by Li et al observed the expression of GLUT-1 in a total of 25 cases examined of head and neck cancer, and the expression was mainly found in the membrane of the cell [20]. This is parallel to our study.

In this study, we noticed that the intensity of staining progressively increased from Grade I to Grade III which was statistically significant ($p<0.0005$). Dunnett's test (1967) was done with Bonferroni correction for multiple comparisons (Table 1).

Spearman correlation coefficient was employed to study the relation between tumor grade and age, while the Rank Biserial correlation test was used to study the relationship between tumor grade and gender (Table 2).

Spearman correlation coefficient was used to study the relation between the intensity of staining and age, while Rank Biserial correlation test was used to study the relationship between the intensity of staining with gender. That was carried out in each of the different tumor grades, in addition to the sample of the study as a whole. The tests did not show a correlation that is statistically significant between the intensity of staining with both age and gender (Table 3).

In the present study, there was an absence of GLUT-1 expression in the center of keratinized area of keratin pearls, indicating the existence of differentiated mature cells in these arears (Figure 4). The greater intensity of GLUT-1 expression refers to the gravity of the cancer. These results also conformed to several other studies [21-25].

Table 1. Pairwise comparison of coloring intensity.

Tumor Degree (average rank)		Test statistic	Adjusted P
1st (12.90)	2nd (20.47)	-7.567	0.279
	3rd (35.63)	-22.733	0
2nd (20.47)	3rd (35.63)	-15.167	0.002

Table 2. Correlation between tumor degree with age and gender.

Tumor Degree	Correlation with Tumor Degree		
	r	p	Significance
Age	-0.344*	0.021	Negative moderate correlation
Gender	0.173**	0.255	Non-significant
* Spearman correlation coefficient			
** Rank Biserial correlation coefficient			

Table 3. Spearman correlation coefficient between staining intensity with age and gender.

	Tumor Degree	Correlation with coloring intensity		
		r	p	Significance
Age	1st	0.052	0.853	Non-significant
	2nd	0.314	0.255	Non-significant
	3rd	0.052	0.853	Non-significant
	Total	-0.172	0.258	Non-significant
Gender	1st	-0.302	0.275	Non-significant
	2nd	-0.073	0.797	Non-significant
	3rd	-0.123	0.662	Non-significant
	Total	0.021	0.89	Non-significant
	3rd	0.123	0.662	Non-significant
	Total	0.095	0.533	Non-significant

GLUT-1 staining localization was also evaluated in this study, and we noticed that in all grades of OSCC, membranous expression prevailed. Our results comply with Harshani et al [19] and Angadi et al [26]. Nevertheless, in contrast to this, and with the rise in histopathological grading, a change occurs in membranous pattern of expression to cytoplasmic, and then to both as observed by Panda et al [21], Azad et al [22], and Vasconcelos et al [27].

Upon evaluating the correlation between the GLUT-1 score and carcinoma grades, a positive correlation was detected ($p<0.0005$). Therefore, GLUT-1 demonstrates to be a valuable accompaniment to histopathological grading. Kunkel at al [14] and Ayala et al [17] in a study they conducted on OSCC cases suggested that GLUT-1 can be used as a negative indicator for prognosis. Eckert et al [15] suggested that GLUT-1 expression represents an independent marker for regular assessment of OSCC. However, Tian et al [28] Kim and Kim [29] and Choi et al., [30] in their studies on GLUT-1 expression on OSCC mentioned no significant correlation between the GLUT-1 protein immunostaining pattern and tumor histopathological differentiation.

The clinical importance of this study may be justified on the basis that OSCC is among the most current malignant oral tumors with rising incidences. However, there are many treatment types such as radiotherapy, surgical resection, and chemotherapy, but OSCC patients' long-term survival remains weak. Thus, pretreatment types like the identification of tumor hypoxia and the observation of prognostic markers can be helpful.

Conclusion

The current study has found that GLUT-1 can be used as a prognostic marker for histological grades of OSCC, and that the overexpression of GLUT-1 may represent a possible indicator of the tumor's offensive behavior. However, further studies in this regard will absolutely help in achieving the objective.

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