Original Article

COMPARISON OF GLUT1 EXPRESSION IN ORAL SQUAMOUS CELL CARCINOMA WITH NORMAL MUCOSA

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ABSTRACT

Objective: In cancerous cells transport of glucose across the cell membrane is the first rate-limiting step, facilitated by a family of 14 GLUTs. Amongst them GLUT1 is the most important member. The aim of this study was to assess and compare GLUT1 status of oral squamous cell carcinoma (OSCC) patients with normal controls and correlate its expression with grade and stage of the tumor.

Material & Methods: This prospective cross-sectional study consisted of 60 subjects of which 30 were OSCC and 30 were healthy controls. Sampling was done using consecutive convenient sampling from June 2015 to February 2016. Grading and staging of all 30 cases of OSCC was carried out. Immunohistochemical technique was used for analyzing GLUT1 expression in OSCC cases and 30 normal controls. The comparison of scores was performed with Fisher's Exact test.

Results: Over expression of GLUT1 was detected in 17 (56.67 %) cases of OSCC as compared to normal mucosa with expression in basal layer only (p=0.016). Statistically insignificant results (p=0.571) were obtained with histopathological grades while significant GLUT1 expression (p=0.036) was found with respect to different clinical stages of the tumor.

Conclusion: A statistically significant relationship of GLUT1 expression was observed in OSCC cases which expressed more strongly with advanced clinical stages. However, no significant results could be obtained when GLUT1 expression was compared with grade of the tumor.

Key Words: GLUT1, Oral squamous cell carcinoma, Immunohistochemical stain.

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INTRODUCTION

Oral cancer (OC) is the 15th commonest malignancy, accounting for about 300,000 new cases annually [1]. Oral squamous cell carcinoma (OSCC) is the commonest histological type of all oral malignancies accounting for more than 90 % of such cases [2,3].

The prevalence of OC shows wide geographic variations, with highest incidence observed in South and Southeast Asia, areas of Eastern Europe (Hungary, Slovenia and Slovakia), Western Europe (France), Latin American countries (Brazil and Uruguay) and Caribbean regions [1,3].

In Asian countries, OC ranks 6th commonest malignancy contributing about 25 % to the overall cancer burden [3]. Highest incidence has been observed in countries like Pakistan, India, Sri-Lanka, Taiwan, Bangladesh and Thailand. However, now a decreasing trend has been observed in Sri-Lanka and Philippine [4]. In Pakistan, it contributes 5 % to the total cancer burden with a male to female ratio of 2:1 [5,6].

In malignant cells increased uptake of glucose is utilized in aerobic glycolysis for rapid cell division and is associated with over expression of GLUT1 [7]. It has been reported that the over expression of GLUT1 in OSCC is an indicator of poor prognosis. The increased expression can be used for predictive and prognostic purposes [8,9].

The facilitative glucose transporter proteins called GLUT family is among the major super families of the membrane-associated carriers that mediate the transport of glucose across the plasma membrane, having 14 known members in humans [10-12]. GLUT1 is encoded by SLC2A1 gene (solute linked carrier 2A1) which is over expressed in cancer cells [13]. Studies carried out in different regions have shown variation in its expression [8,9,14]. Rastogi and coworkers (2007) reported that anti-GLUT1 antibody reduces cell proliferation and induces apoptosis in cancer cells via anti-GLUT1 antibody synergism with anticancer drugs like paclitaxel, cisplatin and fasentin which is also a GLUT inhibitor [15]. GLUT1 (glucose transporter) has a pivotal role

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in immune-diagnosis and staging. Its prognostic and predictive role can be used for personalized treatment modalities [9,14,16,17].

The aim of this study is to assess and compare GLUT1 status of OSCC patients with normal controls and correlate its expression with histological grade and clinical stage of the tumor.

MATERIAL AND METHODS

This prospective cross-sectional study consisted of 60 subjects. Out of 60 total samples, 30 were OSCC and 30 were healthy controls. Consecutive convenient sampling from June 2015 to February 2016 was done. This study was conducted at Department of Pathology, Peshawar Medical College, Peshawar, Department of Oral Surgery Pakistan Institute of Medical Sciences, Islamabad, Department of Pathology, Pakistan Institute of Medical Sciences, Islamabad and Excel Labs, Islamabad.

The study was approved by the Institutional Ethical Committee. Informed consent from the patients was obtained before recording the data on a predesigned proforma. The integrity and impartiality of the research was ensured according to the Ethical Principles of Medical Research involving human subjects in accordance with the Declaration of Helsinki 1964.

Histologically diagnosed cases of OSCC, irrespective of age and gender were selected as subjects. Individuals with histologically declared normal mucosa were selected as controls. Subjects with previous history of receiving chemotherapy, radiotherapy, and immunotherapy, history of other types of cancer, alcoholics and smokers were excluded from this study [14,19,20].

Two sections each 4 µm thin from cases and controls were taken for both routine Hematoxylin and Eosin (H&E) and immunohistochemistry (IHC) staining at Histopathology Lab, Peshawar Medical college. Stained sections were observed by two histopathologists independently to assess the intensity and percentage scores. Human erythrocytes were utilized as a positive internal control.

GLUT1 expression was assessed by immunoreactive score (IRS) as adopted by Ohba *et al.* (2007) based on the presence or absence of the stain in cytoplasm and / or cell membrane and / or nucleus [9,21]. The IRS was a product of percentage of positive cells and intensity of staining. The percentage of positive cells were counted as follows:

- 0 pt – no cells with positive reaction
- 1 pt – up to 10 % positive cells
- 2 pt – 11–50 % positive cells
- 3 pt – 51–80 % positive cells; and
- 4 pt – over 80 % positive cells

The intensity of the staining reaction was scored as

- 0 pt.– no reaction colour
- 1 pt – reaction colour of low intensity
- 2 pt – moderately intense reaction colour
- 3 pt – intense reaction colour

The final score represented a product of the values, ranging from 0 to 12 points

- 0 pt – negative
- 1–5 pt – weakly positive; and
- 6–12 pt – strongly positive

Statistical package for social sciences (SPSS version 19) was used for the analysis of observed data. Comparison between the GLUT1 expression and categorical variables i.e. grade and stage were analyzed for statistical significance by applying Fisher's Exact test. Probability value of ($p \leq 0.05$) was considered as significant.

RESULTS

In this study, immunohistochemical expression of GLUT1 was observed in normal healthy mucosa (n=30) and OSCC cases (n=30). Out of 30 cases of OSCC 18 were females while 12 were males. Age distribution was between 34 and 80 years with 4 patients below the age of 40, while 26 were at or above 40 years of age. Out of 30 normal controls, 13 were females while 17 were males. Their age distribution was between 30 and 73 years with 7 patients below the age of 40 years, while 23 above 40 years of age. The expression of GLUT1 was unequivocally positive in the basal layer of normal mucosa. The intensity in normal mucosa gradually decreased near the surface and was least appreciable in the uppermost squamous epithelial cells. The keratin layer failed to express the stain (Figure-1).

In OSCC cases, GLUT1 expression was most prominent in cell membranes and intercellular bridges followed by cytoplasmic expression. No nuclear staining was observed. Well-formed keratin pearls failed to express the stain.

The comparison of GLUT1 expression in normal mucosa versus OSCC patients showed that staining was strong in 08 out of 30 normal cases whereas 17 cases of OSCC predominantly

expressed strong staining. Nine normal cases and 10 OSCC cases showed moderate staining. Normal cases showed more weak staining (n=10) as compared to OSCC cases (n=3). Among normal cases, only 03 revealed no expression. Our study showed positive correlation between GLUT1 expression and OSCC with a significant *p* value of 0.016 (Table 1).

Table-1: GLUT1 expression in OSCC patients and normal healthy controls.

IHC Scores	Normal Mucosa (%)	OSCC (%)	Total
Score 0	3 (10)	0 (0)	3
Score1	10 (33.33)	3 (10)	13
Score2	9 (30)	10 (33.33)	19
Score3	8 (26.67)	17 (56.67)	25
Total	30 (100)	30 (100)	60

Fisher's Exact test; p value=0.016, n=number of cases

Table-2: GLUT1 expression in relation to different clinical stages of OSCC patients.

IHC Scores	Stage I (%)	Stage II (%)	Stage III (%)	Stage IV (%)	Total
Score 0	0 (0)	0 (0)	0 (0)	0 (0)	0
Score 1	0 (0)	0 (0)	0 (0)	3 (100)	3
Score 2	0 (0)	1 (10)	5 (50)	4 (40)	10
Score 3	5 (29.41)	0 (0)	9 (52.94)	3 (17.64)	17
Total	5 (16.67)	1 (3.33)	14 (46.66)	10 (33.33)	30

Table-3: GLUT1 expression in relation to different clinical stages of OSCC patients.

IHC Score	Grade I (%)	Grade II (%)	Grade III (%)	Total
Score 0	0 (0)	0 (0)	0 (0)	0
Score 1	2 (66.67)	0 (0)	1 (33.33)	3
Score 2	8 (80)	1 (10)	1 (10)	10
Score 3	15 (88.23)	1 (5.88)	1 (5.88)	17
Total	25	2	3	30

Fisher's Exact test; p=0.571>0.05

In all the cases of stage I (n=5), intensity of staining of GLUT1 was strong. There was only 01 case with stage II that showed moderate staining. Out of 14 cases of stage III, 05 showed moderate staining, while in 09 cases strong staining was observed. There were 10 cases with stage IV, out of which 03 cases showed weak staining; 04 moderate and 03 strong staining. The pattern of GLUT1 expression revealed significant correlation with respect to clinical staging of OSCC. (*p*=0.036 by applying Fisher's Exact test) (Table 2).

Out of 25 cases with Grade I, 02 cases showed negative expression, 08 moderate and 15 were strongly positive (Figure 2). In Grade II moderate and strong expression was seen in only 01 case each. In Grade III 01 case each was seen with weak, moderate and strong expression. Statistically insignificant (p=0.571 > 0.05) results were obtained while comparing histopathological grades with immunohistochemical scores of GLUT1 in patients of OSCC (Table-3).

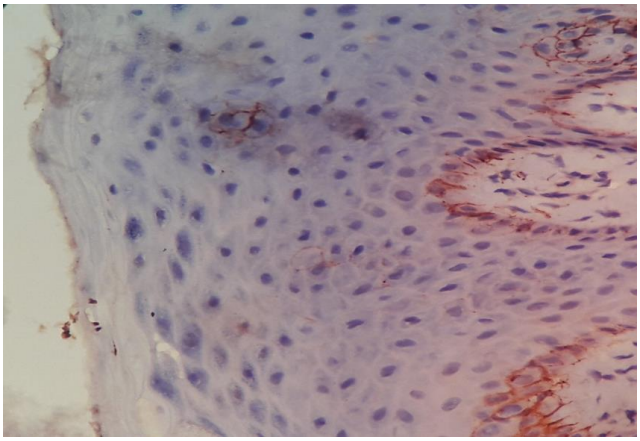

Figure-1: Normal mucosa. Negative GLUT1 staining 40X.

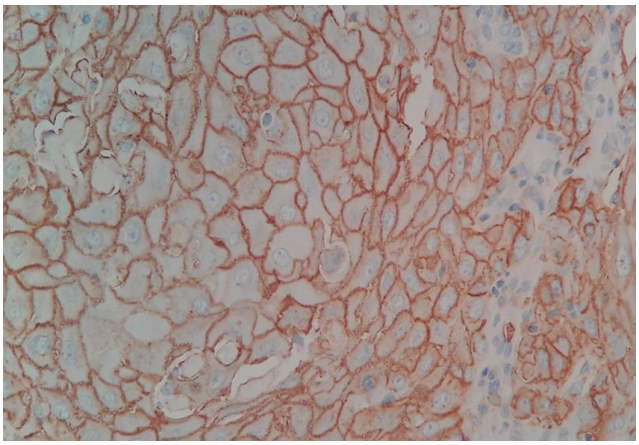

Figure-2: OSCC. Strong GLUT1 immunoreactivity (cytoplasmic and membranous); 40X.

DISCUSSION

Our results revealed a positivity of GLUT1 expression in 100 % of OSCC cases and 90 % positivity in normal controls. The statistical correlation of OSCC cases versus normal mucosa was significant (*p*=0.016) with a strong GLUT1 expression of score 3 in 56.67 % of OSCC cases. This is in agreement with a study conducted by Ayala *et al*. (2010), who found that GLUT1 expression was strong in 50.3 % OSCC cases and weak in the normal adjacent epithelium [14].

The studies performed in India and Portugal have also found a highly significant correlation of GLUT1 expression between normal and OSCC cases

with more than 90 % over expressed. Their findings are consistent with our results.

In our study, staining was predominantly membranous with no nuclear staining which is identical to the study done by Harshani and coworkers in 2014, in which all cases showed membranous staining while only one case presenting with nuclear staining. However, in this respect our study contrasted with Ayala and colleagues in 2010, who obtained 50.3 % cases as membranous stained and 49.7 % as nuclear stained.

The current study revealed a positive correlation between GLUT1 expression and OSCC TNM staging with *p* value of 0.036 using Fisher's Exact test, which is in favor of works performed in India by Angadi et al in 2015, Azad et al in 2016, Choi et al in 2007, Harshani *et al*. (2014), Kunkel *et al*. (2002) [9,19,,23-25]. However, it is in contrast to the study done by Ohba and colleagues as well as Demeda and coworkers, who evaluated the association between GLUT1 expression with respect to the depth of OSCC, they found no positive correlation between GLUT1 expression and the tumor TNM staging [21,26].

According to our study, no significant correlation between GLUT1 staining pattern and histopathological grades of OSCC was observed. Similar results have been revealed by the work conducted by Kunkel *et al.* (2002), Choi *et al.* (2007), Demeda *et al*. (2014) and Angadi *et al.* (2016), where GLUT1 immunohistochemical staining was determined in OSCC cases and no positive correlation was found between GLUT1 expression and tumor grade [23-26].

However, regarding correlation between GLUT1 immunostaining and tumor grade the studies done by Harshani and coworkers as well as Azad and his colleagues obtained a statistically significant association in contrast to our study.

CONCLUSION

A statistically significant relationship of GLUT1 expression was observed in OSCC cases and more strongly expressed with advanced clinical stages. However, no significant results could be obtained when GLUT1 expression was compared with grade of the tumor.

AUTHORS CONTRIBUTION

Khalid Usman: Director and incharge of project
Sadaf Alam: Paper writing and statistical Analysis
Farzana Salman: Data collection and paper writing
Muhammad Mumtaz Khan: Principal author review of manuscript and proof reading
Munazza Khattak: Entry of DATA in SPSS
Abbas Saleem & Zafar Ali Khan: Literature review

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