ORIGINAL ARTICLE

Glucose Transporter 1 Overexpression in Oral Squamous Cell Carcinoma

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ABSTRACT

Objective: To determine association of immunohistochemical over expression of GLUT 1 (Glucose transporter 1) in oral squamous cell carcinoma (SCC) with histopathological grade and smoking.

Study Design: Descriptive cross-sectional study.

Place and Duration of Study: Pathology Department, King Edward Medical University (KEMU), Lahore, from January 2018 to July 2018.

Methodology: Paraffin blocks of diagnosed cases of oral SCC presenting at Pathology Department, KEMU, were selected for immunostain GLUT 1. Tumor was graded by WHO 2010 grading. Immunoreactive score (IRS) was calculated for GLUT 1, by multiplying proportion and intensity score of stain. Data was analysed by SPSS 21. Chi-square test was used to measure correlation between GLUT 1 staining, smoking and grade of tumor. P≤0.005 was taken as significant.

Results: A total of 60 biopsies were included in the study. GLUT 1 was positive in 52 (86.6%) and negative in 8 (13.3%) biopsies. When differentiation of tumor was compared with GLUT 1 positivity with the help of Chi-square test p<0.001 and 95% CI, out of 52 positive biopsies 32 (61.5%) were well, 18 (34.6%) were moderately and 2 (3.8%) were poorly differentiated. GLUT 1 was positive in 43 (82.7%) and only 9 (17.31%) of non-smokers. GLUT 1 was negative in 7 (87.5%) smokers and positive in only 1 (12.5%) of smokers.

Conclusion: GLUT 1 is positive diffusely in oral SCC with higher expression in lower grades of tumor. As the tumor loses squamous differentiation, it also loses GLUT 1 receptors and thus expression. Smoking has no significant relation with tumor differentiation or GLUT 1 expression.

Key Words: *GLUT 1, Smoking, Squamous cell carcinoma.*

INTRODUCTION

Most common malignancy, around 95%, of oral cavity is squamous cell carcinoma (SCC). It is the fifth most common cancer worldwide.[1] Incidence of oral cancer is around 3% of all cancers in developed world; but in Asian countries like India and Bangladesh, its incidence is near 40%. In Pakistan, prevalence of SCC is reported to range from 8-22% of oral malignancies, making it the second most common cause of morbidity and mortality.[2] Oral SCC develops as a result of tobacco exposure, immunodeficiency, multiple mutations, genetic and epigenetic changes. Cells primarily use glucose as energy source which converted to water and carbon dioxide to produce ATP (adenosine triphosphate) in the presence of oxygen by the process of oxidative phosphorylation. In the absence of oxygen, gluoce is converted to lactate with the help of anaerobic glycolysis even in presence of ample oxygen.[2,3] Tumor cells rapidly consume glucose using glycolytic pathway and thus develop a state of hypoxia around them. Glucose transporter 1 (GLUT 1) is a protein located on cell membrane of mammalian cells.[3] It helps in transport of glucose across plasma membrane.[4] More glucose uptake requires more glucose transporters like GLUT 1, which decreases apoptosis and promotes tumor survival. Since cancer cells divide rapidly and exhibit glucose hunger, it show increase expression of GLUT 1 in all layers of epithelium as compared to normal epithelium which shows mild basal (less than 1/3) expression with the help of immunohistochemical stain.[5] GLUT 1 over expression has been observed in many cancers like pancreas, stomach, colon, lung, stomach, breast, endometrium, and especially in SCC.[6] Increased lactate production in tumor cells leads to increased tumor acidification, resulting in aggressive behaviour like invasion and metastasis. Increased GLUT 1 over-expression in tumors is a marker of poor prognosis either due to early lymph node metastasis, invasion or recurrence.[7] Hypoxic tumors have high levels of GLUT 1 and they show poor response to surgery and radiotherapy than non-hypoxic tumors.[8]

Previous studies, done in Pakistan, have not analysed GLUT 1 overexpression with grades of oral SCC and smoking context. In addition, some studies have shown cytoplasmic and some have revealed nuclear positivity of GLUT 1. This study was conducted to correlate immunohistochemical overexpression of GLUT 1 in oral squamous cell carcinoma with histopathological grade and smoking.

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METHODOLOGY

It was a descriptive cross-sectional study conducted at Department of Pathology, in collaboration with Maxillofacial Department, King Edward Medical University, Lahore, from January 2018 to July 2018. The study was approved by Ethical Approval Committee of KEMU. A total of 60 patients were included with the help of non-probability purposive sampling. All oral squamous cells carcinoma biopsies received at Pathology Department, King Edward Medical University. Patients who did not give consent, autolysed or inadequate oral biopsies were excluded from the study. Paraffin blocks of diagnosed cases of oral SCC were selected from January 2018 to July 2018, for immunostain GLUT 1, using avidin biotin technique. Sections were cut at 3 micrometer thickness, incubated with primary antibody GLUT 1 (1:100 dilutions) at 37°C for 1 hour. PBS buffer was used for washing and secondary antibody was applied for ½ hour at 37°C. Slides were then counter-stained with hematoxylin, dehydrated, mounted and examined by two histopathologists. GLUT 1 staining was evaluated on the basis of presence or absence of immunostains in cell membrane/cytoplasm/nucleus at suprabasal level. Basal layer of epithelium staining was taken as positive internal control. 100 cell focus with highest intensity of color was counted and percentage of positive cells (intensity and proportion of staining) was calculated. Proportion score was calculated as 0: 0-10%, 1: 11-25%, 2: 26-50%, 3: 51-75%, 4: 76-100% of cells. Intensity score was measured as 0: Negative, 1: Mild/ Weak positive, 2: Moderate, 3: Strong. Immuno-reactive score (IRS) was calculated by multiplying proportion and intensity score and measured as IRS <8: weak positive; and IRS >8: strong positive.

Data was analysed by SPSS 21. Chi-square test was used to determine association among GLUT 1 staining, smoking and grade of tumor. P <0.005 was taken as significant. Mean and ±SD was calculated for quantitative variables like age. Frequencies and percentages were calculated for qualitative variables like gender, grade of tumor, intensity score of GLUT 1, and smoking. Association between smoking and differentiation of tumor was analysed with the help of cross-tabulation, keeping the p-value <0.001 and contingency coefficient of .727.

RESULTS

A total of 60 biopsies were included in the study. Mean age of the group subjects was 53.45 ±14.3 years. Males

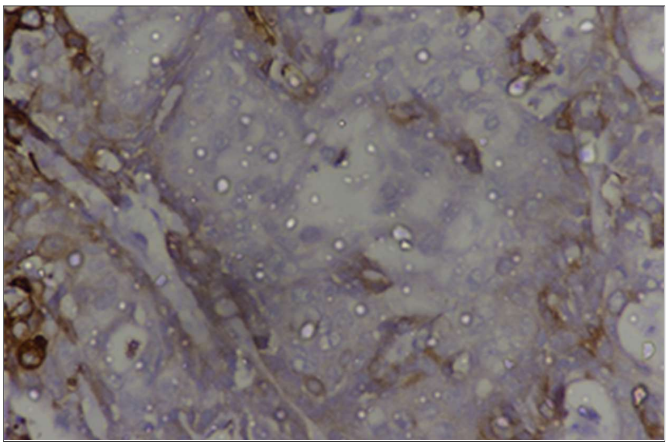

Figure 1: Negative GLUT 1 staining.

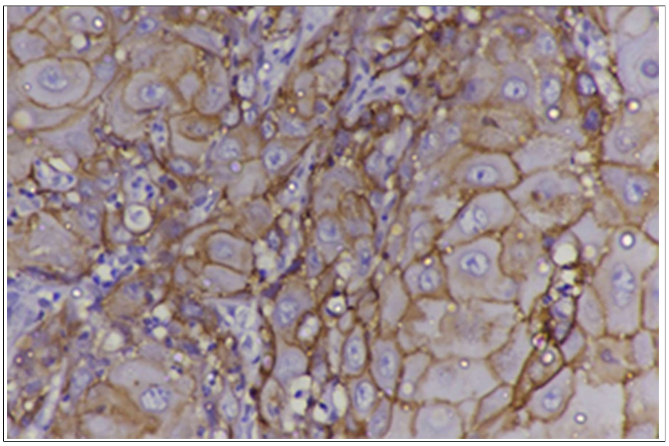

Figure 3: Moderate GLUT 1 staining.

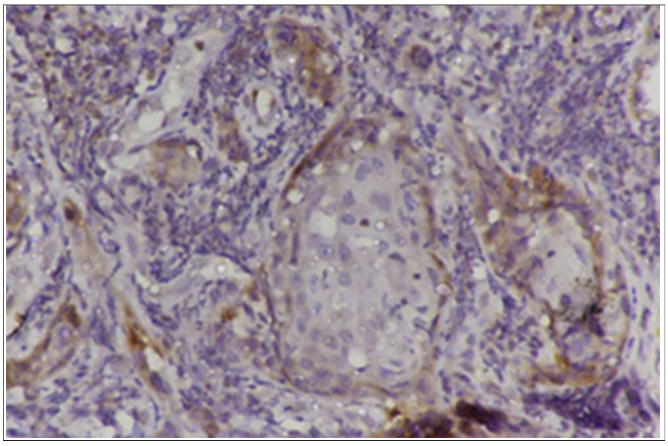

Figure 2: Mild GLUT 1 staining.

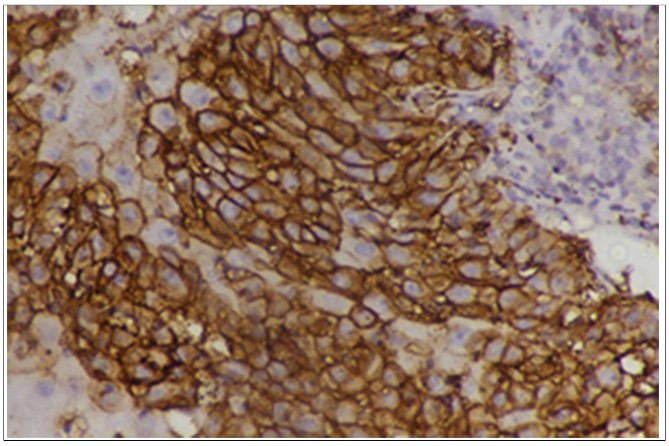

Figure 4: Strong GLUT 1 staining.

were 43 (71.6%), and 17 (28.3%) were females. 10 (16.6%) were smokers (for last five years) and 50 (83.3%) were non-smokers. Differentiation of squamous cell carcinoma showed well differentiation in 38 (63.3%), moderate in 19 (31.6%) cases and poor in 3 (5.1%) cases. GLUT 1 was positive in 52 (86.6%) and negative in 8 (13.3%) biopsies (Figure 1). Intensity of GLUT 1 positivity was weak in 20 (38.46%) cases (Figures 2 and 3) and strong positive in 32 (61.5%) cases (Figure 4). When differentiation of tumor was compared with GLUT 1 positivity with the help of Chi-square test p <0.001, out of 52 positive biopsies, 32 (61.5%) were well, 18 (34.6%) were moderately and 2 (3.8%) were poorly differentiated. GLUT 1 was positive in 43 (82.7%) and only 9 (17.31%) of non-smokers. GLUT 1 was negative in 7 (87.5%) smokers and positive in only 1 (12.5%) of smokers.

Among non-smoker patients samples (n: 50/83.3%), 31 (62%) were well, 18 (36%) were moderate and only 1 (2%) was poorly differentiated. Smokers' (n: 10/16.6%) biopsies exhibited well differentiated morphology in 7 (70%), moderate in 1 (10%) and poor in 2 (20%) cases only.

DISCUSSION

Cells primarily use glucose as energy source which converts to water and carbon dioxide to produce ATP (adenosine triphosphate) in the presence of oxygen by the process of oxidative phosphorylation. In the absence of oxygen, gluoce is converted to lactate with the help of anaerobic glycolysis even in presence of ample oxygen.[2,3] Increased lactate production in tumor cells leads to increased tumor acidification, resulting in aggressive behaviour like invasion and metastasis.[7] Cancer cells exhibit glucose hunger as they require energy to rapidly proliferate. Glucose transporters (GLUTs) are translocated from intracellular nearly undetectable levels to plasma membrane in cancer cells. GLUT 1 can alter glucose influx under certain conditions like mitosis, meiosis, malignant transformation because these conditions have higher metabolic requirements. Hypoxic tumors have high levels of deregulated GLUT 1 and they show poor response to surgery and radiotherapy than non-hypoxic tumors.[8] GLUT 1 overexpression also causes chemoresistance.

Many studies have shown to decrease chemoresistance significantly (p <0.01) when GLUT 1 expression is decreased in cancer cells, thus showing its therapeutic implications, especially in head and neck cancers.[9] Normal presence of GLUT 1 in basal layer of epithelium suggests that proliferation of immature epithelium is associated with transport of glucose from basement membrane to upwards in epithelial layers. As epithelium matures in suprabasal layers, expression of GLUT 1 decreases and vanishes ultimately. This indicates that cell maturity and decreased membrane staining may be an indicator of low proliferative potential and thus favourable prognosis. On the contrary, high GLUT 1 expression is associated with higher grade, advanced tumor stage, metastasis and thus poor survival.[10] Tumor hypoxia, initially leads to unmasking of GLUT proteins. Later on, there is translocation of glucose transporters from cytoplasmic vesicles to plasma membrane and increase synthesis of GLUT 1 mRNA. This process is directly propotional to duration and extent of hypoxia. Co-localisation of GLUT 1 with golgi complex leads to combined membranous and cytoplasmic overexpression.[8]

According to this study results, GLUT 1 was positive (suprabasal epithelium) in membranous pattern only in 86.6% (n: 52) and negative in 13.3% (n: 8) biopsies. This is in contrast to findings of Azad who reported cytoplasmic and combined (cytoplasmic + membranous) pattern of staining.[11] As far as percentage of positive cells is concerned, studies have shown 100% positivity as narrated by Angadi, 96% by Harshani, and 70% by Demeda (p <0.05).[5,12,13]

GLUT 1 is stronger in central areas of tumor as compared to peripheral region. Centre of tumor and necrotic zones are located away from blood vessels and express hypoxic zones that upregulate GLUT 1. Intensity of GLUT 1 positivity was strong positive in 61.5% (n:32) and weak positive in 38.46% (n:20) in the present study. Huan reported 85.82% strong positivity and 52.78% cases to be weak positive for GLUT 1 in cervical SCC.[14] Intensity of GLUT 1 is variable in various studies. Li X and Harshani have reported strong intensity in all cases.[10,12] Meier and Demeda observed weak to negative staining in oral canine SCC and human SCC, respectively.[15,13] Many studies like these by Angadi and Abdou (p=0.03) *et al*. have shown direct relation between staining and grade or tumor differentiation.[5,16] In this study, when differentiation of tumor was compared with GLUT 1 positivity, with the help of Chi-square test p <0.005 and 95% CI, out of 52 positive biopsies 32 (61.5%) were well, 18 (34.6%) were moderately and 2 (3.8%) were poorly differentiated. Positivity of GLUT 1 reduced as the grade increased, indicating that they are inversely proportional to each other. Demeda, Meier and Airley have reported no correlation between grade and GLUT 1 positivity.[13,15,17] The present study concurs with results of Vanconcelos and Brands.[18,19]

According to the present findings, GLUT 1 was present in 96.1% of well and moderately differentiated (low grade) and 3.8% positive in poorly differentiated (high grade) tumors. It is infer that as the tumor loses its squamous differentiation, it loses GLUT 1 staining as well. Azad found strong correlation between tobacco users and GLUT 1 staining.[11] There was no significant correlation between these two. GLUT 1 was positive in 17.31% (n=9) of smokers and 82.7% (n=43) of non-smokers. This contrast could be due to small number of smoker

patients in this study or other factors are responsible for dysplasia and anaplasia in oral cancers of the local population, since many studies have indicated the role of other GLUT members in oral cancers.[20]

CONCLUSION

GLUT 1 is positive diffusely in oral SCC with higher expression in lower grades of tumor. As the tumor loses squamous differentiation, it also loses GLUT 1 receptors and thus expression. Smoking has no significant relation with tumor differentiation or GLUT 1 expression.

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