INTRODUCTION

Glut-1 is a glucose transporter protein which is one of the 14 members of the mammalian facilitative glucose transporter family. Glucose uptake in nearly all cells is mediated by Gluts. Glut-1 positivity in malignant cells revealed by immunohistochemistry (IHC) indicates increased proliferative activity, energy requirements and aggressive behavior. The influence of Glut-1 on prognosis and its use as a biomarker may be a manifestation of tumor hypoxia and the adaptive upregulation of anaerobic glycolysis that may ultimately promote tumor cell survival, suggesting that Glut-1 may be considered to be a negative biomarker of prognosis in patients with head and neck squamous cell carcinoma (HNSCC). Detection of these metabolic changes may be used to provide diagnostic, therapeutic and prognostic information.

The aim of our study was to evaluate the expression of Glut-1 in patients with different clinical stages and histopathological grades of OSCC.

MATERIALS AND METHODS

In the present study, 30 patients of OSCC and 30 normal subjects (controls) were included. After obtaining the informed consent from the patients, the history and clinical findings of each patient was recorded. These cases were staged clinically based on Tumor Node Metastasis (TNM) classification and for histological grading Broder’s grading system (1927) was followed. These cases were confirmed by corresponding Haematoxylin and Eosin sections [Figure 1]. The control group included 30 apparently normal persons who visited our Dental College for the purpose of extraction of impacted third molars. The traumatized soft tissue which was obtained along with the extracted tooth was fixed, processed and stained with routine immunohistochemical procedure using GLUT-1 primary antibody.

Immunohistochemical staining

Immunohistochemical staining was performed using Glut-1 antibody.
Three micrometer paraffin embedded sections were used for staining. The sections were dewaxed in xylene, rehydrated through decreasing concentrations of alcohol. Immunohistochemical staining for Glut-1 was done using an avidin-biotin technique. The sections were micro waved for 4 cycles of 5 minutes each in Tris-buffer, pH 9.0-9.2 for antigen retrieval. The sections were allowed to cool and endogenous peroxidase activity was blocked by immersion of slides in 3% H2O2 and then incubated in power block. The sections were then incubated in primary antibody Glut-1 (1:100 dilution) at 37oC for an hour. After washing with PBS buffer, the secondary antibody was applied for 30 min at 37oC. The staining for Glut-1 was visualized with DAB chromogen. Specimens were counterstained with Mayers hematoxylin then dehydrated in increasing concentrations of alcohol and mounted with cover slip using DPX.

Esophageal squamous cell carcinoma was taken as positive control and negative controls were obtained by omitting the primary antibody [Figure 2].

Glut-1 staining was evaluated on the basis of presence or absence of staining in the cell membrane/nucleus/cytoplasm. Random fields were chosen and 300 cells were counted. The percentage of positive cells were then calculated and graded.

Two observers independently evaluated the staining, their intensity and the average of the observations were taken. The intensity was graded in all the cases from 0–3, that is, with ‘0’ to represent negative staining (less than 10% positive tumor cells), 1 (10–25% positive tumor cells), 2 (25–50% positive tumor cells) and 3 (more than 50% positive tumor cells) to represent mild, moderate and intense staining respectively depending on the percentage of tumor cells that expressed the protein. Data were analyzed statistically using the Chi-square test. A P value of less than 0.05 was considered to be significant.

RESULTS

Table 1 shows the clinical details of the 30 patients diagnosed histopathologically as OSCC with different clinical stages and histopathological grades.

Immunohistochemical expression of Glut-1 was studied in normal mucosal tissues (N = 30) and OSCC cases (N = 30). Normal mucosal epithelium showed undetectable or weakly detectable Glut-1 expression in supra basal layers, thus a predominant basal staining was seen. [Figure 3].

Out of the 30 normal cases, 13 were males and 17 were females. The age distribution was between 18 years and 67 years. Out of them 12 cases were less than 40 years and 18 cases were more than 40 years.

Table 2 shows a comparison of the expression of Glut 1 in normal persons and in patients with OSCC. Intensity of staining was observed to be 0 in 18 normal cases, 1 in 5 normal cases, 2 in 4 normal cases, 3 in 3 normal cases. Intensity of staining was observed to be 0 in 1 OSCC case, 1 in 10 OSCC cases, 2 in 14 OSCC cases, 3 in 5 cases of OSCC. A significant difference was observed between normal patients and in patients with OSCC patients with respect to immunohistochemistry scores (Chi-square test; P = 0. 00004).
Out of 30 cases of OSCC; 29 cases expressed cytoplasmic staining with predominant membrane staining pattern whereas only one case expressed nuclear staining.

Table 3 shows the comparison of expression of Glut-1 with respect to clinical staging of OSCC. Out of the 10 cases with stage I, intensity of staining was observed to be 0 in 1 case, 1 in 8 cases, 2 in 1 case. Out of 6 cases with stage II, intensity of staining was 1 in 1 case, 2 in 5 cases. Out of 12 cases with stage III, intensity of staining was 1 in 1 case, 2 in 8 cases and 3 in 3 cases. Out of 2 cases with stage IV, intensity of staining was 3 in 2 cases. A significant difference was observed between clinical staging with respect to immunohistochemistry scores. (Chi-square test; \(P=0.0004\)).

Table 4 shows the comparison of expression of Glut-1 with respect to histopathological grades of OSCC. Out of the 14 cases with grade I, intensity of staining was observed to be 0 in 1 case, 1 in 9 cases, 2 in 4 cases. Out of 12 cases with grade II, intensity of staining was 1 in 1 case, 2 in 10 cases, and 3 in 1 case. Out of 4 cases with grade III, intensity of staining was 3 in 4 cases. A significant difference was observed between histological grades with respect to immunohistochemistry scores. (Chi-square test; \(P=0.00004\)).

**DISCUSSION**

Presently TNM classification and histological typing, considered as prognostic markers are not sufficient for exact prediction of prognosis in OSCC. Glut-1 expression is seen...
in patients with HNSCC; however the prognostic value of this parameter has not been analyzed systematically for this tumor type. The current study reveals that Glut-1 can be used as a prognostic marker or as a negative biomarker in OSCC.

Glucose transporters which are membrane proteins active in the transport of hexoses such as glucose and fructose across plasma membranes are divided into two families: Facilitative glucose transporters (Glut family) and Na⁺ coupled glucose transporters (SGLT family). Glut-1 is also called as erythrocyte, brain or Hep G2-type glucose transporter. Glut-1 is a glucose transporter protein with high affinity and clear potential to provide cellular growth advantages. Over expression may play a role in survival of tumor cells by providing adequate energy supply which supports their high metabolic rate and fast growth in an environment that often is less than ideal from a physiologic stand point or not natural. Direct link is seen between Glut-1 over expression and malignant transformation process. Enhancement of glucose utilization, especially of glycolytic (anaerobic) metabolism is widespread characteristic of malignant cells. Function and expression of Glut-1 is regulated by number of physiological and patho-physiological conditions.

Altered expression of glucose transporter protein has been described in different tissues under various conditions such as cells undergoing transformation by oncogenes, hypoxia and exposure to insulin. It is a potential endogenous marker of hypoxia. Expression of Glut-1 transporter protein is induced by certain oncogenes such as ras and src and regulated by growth factors such as platelet-derived growth factor and epidermal growth factor. Changes in Glut-1 expression and rates of glucose transport are affected by growth rates, transformation and malignancy.

The glycogen content in both normal mucosa and pre neoplastic lesions was always inversely correlated with Glut-1

**Table 3: Comparison of expression of Glut-1 with respect to clinical staging of Oral Squamous Cell Carcinoma**

| IHC score | Stage I | %     | Stage II | %     | Stage III | %     | Stage IV | %     | Total | %     |
|-----------|---------|-------|----------|-------|-----------|-------|----------|-------|-------|-------|
| Score 0   | 1       | 100.00| 0        | 0.00  | 0         | 0.00  | 0        | 0.00  | 1     | 3.33  |
| Score 1   | 8       | 80.00 | 1        | 10.00 | 1         | 10.00 | 0        | 0.00  | 10    | 33.33 |
| Score 2   | 1       | 7.14  | 5        | 35.71 | 8         | 57.14 | 0        | 0.00  | 14    | 46.67 |
| Score 3   | 0       | 0.00  | 0        | 0.00  | 3         | 60.00 | 2        | 40.00 | 5     | 16.67 |
| Total     | 10      | 33.33 | 6        | 20.00 | 12        | 40.00 | 2        | 6.67  | 30    | 100.00|

Chi-square=30.0213, df=9, P=0.0004, Chi-square test, P=0.0004, Significant

**Table 4: Comparison of expression of Glut-1 with respect to different histopathological grades of Oral Squamous Cell Carcinoma**

| IHC score | Grade I | %     | Grade II | %     | Grade III | %     | Total | %     |
|-----------|---------|-------|----------|-------|-----------|-------|-------|-------|
| Score 0   | 1       | 100.00| 0        | 0.00  | 0         | 0.00  | 1     | 3.33  |
| Score 1   | 9       | 90.00 | 1        | 10.00 | 0         | 0.00  | 10    | 33.33 |
| Score 2   | 4       | 28.57 | 10       | 71.43 | 0         | 0.00  | 14    | 46.67 |
| Score 3   | 0       | 0.00  | 1        | 20.00 | 4         | 80.00 | 5     | 16.67 |
| Total     | 14      | 46.67 | 12       | 40.00 | 4         | 13.33 | 30    | 100.00|

Chi-square=34.5563, df=6, P=0.00001, Chi-square test, P=0.00001, Significant
expression; exhibiting intense glycogen storage in normal and reduced glycogen storage in cells with different degrees of dysplasia associated with increased Glut-1 expression. Such localization pattern of glycogen was inversely correlated with the extension of proliferating compartment showing an association between glycogen storage and tumor cell differentiation in SCC.\cite{13}

This study was done to evaluate the expression of Glut-1 in normal and OSCC cases with different clinical stages and
histopathological grades to determine the role of Glut-1 as a prognostic marker.

A study was conducted by Burstein DE et al., to evaluate the expression of Glut-1 in head and neck squamous intraepithelial neoplasia. Results showed negative or weak expression of Glut-1 in normal epithelium, and Glut-1 immunostaining was detected in cell layers above parabasal layer and that expression increased with the increased grading of squamous intraepithelial neoplasia.[14]

A study was done by Ayala FR et al., to investigate expression of Glut-1 in OSCC in which results showed that 50.3% cases showed membrane staining pattern and 49.7% showed nuclear expression. In the present study only 1 case showed nuclear staining whereas the rest showed membrane staining pattern.

In the same study Ayala FR et al., investigated Glut-1 expression in OSCC. They compared the results with normal adjacent hyperplastic epithelium and the results showed that Glut-1 staining in non-neoplastic squamous epithelium was undetectable or weakly detected in suprabasal layers and predominant basal staining was seen. Similar results were seen in our study where only 13 normal cases showed positive staining and a predominant basal staining was seen.

Study was conducted by Ohba S et al., on Glut-1 expression in invasive front to associate with depth of OSCC and prognosis; correlation with clinical characteristics showed that there was no positive correlation of Glut-1 expression with Tumor status or Node status. In contrast our study showed positive correlation between Glut-1 expression and TNM staging with P value of 0.0004 in Chi-square test.

Study was done by Tian M et al., to investigate expression of Glut-1 in OSCC, results showed that there was no correlation between staining pattern and tumor differentiation or T grade classification. Similar finding were seen in another study conducted by Airley et al., where Glut-1 immunohistochemical staining was carried out in many tumors and normal tissue types and the results showed no correlation between Glut-1 and grade of differentiation, which contrasts with the previous studies, where Glut-1 appears to correlate with histological differentiation of OSCC.[3] In our study we found correlation of Glut-1 staining with histological grades that was statistically significant with a P value of 0.00001 in Chi-square test.

The present study showed a significant statistical difference with P value of 0.00004 in Chi-square test between expression of Glut-1 in normal and OSCC cases. Our results coincided with the findings of a previous study done by Reisser C et al., to evaluate expression of Glut-1 in normal, pre neoplastic, neoplastic mucosal lesions of head and neck, where they observed weak expression of Glut-1 in normal mucosa and strong expression in HNSCC.[13]

A study was conducted by Mellanen P et al., on immunoexpression of glucose transporters 1–4 in head and neck tumors. The results showed that Glut-1 mediated facilitative glucose transport is involved in increased glucose metabolism of head and neck cancer.[17] Another study was done to determine expression of Glut-1 in association with increased glucose metabolism and prognosis in patients with OSCC by Kunkel M et al. The study supported that the significance of Glut-1 over expression which was associated with shorter survival and it was concluded that Glut-1 can be used as predictive marker or negative biomarker of prognosis in patients with OSCC.[18] This finding was further supported by other studies by Schutter HD et al., where they stated that Glut-1 can be correlated independently with prognosis.

Eckert AW et al., carried a study to determine expression of staining in comparison with clinico-pathological data. Results showed increased detection of Glut-1 in OSCC and suggested that Glut-1 expression is an independent marker for routine assessment of OSCC.[19] Recent study carried out to estimate prognostic value of Glut-1 by Ayala FR et al., in OSCC showed that Glut-1 can be used as an indicator of poor prognosis in OSCC cases.[15]

The present study shows that the intensity of staining of Glut 1 varied with different clinical staging and histopathological grades of OSCC with severe intensity in Stage IV and poorly differentiated OSCC. Statistical significant results with respect to the intensity of staining were observed between clinical stages and histopathological grades of OSCC.

CONCLUSION

The present study shows that increased expression of Glut-1 is an early event in development of OSCC and that it can be used as an prognostic marker. There was a statistically significant increase in Glut-1 staining as the clinical staging progressed from stage 1 to –4 and as the histopathological grading progressed from grade I to grade III. The enhanced expression of this protein indicated increased glycolytic activity of the tumor cells with an increase in the stage or grade of the tumor. Hence, Glut-1 expression may be used as a prognostic biomarker in OSCC. However, studies of Glut-1 expression in larger samples need to be conducted for more conclusive results.

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