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Dr. Sanjay Mishra MD
Assistant Professor,
Department of Radiotherapy,
IMS and SUM Hospital,
Siksha 'O' Anusandhan
Deemed to be University,
Bhubaneswar, Odisha, India

Dr. Shaikh Ifterkhar
Assistant Professor,
Department of Radiotherapy,
IMS and SUM Hospital,
Siksha 'O' Anusandhan
Deemed to be University,
Bhubaneswar, Odisha, India

Corresponding Author:
Dr. Sanjay Mishra MD
Assistant Professor,
Department of Radiotherapy,
IMS and SUM Hospital,
Siksha 'O' Anusandhan
Deemed to be University,
Bhubaneswar, Odisha, India

GLUT-1 biomarker in predicting prognosis of VC and OSCC

Dr. Sanjay Mishra MD and Dr. Shaikh Ifterkhar

Abstract
Introduction: GLUT-1 is an endogenous marker of hypoxia which has been identified in diverse human tumors and there is evidence that GLUT-1 is associated with tumor aggressiveness in head and neck squamous cell carcinoma.
Aim: Evaluation and comparison of GLUT-1 expression in verrucous carcinoma & various grades of squamous cell carcinoma.
Objective: Our study aims to find the correlation of GLUT-1expression with the clinical and histopathological features of various lesions included in the study which are verrucous carcinoma and various grades of squamous cell carcinoma. It also aims at evaluating the aggressiveness of each individual lesion.
Materials & Method: Study group - Histopathologically diagnosed 10 verrucous carcinoma (VC) cases, 30 oral squamous cell carcinoma (OSCC) cases, 10 each of well differentiated, moderately differentiated and poorly differentiated were selected. Only primary carcinoma cases were considered. For control group- 10 biopsy specimen of normal oral mucosa that are taken during minor oral surgical procedures with patient consent. Immunohistochemisrty method was employed to evaluate the GLUT-1 expression in the various tissue sections.
Results: GLUT-1expression is increased VC and OSCC in comparison to normal mucosa. Its expression in various OSCC cases is correlated with the aggressiveness, local metastasis.
Conclusion: A better prognosis can be obtained by modifying treatment planning in accordance with the requirements of each lesion. Immunoexpression of GLUT-1 of tumour cells (VC & OSCC) can act as an adjunct in predicting the aggressiveness of each lesion.

Keywords: Glut-1, squamous cell carcinoma, verrucous carcinoma

Introduction
Glut-1 is a hypoxic tumor marker which is used to determine the metabolic activity of tumor cells. Our study comprises of immunohistochemical comparison of Glut-1 expression in normal mucosa, verrucous carcinoma & all the three histological grades of oral Squamous cell carcinoma [1-3]. There are a variety of cell and tissue molecular biomarkers that can provide information in addition to that available from clinical examination and histological studies, as well as prognostic tumour biomarkers linked to the clinical outcome of oral squamous cell carcinoma (OSCC) [2-5].
Similar to CA IX and HIF-1 alpha, GLUT-1 has been found in a variety of human cancers. It has also been hypothesised that GLUT-1 may be an endogenous marker of hypoxia that correlates with aggressiveness in head and neck squamous cell carcinoma (HNSCC) [6-8]. Sugars are the main source of metabolic energy in mammalian cells [8-10]. The entry of sugars into cells, however, requires membrane-associated carrier proteins since the plasma membrane is impermeable to polar molecules. To facilitate its translocation, GLUT transporters take advantage of the sugar concentration gradients that already exist between the plasma membrane's internal and external sides [11-14]. SCC is a type of cancer that has a unique histology. It results from unchecked proliferation of epithelial cells or cells with specific cytological or tissue architectural features of squamous cell differentiation, such as the presence of keratin, tonofilament bundles, or desmosomes, structures involved in cell-to-cell attachment [15]. Verrucous carcinoma is a low grade histopathological variant of Squamous cell carcinoma with intact basement membrane and pushing border rete ridges and intraepithelial keratinisation [16]. It is considered a potentially malignant disorder as often it has been seen that it eventually leads to Squamous cell carcinoma. Some of the studies are carried out in evaluating the expression of GLUT-1 in epithelial dysplasia and carcinoma but not many have been carried out in verrucous carcinoma [17-19]. Our study is evaluating

the expression of Glut-1 in verrucous carcinoma and the various grades of squamous cell carcinoma.

Materials and Methods

Materials included Glut 1 antibody and IHC kit from Path N Situ. Positively charged slides and the other materials required for IHC were obtained. The study group comprised of 10 well differentiated OSCC, 10 moderately differentiated OSCC, 5 poorly differentiated OSCC, 10 VC and 5 normal epithelium tissues. Formalin fixed paraffin embedded tissue blocks were taken of the earlier diagnosed cases from the institution. Patients with immunocompromised diseases or who are already undergoing treatment were excluded. IHC was performed with the given tissue sections using Glut- 1 antibody (Fig.1, 2, 3, 4, 5). The slides were studied and scored on the basis of stained cells with glut-1.

Immunoexpression of Glut-1

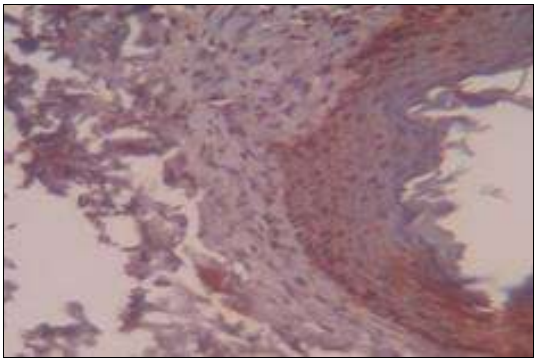

Fig 1: Normal mucosa

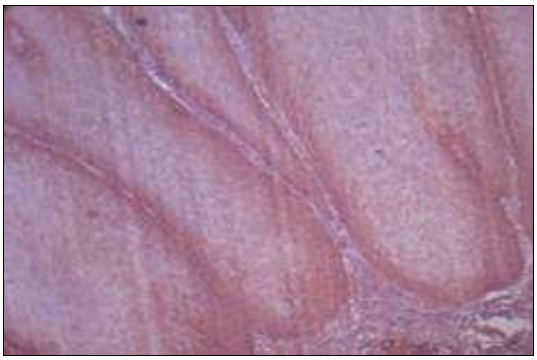

Fig 2: Verrucous Carcinoma

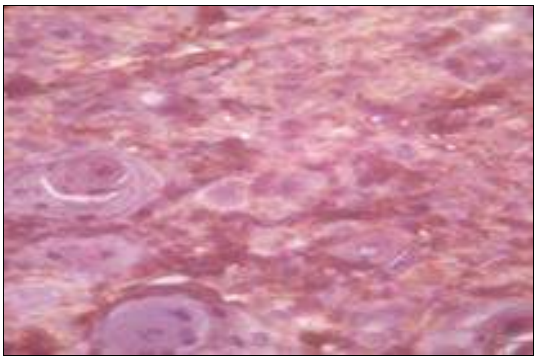

Fig 3: WDSCC

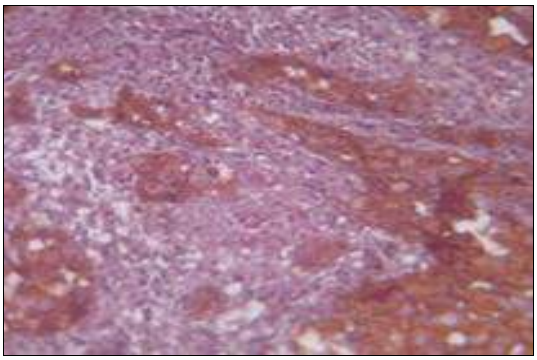

Fig 4: MDSCC

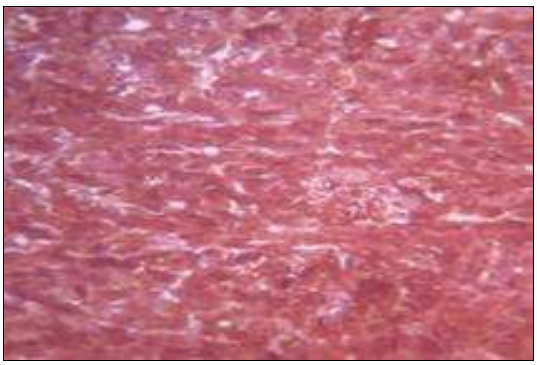

Fig 5: PDSCC

Findings

When clinical parameters like age, sex, site, habit and TNM staging of OSCC cases were compared, there was significant association of Glut-1 expression with age group of patients (P=0.01, table 2) whereas in VC Glut-1 expression was significantly associated with habits of patients (P=0.02, table 1). On comparing the expression of Glut-1 in normal, VC and different grades of OSCC it was seen that mean value of Glut-1 expression increased in VC and different grades of OSCC in comparison to normal tissue.

Results clearly indicated that Glut-1 expression showed mixed results in relation to the various clinical parameters like age, sex, site, habit etc. whereas histopathologically its expression was directly proportional to the aggressiveness of the lesion. Its expression increased from normal epithelium to verrucous carcinoma and in various grades of squamous cell carcinoma progressively along with the grade of OSCC. P was found to be highly significant histopathologically. (Table 3; P=0, $p<.05$)

Table 1: Glut-1 expression in VC (in terms of clinical parameters)

Clinical Parameters		VC cases	% of Cases	Glut 1 expression	P value
Age	<50	2	20%	14	0.111
	>50	8	80%	40	
Sex	M	6	60%	31	0.597
	F	4	40%	23	
Site	B.M	5	50%	27	0.919
	A.M	3	30%	17	
	A.R	2	20%	10	
habit	Smoking	5	50%	27	0.024 $p<.05$
	Chewing	3	30%	12	
	Both	2	20%	15	

Table 2: Glut-1 expression in OSCC (in terms of clinical parameters)

Clinical Parameters		OSCC cases	% of Cases	Glut 1 expression	P value
Age	<50	5	20%	58	0.013, p<0.05
	>50	20	80%	187	
Sex	M	20	95%	232	0.796
	F	5	5%	13	
Site	B.M	6	24%	55	0.708
	A.M	6	24%	54	
	Tongue	6	24%	61	
	A.R	2	8%	17	
	Lip	1	4%	8	
	Retromolar Pad	2	8%	20	
	GB sulcus	2	8%	20	
Habit	Smoking	5	20%	58	0.1
	Chewing	13	52%	121	
	Both	5	20%	46	
	None	2	8%	10	
TNM	I	5	20%	49	0.718
	II	10	40%	103	
	III	8	32%	74	
	IV	2	8%	19	

Table 3: Glut-1 expression in normal and different lesions

Cases	Frequency	Total Score	Mean of Glut-1 expression	SD	P value
Normal	5	6	1.2	1.1	0.00 (p<0.05)
VC	10	54	5.4	1.58	
WDSCC	10	88	8.8	1.93	
MDSCC	10	103	10.3	1.33	
PDSCC	5	54	10.8	2.05	

Discussion

The predictive value of GLUT-1 expression has not been comprehensively examined in OSCC, despite significant research on this parameter in HNSCC. 16, 18 Verrucous carcinoma has not been the subject of many investigations before. Malignant cells frequently exhibit enhanced glucose uptake, particularly in glycolytic (anaerobic) metabolism. Comparing malignant cells to their benign/normal counterparts *in vitro* and *in vivo*, malignant cells exhibit increased glucose absorption and utilisation [9-11]. The higher energy needs of rapidly reproducing cells are maintained in part by glycolytic metabolism, which is also thought to be a significant adaptive modification required to combat the unfavourable micro environmental conditions prevalent in tumours. [13-15]. Because of this, these metabolic alterations have historically been employed to offer diagnostic and prognostic information. [15-16]. The increased glucose uptake in tumours is mostly due to enhanced facilitative glucose transporter function, which is regulated by a number of oncogenes and growth factors. 17 One of the proteins that is up regulated in hypoxic circumstances is facilitative glucose transporter (GLUT-1). The differentiation and invasion processes may be responsible for the GLUT-1 staining that develops in the outer epithelial layers. However, the presence of GLUT-1 staining in central tumour nests far from blood arteries may indicate the presence of GLUT-1 produced by hypoxia. It is debatable how GLUT-1 affects OSCC. Numerous studies have found a link between GLUT-1 expression and the development of numerous tumours, including head and neck, esophageal, lung, and other cancers. 18 However, nothing is known about how OSCC expresses itself in different histological grades. In order to examine GLUT-1 expression in normal mucosa, different histological grades of OSCC, and verrucous carcinoma-a low grade histopathological variant of squamous cell carcinoma-the current study was conducted [19]. The purpose of the current study is to evaluate the validity of GLUT-1 as a promising prognostic marker by examining the expression of GLUT-1 in normal mucosa and various grades of OSCC and verrucous carcinoma. The current study found a statistically significant difference in GLUT-1 expression between normal mucosa and OSCC cases, as well as weak or negative GLUT-1 expression in normal epithelium. GLUT-1 immunostaining was also found in cell layers above the parabasal layer, and its expression increased as OSCC grading increased [16-19]. In the current investigation, normal mucosa had localised to homogeneous GLUT-1 expression in the basal compartment and a few locations of the suprabasal cell layer, which was consistent with the findings of Reisser *et al*., who found that normal mucosa expressed Glut-1 less strongly than OSCC. This result is consistent with research by Ayala *et al*. [20] and Burnstein *et al*. [21]. We observed lower levels of GLUT-1 expression in the keratinized pearl region, which is consistent with the pro-stromal pattern of Glut-1 expression that is low in central differentiated sections of WDSCC and is negatively correlated with the glycogen content, as observed by Angadi V. C. *et al*. [12]. The findings of Harshani *et al*. [22], who found a positive association between GLUT-1 expression and TNM staging, were validated by Yamada *et al*. [23] 's findings that GLUT-1 expression was higher in stage III instances of PDSCC. In OSCC patients, Demeda *et al*. [24] could not find any correlation between GLUT-1 expression and tumour size or clinical stage of the tumour. In contrast to the study conducted by Azad N. *et al*., who compared the GLUT-1 expression between the tobacco & non tobacco users in OSCC cases and found that there was more expression of GLUT-1 in tobacco user groups, we did not find any significant correlation of TNM staging with GLUT-1 expression when comparing overall OSCC cases with clinical staging of the tumour [17]. Various additional carcinomas, including rectal carcinoma [28], colorectal carcinoma [27], bladder carcinoma [28], GLUT-1 expression has also been linked to poor overall survival in pancreatic carcinoma, esophageal carcinoma 29, laryngeal carcinoma, and other cancers. And stomach carcinoma [26], With gastric cancer 26, The energy metabolism of tumour cells has been examined in order to comprehend tumour biology. Many authors (Ayala F R *et al*. [20], Kunkel M *et al*. [4], Ohba S *et al*. [11], Schutter H D *et al*. [31], Eckert A W *et al*. [9], Grimm M *et al*. [32], Harshani J M *et al*. [22], Li C X *et al*. [33]) came to the conclusion that GLUT-1 can act as a predictive marker in head & neck squamous cell carcinoma, It was also suggested by Kunkel M *et al*. [4] and Ayala F R *et al*. [20] that Glut-1 over expression is linked to shorter survival Additional research by Schutter H. D. *et al*. [31] and Eckert A. W. *et al*. [15] (survival p=0.001) shown that Glut-1 can be utilized as an independent marker in routine OSCC assessment.

Conclusion

According to the findings of the current investigation, GLUT-1 expression may significantly rise and act as a promising immune marker for doctors to use in assessing the behaviour of VC and OSCC. Verrucous Carcinoma's

aggressiveness can be evaluated using GLUT-1, and it can also be used to identify which lesions are more likely to progress to malignancy. GLUT-1 serves as an adjuvant in determining the specific prognosis of each case in OSCC.

Conflict of Interest
There is no conflict of interest

Source of funding
Nil

Ethical clearance
Approved from Institutional Ethics Committee

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