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Original Research Article

UTILITY OF S100 IMMUNOSTAIN FOR DETECTION OF NEURAL INVASION IN ORAL SQUAMOUS CELL CARCINOMA COMPARED TO HEMATOXYLIN –EOSIN STAIN

Irene Thomas[1], Savithri M.C.[2], Sreeja Raju[3]

[1]*Assistant Professor, Department of Pathology, Sree Narayana Institute of Medical Sciences, Kunnukara, Kerala, India*
[2]*Professor, Department of Pathology, Amala Institute of Medical Sciences, Thrissur, Kerala, India*
[3]*Assistant Professor, Department of Pathology, Amala Institute of Medical Sciences, Thrissur, Kerala, India*

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Corresponding Author:
Dr. Irene Thomas,
Assistant Professor, Sree Narayana Institute of Medical Sciences, Kunnukara, Kerala, India
Email: irenethomas06@gmail.com

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ABSTRACT

Background: Oral cavity cancer is the sixth most common cancer in the world. Oral squamous cell carcinoma (OSCC) constitutes 95% of all oral cancers. Neural invasion (NI) is one of the most important clinicopathological feature that determine the prognosis in patients with OSCC. This study is done to evaluate the efficacy of S100 immunostain in the detection of neural invasion in histology sections of OSCC compared to haematoxylin-eosin stain and subtype the NI based on its severity, location and focus.

Materials and Methods: This was a descriptive study conducted in the Department of Pathology, Amala Institute of Medical Sciences, Thrissur over a period of 18 months. The study included 62 cases of OSCC which satisfied the inclusion and exclusion criteria. Microscopic examination of the Hematoxylin and Eosin (H&E) sections and S100 immunostained slides were done. Cases with NI were further subtyped into epineural association, perineural invasion and endoneural invasion based on severity.

Results: Examination of H&E slides revealed 20 cases (32%) of NI whereas in S100 stained slides the number of cases increased to 33 (53%). This is statistically significant with a chi square test p value of <0.05%. Severity of NI was also better evaluated with S100 stain.

Conclusion: Our study showed that the incidence and accuracy in detecting NI which is an important prognostic factor in OSCC, was enhanced using S100 immunomarker.

Keywords: Oral squamous cell carcinoma, neural invasion, S100 immunostain.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) constitutes majority of cancer cases in India and is associated with increased morbidity and high rate of recurrence.[1,2] The oral cavity extends from the skin-vermilion junction of the lips to the junction of hard and soft palate above to the line of circumvallate papillae below and to the anterior tonsillar pillars laterally. It includes mucosal lip, buccal mucosa, lower and upper alveolar ridges, retromolar gingiva, floor of the mouth, hard palate and anterior two-thirds of the tongue. Among them tongue is the most common site in the Western world whereas in South Asian countries it is more prevalent in buccal mucosa.[3] Tobacco smoking and alcohol intake are commonly implicated in the causation of OSCC. However recent literature describes other risk factors including human papilloma virus (HPV) infection. Staging of tumor is of paramount importance in predicting the outcome but several other factors are important in prognostication. Neural invasion (NI) is one of the well-known routes of cancer spread especially in head and neck, prostate and pancreatic cancers. It indicates poor disease-free survival.[4,5] NI is considered as a homogenous entity in the clinical setting, even though different histologic patterns of tumour cells interaction with nerve tissue has been identified. Tumour spread by neural invasion is considered as one of the prognostic factors as per

American Joint Committee for Cancer (AJCC) staging manual eighth edition.[6] This study proposes to identify neural invasion in OSCC using routine haematoxylin-eosin (H&E) stain and S100 immunostain and compare the results.

MATERIALS AND METHODS

This study is a descriptive study enrolling histology proven cases of OSCC. The study period was from 01 January 2018 to 30 June 2019. During this period a total of 62 cases were studied after getting approval from the Institutional Research and Ethical Committee. Cases which received neoadjuvant treatment were excluded.

The cases were reviewed and morphologically classified according to differentiation.Two paraffin tissue blocks containing tumour proper were selected for each case. IHC for S100 was performed using Mouse Monoclonal Anti S100 Antibody (15E2E2)

Neural invasion was evaluated based on criteria defined by Leibig et al. According to him, tumour in close proximity to a nerve and involving at least one-third of its circumference or tumour infiltrating any of the three layers of nerve sheath is considered NI [4].

NI was further differentiated into three according to criteria developed by Leibl et al: epineural tumour association (ENA), perineural invasion (PNI) and endoneural invasion (ENI) [7].

ENA - Tumour directly touches the epineural sheet without infiltrating the perineurium.

PNI - Tumour cells within the perineural space.

ENI - Tumour cells infiltrate into the endoneurium within the nerve fascicles.

NI was subcategorized based on location into intratumoural and extratumoural based on Miller et al. criteria (8). For every focus of NI identified, distance to tumour edge in millimeters was assessed with tumour edge being zero. A positive value indicated as extratumoural and a negative value indicated extratumoural NI. Based upon the number of nerves involved it was again divided into focal (single nerve involvement) and multifocal (multiple nerve involvement)

Positive S100 staining pertains to cytoplasmic and nuclear staining.

Statistical analysis: Data was entered in MS Excel worksheets and analyzed using SPSS version 23.0 statistical software. Categorical data in two methods will be analyzed using Chi square test.

RESULTS

In this study, the patient age ranged from 30 to 89 years, with the maximum number of cases in this study between the age group of 60-69 (32.3) [Figure 1]. Out of 62 patients 50 (80.6%) were males and 12 (19.4) were females. 33 (53.2%) patients were smokers and 29 (46.8%) patients were non-smokers. Among 62 patients 25 (40.3%) were alcohol users and 37 (59.7%) were teetotallers. Only 6.5% were using smokeless tobacco. Out of 62 cases, maximum number of cases were seen in tongue- 38 (61.3%) [Figure 2].

There were 45 (72.6%) cases of well differentiated SCC, 16 (25.8%) cases of moderately differentiated SCC and 1 (1.6%) case of poorly differentiated SCC. Among 62 cases, 20 cases (32%) showed NI [Figure 3]. S100 immunostaining increased the number of cases with NI to 33 (53%) (Table 1). There was no association between NI and tumour stage (p value- 0.273) [Table 2]. There was no statistically significant association between NI and lymph node/distant metastasis. Tumour differentiation also did not show any statistically significant association with NI.

Table 1: Comparing NI detected using H&E and S100 immunostain

H&E	S100		Total	P value (Chi square test)
	Negative	Positive		
Negative	29	13	42	0.0001
Positive	0	20	20	
Total	29	33	62	

Table 2: Association between tumour stage and NI

Tumour stage	S100		Total	P value (Fisher exact test)
	Negative	Positive		
T1	11(64%)	6(36%)	17	0.273
T2	10(38%)	16(62%)	26	
T3	2(28%)	5(72%)	7	
T4	6(50%)	6(50%)	12	
Total	29	33	62	

Table 3: Comparing severity of NI in H&E and S100 immunostained slides

NI	H&E	S100	P value (Chi square test)
Negative	42	29	0.0001
ENA	10	13	
PNI	9	14	
ENI	1	6	

Severity of NI is graded as ENA, PNI and ENI of which ENI is considered most severe. More ENI positive cases could be detected with S100 stain compared to H&E and was statistically significant (P

value 0.0001). [Table 3]. Among 33 cases, 24 cases showed intratumoural NI, 7 cases with extratumoural NI and 2 cases with both intra and extratumoural NI. Out of 33 cases with NI, 16 (48.5%) were showing focal infiltration and 17 (51.5%) cases with multifocal infiltration.

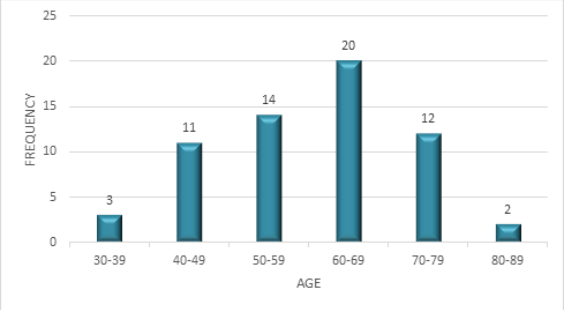

Figure 1: Distribution of patients based on age

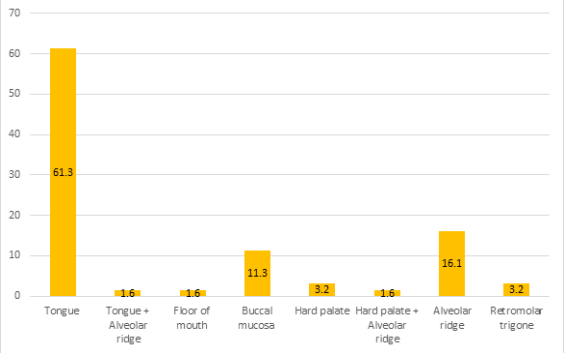

Figure 2: Distribution of patients according to site involved

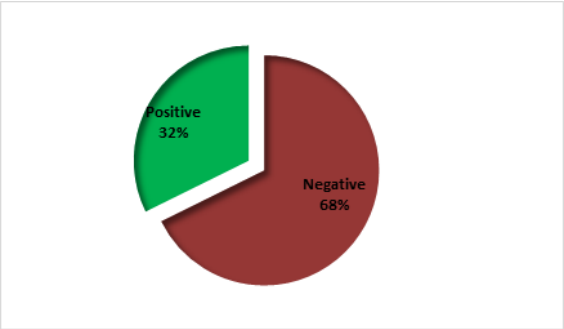

Figure 3: Distribution of cases based on NI in H&E stained slides

DISCUSSION

Oral squamous cell carcinoma (OSCC) is one of the most common malignancy in India and constitutes the sixth most common cancer worldwide.[9] It has been studied extensively in literature as it is a very aggressive tumour and is associated with severe morbidity and recurrence. Many clinicopathologic factors that affects the prognosis of OSCC has been described in literature and neural invasion (NI) is one among them. NI is a distinct pathological entity that can be seen in the absence of lymphatic or vascular invasion. Tumour can spread to distant sites through nerves and can be the sole source of metastatic spread. Treatment options for OSCC varies with the presence of NI and it indicates the need for adjuvant radiotherapy/ chemotherapy. Since it is an important factor in determining the prognosis and treatment of the patient, detection of NI is very crucial. In this study, we evaluated the efficacy of S100 immumostain over haemotoxylin-eosin stain in the detection of NI in OSCC.

Various definitions have been proposed in literature for definition of NI. NI detection in our study was based on the method proposed by Leibig et al. as tumour infiltrating any of the three layers of nerve sheath.[4] We categorized them according to severity into epineural association (ENA), perineural invasion (PNI) and endoneural invasion (ENI) as per criteria put forward by Leibl et al.[7] Tumours were further subclassified into intratumoural (IT) and extratumoural (ET) based on criteria specified by Miller et al.

OSCC is known to have increased incidence in elderly males. In the current study, the patients' age at the time of diagnosis ranged from 30 to 89 years and among them, the maximum number of cases was seen between the age group of 60-69 (32.3%). In a study conducted by Gul et al. on prevalence of OSCC it was found that majority of reported cases belonged to an age range of 40 to 70 years.[10] In another study by Tandon et al, the mean age was 51.35 ±14.39 years and 55.35 ± 8.87 years in males and females respectively.[11] Of the 62 patients in this study, there were 50 (80.6%) men and 12 (19.4%) women with a male to female ratio of 4.1:1. S. Nair et al. in their study observed that in squamous cell carcinoma of oral cavity the male: female ratio was 2.83:1.[12]

Tobacco use and alcohol consumption are well accepted risk factors for OSCC. A smoker has 10 times risk as compared to a non-smoker and the risk depends on the duration and quantity of smoking.[13,14] In our study, 33 (53.2%) patients' were smokers and 29 (46.8%) were non-smokers. There were 25 (40.3%) alcohol users and 37 (59.7%) teetotallers. Four (6.5%) patients were using smokeless tobacco. Zheng et al. reported that tobacco consumption was strongly associated with OSCC whereas there was no trend in risk associated with tobacco for adenocarcinomas and other histologic types of oral cancer. He also showed that intake of alcohol increases the risk of oral cancers with a significant trend with increasing dose ($p<0.002$).[15]

In a case control study by Blot et al. it was observed that tobacco smoking and alcohol drinking separately increase the risk of oral and pharyngeal cancer. He also showed that cessation of smoking was associated a reduced risk of oral cancers and the risk varied with type of tobacco intake and alcoholic beverage.[16]

In our study the majority of cases affected tongue (61.3%) and the second most common site was alveolar ridge (16.1%) followed by buccal mucosa (11.3%). 2 (3.2%) cases each were seen in hard palate and retro molar trigone and one each (1.6%) in floor of mouth and alveolar ridge. One case each with combined involvement of tongue + alveolar ridge and

hard palate + alveolar ridge was reported. In a study by Asio et al. in 384 patients, the maximum number of cases were reported in tongue (34.1%) followed by palate (13.5%) and buccal mucosa (13.3%).[17] In an Indian study Tandon et al. observed buccal mucosa (45.92%) and gingiva-buccal sulcus (31.63%) as the commonest sites in variance with our statistics.[11]

Tumour differentiation in our cases was as follows: 45 (72.6%) were well-differentiated OSCC, 16 (25.8%) were moderately-differentiated OSCC and 1 (1.6%) was poorly-differentiated OSCC. There was no significant association between tumour differentiation and NI (p value- 0.063). This result was in agreement with the Indian study by Manjula et al. in which no significant association was seen between histologic grade and NI status detected in H&E stained slides (p value- 0.239). Kevin et al also revealed similar scenario in cases with NI in S100 immunostained slides emphasizing the fact that NI can be seen even in well differentiated tumours.[18]

The tumor stage was, T1 in 17 cases (27.4%), T2 in 26 cases (41.9%). T3 in 7 (11.3%) cases and T4 in 12 (19.4%) cases. Cases showing NI (using S100 stain) were 6/17 (36%), 16/26 (62%), 5/7 (72%) and 6/12 (50%) in T1, T2, T3 and T4 stages respectively. There was no statistical significance with pathological tumor stage even though percentage of NI was more in T2 and T3 cases. This result was in agreement with the Indian study by B. Varsha et al. in which no significant association was seen between NI and pathological tumour stage (2). Only 22 (35.5%) cases show nodal involvement and 40 (64.5%) cases didn't show any nodal metastasis. Of these, 12 (54.5%) node positive cases and 21 (52.5%) node negative cases showed NI in S100 immunostained slides. There was no statistical significance (p value- 0.988). This was in concordance with results obtained by Kurtz et al. where 17/33 cases with NI show metastasis to lymph node whereas 16/33 cases with NI didn't show any lymph node involvement (p value- 0.79).[18] Lim et al in his study also failed to observe any statistically significant correlation (p value >0.99) between NI and lymph node metastasis in OSCC involving the tongue.[19] In contrast to this, Viswanatha et al. observed statistically significant association between lymph node metastasis and NI (p value <0.001) in oral tongue carcinoma. In his study, 63% of cases had NI and among them 42% had lymph node metastasis while in the remaining 37% who had no NI, none of them had positive nodal metastasis which was statistically significant.[20] Four (93.5%) of our cases showed distant metastasis and 58 (6.5%) didn't show any metastasis. We couldn't find any statistical significance between NI in S100 stained slides and tumour metastasis (p value-0.894). There were no studies in the literature correlating NI and distant metastasis.

In the current study sections of OSCC stained by H&E revealed NI in 20 cases (32.3%) whereas when S100 immunostain was used NI could be identified in 33 cases (53.2%). This was found to be statistically significant with a p value< 0.0001. This result was with in agreement with the findings of a similar study by Kurtz et al. where the neural invasion was reported in only 30 % cases in H&E stained slides initially. More intense scrutiny increased the percentage to 62% and examination of S100 stained slides further increased NI to 82%.[18] This observation was due to the enhanced detection of nerves of small diameters embedded within the tumor. Desmoplastic stroma can look deceptively similar morphologically and S100 immunostain is helpful in differentiation. We found that compared to routine the H&E stained slides, NI could be more easily detected in S100 stained sections. The primary reason is due to the sharp contrast between S100 stained nerves and the hematoxylin counterstain, allowing low power detection of NI. Cavalcante et al. suggested that PGP 9.5 is a more useful immunomarker than S100 in detection of nerve involvement. After comparing the three method's- H&E, S100 and PGP 9.5, he observed that PGP 9.5 identified more cases than H&E and S100 stained slides.[21]

CONCLUSION

Oral cavity cancer being one of the commonest tumour, factors affecting the prognosis is very important. NI has got significant impact on survival and hence in this study we aimed at evaluating the efficacy of S100 immunostain in the detection of neural invasion in histology sections of oral squamous cell carcinoma compared to the routinely used H&E stain. While H&E stain detected NI in only 32.3% of cases, S100 immunostain helped identify NI in 53.2% .This difference in results were statistically significant (p value- 0.0001). Moreover S100 was also useful in assessing the severity of NI. Hence it is concluded that S100 immunostain will help identify more cases with NI which is an important prognostic factor in OSCC.

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