Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 1281-1286 | ISSN:2251-6727

The study of association between Depth of Invasion, Tumor Budding, and Worst Pattern of Invasion with Tumor stage and Lymph node status in Oral Squamous cell carcinoma

Dr. Chaitra N[1], Dr. Amrutha M R[2], Dr. Sathish Chandra M R[3], Dr. Shruthi H[4]*, Dr. Tongbram Purnika[5], Dr. Kodandaswamy C R[6]

[1,4*]Assistant professor, Department of Pathology, Sri Siddhartha Medical College, Agalakote, Tumkur-572107
[2]Associate professor, Department of Pathology, Sri Siddhartha Medical College, Agalakote, Tumkur -572107
[3]Associate professor, Department of Community Medicine, BGS Global Institute of Medical Sciences, Bangalore 560060
[5]Junior resident, Department of Pathology, Sri Siddhartha medical college, Agalakote, Tumkur -572107
[6]Professor and head, Department of Pathology, Sri Siddhartha medical college, Agalakote Tumkur -572107

*__Corresponding author__: Dr. Shruthi H
*Assistant professor, Department of Pathology, Sri Siddhartha Medical College, Agalakote, Tumkur-572107
(Email- shrunagabhushan@gmail.com)

(Received: 27 October 2023 Revised: 22 November Accepted: 26 December)

Introduction

Malignancy of oral cavity is a major health burden in India, which contributes to 1/3rd of the global oral cancers. Annual incidence of oral cavity cancers is 77,000 and death due to these cancers is estimated to be 52,000 per year. Among them, 84-97% of oral cancers are histologically diagnosed as Squamous cell carcinomas, thus accounting to be the most common histologic type. (1)

Detection, grading and staging of carcinoma avails limited data regarding the prognosis of the tumour. Thus the impact of parameters such as depth of invasion, tumour budding and pattern of invasion have been studied to predict the growth and spread of the primary tumour. (2) These parameters can be utilised to certify the tumour as high risk or low risk group. This risk stratification aids in determining appropriate treatment modality to the patient. Patients belonging to histopathologically high risk category need multimodality treatment approach, whereas local surgical resection alone can suffice a low risk category patient. (3)

Depth of invasion or tumour thickness is a measure of the maximum depth of tumour deposit from the basement membrane of the adjacent normal squamous epithelium, in millimetres. (4) The cut-off of 4 mm is used to assess the risk of spread in oral squamous cell carcinomas. (5) Tumour budding is defined as group of less than 5 tumour cells at the invasive front of carcinoma. (3) International Tumor Budding Consensus conference (ITBCC) recommends the assessment of tumour budding as low (0-4 buds/20x), intermediate (5-9 buds/20x) and high (> 9 buds/20x). (6) Analysis and categorisation of Pattern of invasion based on the Bradwein-Gensler system is promising. This system categorises pattern of invasion into 1-5 types, as broad pushing pattern, finger like protrusion, groups of > 15 tumour cells, groups with < 15 tumour cells and tumour cell satellite island at >1 mm distance from tumour-stromal interface, respectively. (7)

This study aims to evaluate the association between depth of invasion, tumour budding and pattern of invasion with the known prognostic indicators like tumour stage and lymph node metastasis in oral squamous cell carcinomas.

Materials and methods

This is a cross-sectional study which includes 50 oral squamous cell carcinoma cases, which included resected specimen of primary tumour with or without lymph node resection. Formalin fixed paraffin embedded tissue blocks were retrieved and stained with hematoxylin & eosin stain. These slides were viewed to assess for tumour depth, budding, pattern of invasion and tumour grade. Sections from lymph node were examined to determine nodal metastatic deposits. Tumour staging was obtained from the previous histopathological reports. Patients who have undergone surgical resection for primary oral cavity malignancy were included in this study. Exclusion criteria includes the patients who have undergone neoadjuvant therapy, patients with synchronous head and neck tumours and cases with extensive necrosis or cystic degeneration.

All the slides were viewed individually by each author and senior pathologists were consulted in case of discrepancies. In the present study, Depth of invasion was measured based on AJCC 8th edition, 2017 definition. International Tumour Budding Consensus conference guidelines were followed to analyse tumour budding. Pattern of invasion was categorised based on the Bradwein-Gensler system and tumour was graded as per Broders grading of squamous cell carcinomas.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 1281-1286 | ISSN:2251-6727

The findings were then correlated with the tumour stage and lymph node status.

Statistical Analysis:
All the data were entered in Microsoft Excel and analyzed using SPSS 20.0. The qualitative study variables were expressed in frequency with percentages. To find the significant difference Yates Chi-square test is used since the frequencies were ≤ 5. A p-value of <0.05 is considered statistically significant.

Results
This is a cross-sectional study done on 50 cases of oral squamous cell carcinoma which included detailed examination of microscopic slides for novel parameters like Depth of Invasion, Tumour budding and Worst pattern of invasion. The relevant clinical details were retrieved from the histopathology request form and the data was recorded and analysed.
In the study we found that the gender-wise incidence showed slight male preponderance (29/50; 58%). Age-wise distribution was studied and found that most common age range was 45-70 years, with the median age of 60 years.
Pathological tumour staging and lymph node metastasis are the notable prognostic parameters of oral cavity malignancies. In our study, pathological staging of primary tumour was done based on AJCC 8th edition criteria. 26/50 (52%) were in stage pT1 accounting for the predominant stage, followed by stage pT2(30 %) and pT3 (18%). Microscopic slides from lymph nodes were examined for presence or absence of metastatic deposits. 34/50 (68%) were categorised as negative (pN0/Nx), while 16 /50 (32 %) were found to be positive for metastatic deposits. (Figure 1) These findings were correlated with new-fangled markers such as depth of invasion, tumour budding and worst pattern of invasion.
Many studies and meta-analysis have shown that the tumour depth of more than 4 mm is associated with increased likelihood of growth and metastasis of the tumour. Thus, in this study, we have used the cut-off of 4 mm to classify the tumours into high risk and low risk for higher stage and metastatic spread. Majority (31/50; 62%) of the tumours showed <4 mm, while 38% cases had more than 4 mm depth of invasion. 100 % of the tumours with stage pT1 showed tumour depth of less than 4 mm. While, majority of pT2 and pT3 (66.6 and 100 %, respectively) had tumour thickness of more than 4 mm. (Table 1) Similar correlation was done with depth of invasion and nodal status. 76.4 % cases with negative nodal status showed tumour depth of < 4 mm, whereas majority (68.7%) of the cases with nodal metastasis showed depth of 5 mm or more. (Table 2)
Tumour budding as analysed and classified based on the ITBCC recommendations. The current study showed that low tumour budding (0-4 buds) was the predominant (56%) category, followed by high (> 9 buds) and intermediate category, showing 26% and 18 %, respectively. 23/26 (88.5 %) of the tumours with pathological stage I showed low tumour budding. (Figure 2) In contrast 77.8% of stage III and 40 % of stage II showed high tumour budding. (Figure 3) Thus, tumours with high tumour budding had preponderance for higher tumour stage. The correlation with tumour budding and lymph node status showed that 27/34(79.4 %) pN0 cases showed low tumour budding. While, 10/16 node positive cases (62.5%) had >9buds/20x (high budding). Thus, this semiquantitative assessment of this parameter shows correlation with the stage and metastatic potential.
Worst pattern of invasion is the futuristic parameter analysed in certain tumours. In this study, we have grouped the pattern of invasion based on Bradwein-Gensler system. Group 1 defined by broad pushing invasive front was seen in 6/50 cases (12%), Group 2 with finger-like invasion in 10/50 (20%), Group 3 with invasive tumour islands having > 15 cells was seen in 24 % cases, Group 4 with invasive tumour islands having < 15 cells in 26 % and Group 5 defined by satellites 1 mm away from tumour was observed in 18 % of cases. Tumours with stage I had predominantly 1, 2 and 3 group patterns of invasion (19.2%, 23.1% and 30.8 %, respectively), whereas tumours with stage II and stage III showed group 4 and 5 patterns. Thus, the tumours with patterns 4 & 5 were predominantly in pT2 and pT3. (Figure 4) Similarly, tumours with WPOI group 4&5, predominantly had metastatic lymph node/s (43.7 and 37.5%, respectively), in contrast to tumours with WPOI 1-3 which showed negative nodal status (17.6%, 29.4% and 26.4 %, respectively).
In addition, tumour was graded using Broders system into three tiers. In this study, we found that the distribution of well, moderately and poorly differentiated tumour grades were comparable among all tumour stages and lymph node positive as well as negative cases. The study results were not statistically significant (p value=0.11 and 0.2489)

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 1281-1286 | ISSN:2251-6727

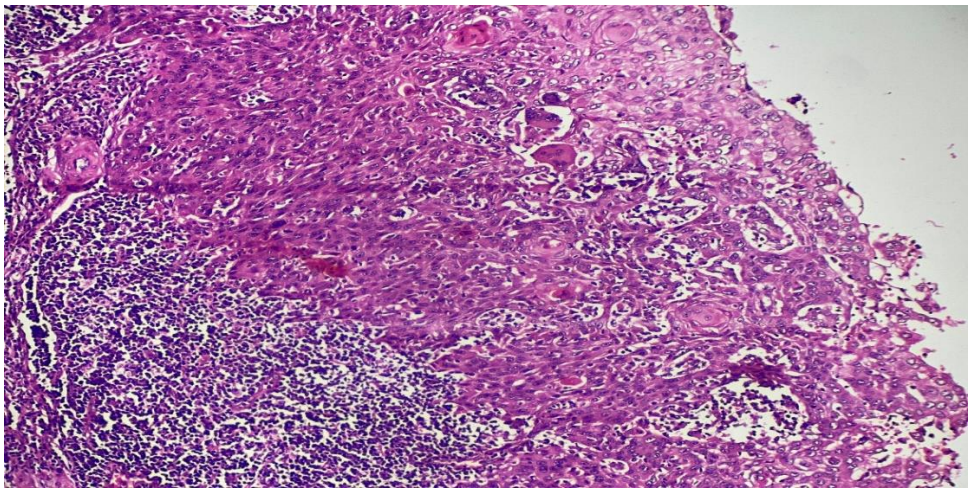

Figure 1 *showing metastatic deposits in lymphnode H&E 400x*

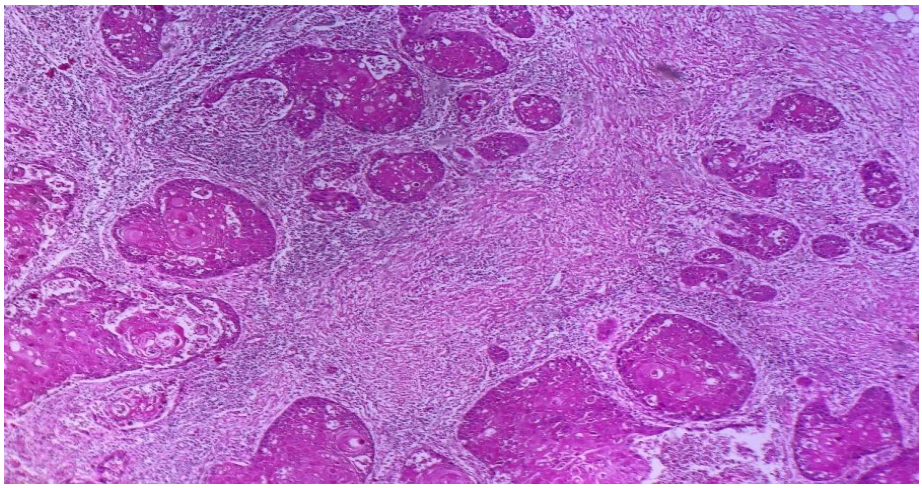

Figure 2 *showing low tumor budding H&E, 200x*

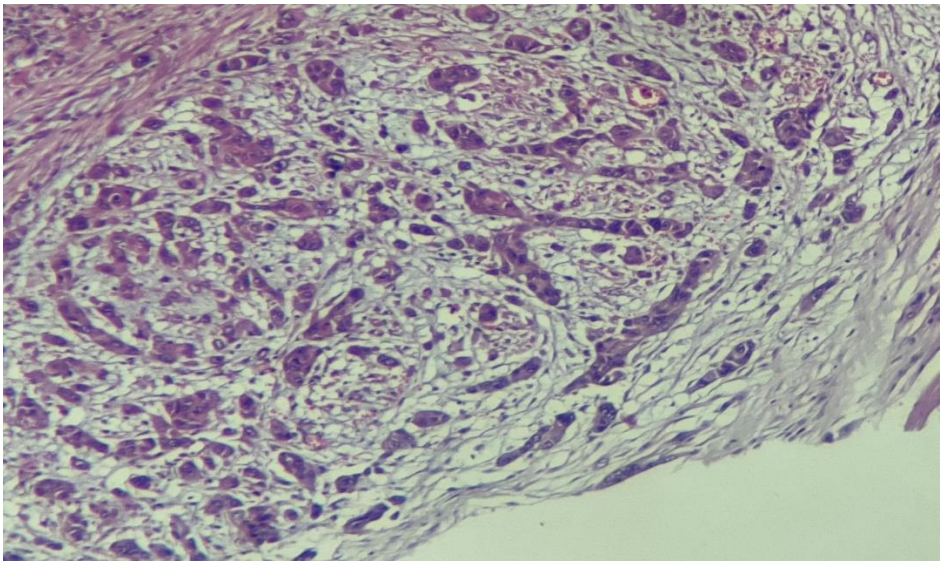

Figure 3 *showing high tumor budding H&E 200x*

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 1281-1286 | ISSN:2251-6727

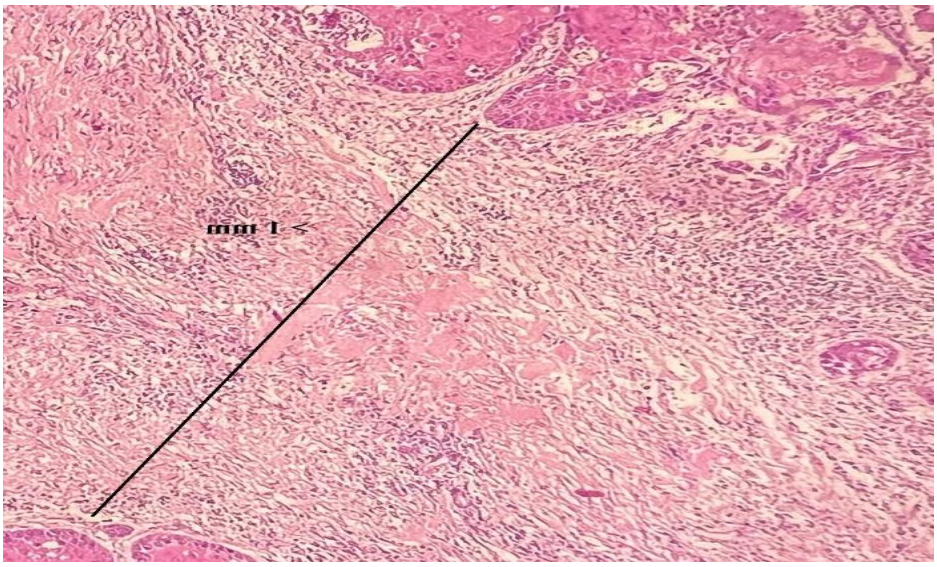

Figure 4 *showing WPOI 5 H&E, 100x*

Table 1: Correlation between the Study parameters and Pathological tumour stage

Study Parameters	Total (n=50)	pT1 (n=26)	pT2 (n=15)	pT3 (n=9)	Yates Chi-square	p-value
TUMOR BUDDING						
Low	28 (56%)	23 (88.5%)	5 (33.3%)	0 (0%)	23.813	0.00008
Intermediate	9 (18%)	3 (11.5%)	4 (26.7%)	2 (22.2%)		
High	13 (26%)	0 (0%)	6 (40%)	7 (77.8%)		
WORST PATTERN OF INVASION(WPOI)						
1	6 (12%)	5 (19.2%)	1 (6.6%)	0 (0%)	-	-
2	10 (20%)	6 (23.1%)	3 (20%)	1 (11.11%)		
3	12 (24%)	8 (30.8%)	3 (20%)	1 (11.11%)		
4	13 (26%)	6 (23.1%)	4 (26.7%)	3 (33.33%)		
5	9 (18%)	1 (3.8%)	4 (26.7%)	4 (44.45%)		
DEPTH OF INVASION						
<4 mm	31 (62%)	26 (100%)	5 (33.3%)	0 (0%)	30.62	0.000 (<0.05)
> 4 mm	19 (38%)	0 (0%)	10 (66.7%)	9 (100%)		
HISTOLOGIC GRADE						
Well Differentiated	19 (38%)	12 (46.2%)	5 (33.3%)	2 (22.22%)	7.539	0.11
Moderately Differentiated	24 (48%)	14 (53.8%)	7 (46.7%)	3 (33.33%)		
Poorly Differentiated	7 (14%)	0 (0%)	3 (20%)	4 (44.45%)		

Table 2: Correlation between the Study parameters and the Lymph node status

Study Parameters	Total (n=50)	pN0/Nx (n=34)	pN1/N2/N3 (n=16)	Yates Chi-square	p-value
TUMOR BUDDING					
Low	28 (56%)	27 (79.4%)	1 (6.25%)	20.555	0.00003
Intermediate	9 (18%)	4 (11.8%)	5 (31.25%)		
High	13 (26%)	3 (8.8%)	10 (62.5%)		
WORST PATTERN OF INVASION (WPOI)					
1	6 (12%)	6 (17.65%)	0 (0%)	10.38	0.0344
2	10 (20%)	10 (29.41%)	0 (0%)		
3	12 (24%)	9 (26.47%)	3 (18.75%)		
4	13 (26%)	6 (17.65%)	7 (43.75%)		
5	9 (18%)	3 (8.82%)	6 (37.5%)		
DEPTH OF INVASION					
<4 mm	31 (62%)	26 (76.47%)	5 (31.25%)	7.621	0.005
> 4 mm	19 (38%)	8 (23.53%)	11 (68.75%)		
HISTOLOGIC GRADE					
Well Differentiated	19 (38%)	16 (47.1%)	3 (18.75%)	2.781	0.2489
Moderately Differentiated	24 (48%)	15 (44.1%)	9 (56.25%)		
Poorly Differentiated	7 (14%)	3 (8.8%)	4 (25%)		

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 1281-1286 | ISSN:2251-6727

Discussion

Oral squamous cell carcinomas account for the major cause of morbidity and mortality among the general population. Oral cavity malignancies include the plethora of tumours arising from mucosal surface of lip, buccal mucosa, gingivo-buccal sulcus, hard palate, tongue and floor of mouth. [4] Certain harmful addictions like tobacco chewing, alcohol consumption, betel quid and gutka pose a higher risk of developing these cancers. High risk HPV virus is also associated with a significant risk of oral Squamous cell carcinomas. [9] Majority of these cases are managed mostly on the basis of clinical staging, which is solely based on the tumour size. [3] Meanwhile, there has been a huge progress in the cancer treatment with invention of multimodality treatment approaches. Likewise, newer pathological parameters have been put forth for the early prediction of prognosis of the tumour, but not in much use in routine practice. In this study, we have evaluated the usefulness of these newer parameters like depth of invasion, tumour budding and worst pattern of invasion by correlating with the well-known prognostic indices like pathological tumour staging and lymph node metastasis.

In our study, 62% of the cases had tumour thickness of less than 4 mm. Depth of invasion showed significant association with pathological stage of the tumour, with 100% pT1 cases showing depth less than 4 mm and majority of higher stages showing increased depth (p value <0.05). Out of which, 76% of node negative cases showed thickness of 4 mm or less. 68% of node positive tumours showed increased thickness. Thus, increased tumour depth had statistically significant correlation with lymphnode metastasis (p value = 0.05). Similarly, the study conducted by Xu B et al., [2] also showed that majority of lymph node negative cases showed depth of invasion <5 mm. [2] Concordantly, the study conducted by Almangush A et al [3] also concluded that the depth of more than 4 mm is significantly associated with mortality of the patients with oral Squamous cell carcinoma. [3]

The current study showed statistically significant association between tumour budding with pathological stage and lymph node metastasis (p value =0.00008 And = 0.00003, respectively). In comparison with our study, Xu B et al., in their study also stated 67.6% node negative tumours to be showing low tumour budding, whereas 55.6 % cases with nodal metastatic tumours showing high tumour budding (more than 9 buds per 20x magnification field). Xu B et al., concluded that semi-quantitative analysis of tumour budding has a superior role in prognostication of early oral tongue Squamous cell carcinoma. [2]

In the present study, Worst pattern of invasion was analysed and showed that patterns 4 and 5 showed significant preponderance for increase in stage and metastatic spread of the tumour (p value=0.0344). The study conducted by E W Odell et al., has also concluded that pattern of invasion has closest correlation with metastasis in small lingual squamous cell tumours. [8] Similarly, the study conducted by Xu B et al., also stated WPOI as independent adverse prognostic factor in malignant oral squamous neoplasm. [2]

As these are the novel parameters, there is limited date available in the literature. Thorough knowledge and utility of these parameters routinely for prognostication is a hope for the future oncology team. Oral neoplasms are one of the challenges for the oral & maxillofacial surgeons. Thus, lesions arising in this location needs precise diagnosis, grading and risk stratification and thus helping in rightful usage of multidisciplinary treatment.

Conclusion

The findings of the study suggest that there is a statistically significant association between the novel parameters such as Depth of invasion, Tumour budding and Worst pattern of invasion with the well-established prognostic indices like pathological tumour staging and lymph node metastasis in oral Squamous cell carcinoma. We conclude that these emerging parameters have an independent role in predicting the adverse outcome of these cancers. Thus, we recommend the use of these parameters in routine histopathological reporting of Oral Squamous cell carcinoma.

References

1. Borse V, Konwar AN, Buragohain P. Oral cancer diagnosis and perspectives in India. Sensors International 2020;1:1-12
2. Xu B, Salama A.M, Valero C, Yuan A, Khimraj A, Saliba M, Zanoni D.K, Ganly I, Patel G. S, Katabi N, Ghossein R. The Prognostic Role of Histologic Grade, Worst Pattern of Invasion, and Tumor Budding in Early Oral Tongue Squamous Cell Carcinoma: a Comparative Study. Virchows Arch.2021;479(3):597-606.
3. Almangush A, Bello I.O, Keski- Santti H, Maklen L.K, Kauppila J.H, Pukkila M, Hangstrom J, Larrene J, Tommola S, Neiminen O, Soini Y, Kosma V-M, Kolvunen P, Grenmen R, Leivo I, Salo T. Depth of Invasion, tumor budding, and worst pattern of invasion: Prognostic indicators in early- stage oral tongue cancer.Wiley Head and Neck Journal.2014; 36(6):811-18.
4. Ridge JA, Lydiatt WM, Patel SG, Glastonbury CM, Gensler MB, Ghossein RA and Shah JP. AJCC cancer staging manual. 8th ed. Lip and oral cavity.American Joint Commission on Cancer: Springer International; 2017:79-94.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 1281-1286 | ISSN:2251-6727

5. Huang S.H, Hwang D, Lockwood G, Math M, Goldstein D.P, O'Sullivan B. Predictive Value of Tumor Thickness for Cervical Lymph-Node Involvement in squamous Cell Carcinoma of The Oral Cavity.American Cancer Society.2009:1489-96.
6. Xie N, Yu P, Liu H, et al. Validation of the International Tumor Budding Consensus Conference(2016) Recommendations in Oral Tongue Squamous Cell Carcinoma. Journal of Oral Pathology and Medicine.2019;48:451-58.
7. Gensler M, Teixeira S.M, Lewis C.M, Lee B, Rolnitsky L, Hille J. J, Genden E, Urken M.L, Wang B.Y. Oral Squamous Cell Carcinoma Histologic Risk Assessment, but Not Margin Status, Is Stongly Predictive of Local disease-free and Overall Survival. The American Journal of Surgical Pathology.2005;29(2): 165-178.
8. Ramakrishnan, P; Pattanayak, M; Arora, A; Singh, A; Asthana, V; Saini, S. Evaluation of POSSUM Scoring Systems in Predicting Postoperative Morbidity and Mortality in Indian Patients Operated for Esophageal Cancer. Bali Journal of Anesthesiology 4(2):p 53-58, Apr–Jun 2020.
9. Glass SH. SCC-general.PathologyOutlines.com website. https://www.pathologyoutlines.com/topic/oralcavityscc. html. Accessed December 7th, 2022.
10. Muller S, Boy S.C, Terrence A. D, Magliocca K.R, Richardson M.S, Sloan P, Tilakaratne W.M, Zain R.B, Thompson L . Data Set for the Reporting of Oral Cavity Carcinomas. Arch Pathol Lab Med.2018:1-8
11. Rhutso Y, Kakoti L.M, Sharma J.D, Kalita M. Significance of Pattern of Invasion in Tongue Squamous Cell Carcinoma- A Retrospective Study From Regional Cancer Center of North-East India. South Asian J Cancer. 2022;00(00):00-00
12. Mishra A, Das A, Dhal I, Shankar R, Bhavya B.M, Singh N, Tripathi P, Daga D, Rai A, Gupta M, sahu G.C. Worst Pattern Of Invasion in oral squamous cell carcinoma is an independent prognostic facrtor.Journal of oral biology and craniofacial Research.2022:12(6);771-76
13. Dolens E, Dourado M, Almangush A, Salo T, Rocha C, Silva S, Brennan P, et al. The impact of histopathological feature on the prognosis of oral squamous cell carcinoma: A comprehensive review and meta nalysis.Frontiers in Oncology.2021:11;784924
14. Lugli A, Zlobec I, Berger MD, Kirsch R and Nagtegaal I. Tumour budding in solid cancers. Nat Rev Clin Oncol.2021: 18; 101-115.
15. Parekh D, Kukreja P, Mallick I, Roy P. Worst pattern of invasion-type 4(WPOI-4) and Lymphocyte host response should be mandatory reporting criteria for oral cavity squamous cell carcinoma: A re-look at the American Joint Committee of Cancer (AJCC) minimum dataset. Indian J Pathol Microbiol 2020;63:527-33.