

ORIGINAL RESEARCH

Analysis of different prognostic histological parameters and related epidemiological factors of oral squamous cell carcinoma: An experience from western UP

¹Dr. Deepti Mishra, ²Dr. Durgesh Kumar, ³Dr. Ankur Verma, ⁴Dr. Naincy Rastogi, ⁵Dr. Shweta Verma

¹Assistant Professor, ^{4,5}Senior Resident, Department of Pathology and Cancer Genetics, Kalyan Singh Superspeciality Cancer Institute, India

^{2,3}Assistant Professor, Department of Surgical Oncology, Kalyan Singh Superspeciality Cancer Institute, India

Corresponding Author

Dr. Deepti Mishra

Assistant Professor, Department of Pathology and Cancer Genetics, Kalyan Singh Superspeciality Cancer Institute, India

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ABSTRACT

Introduction – The most common malignancy of oral cavity is squamous cell carcinoma. Its epidemiology shows marked variation in different geographical regions. Hence the present study is conducted to assess different histopathological feature, which have prognostic and predictive value, which is routinely used by clinicians for planning of multimodality treatment. **Material & methods**- The cross-sectional study was carried out on total of 400 patients with OSCC who attended the surgical oncology unit of Kalyan Singh Super speciality Institute, Lucknow. Neck dissection was performed in all patients with clinico-radiologically/ FNAC proved positive nodes as well as in patients with large tumor and clinically negative nodes. Follow up program was done. SPSS 20.0 version have been used for the statistical analysis of the data. **Results** – A total of 400 cases included in this study, who met the eligibility criteria. Majority of study population (67.16%) were in the age group of less than 50year. The buccal mucosa was the most common site of tumor comprising of 187 cases. Most of the cases exhibited maximum depth of invasion >1cm (199 cases) followed by 0.5-1cm (142 cases). **Conclusion** – Study concluded that there is increasing incidence of OSCC in younger population and male gender who presented late and came with higher stage of disease in this zone of country. There is need of initiating more aggressive community awareness programs to educate individuals, especially in the younger age group.

Keywords – carcinoma, epidemiological factors, metastasis, oral cavity

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INTRODUCTION

Squamous cell carcinoma is the most common malignancy of oral cavity, it accounts more than 90% of oral cavity cancers. Epidemiology of oral cancer shows marked variation in different geographical regions hence it must be studied separately for specific geographical region. Worldwide, incidence of oral cancer higher among males than females. However, the ratio is reversed in India. Most commonly it occurs in older age group, in 6th to 7th decade of life. This devastating disease often leads to disfigurement or low survival rate, either by disease progression or as a consequence of therapeutic procedure. Therefore, different prognostic biomarkers have been developed to stratify patients by risk or response to therapy for this malignancy^{1,2,3}. However, these sophisticated techniques require costly equipments, kits and well-

trained persons and this can be possible in an advanced lab that rarely can be implemented in regular practice^{4,5}. Clinical and histopathological analysis are still the main prognostic parameters, which can routinely be used for planning of multimodality treatment including radiotherapy and chemotherapy in high risk patients.

In this study we tried to highlight the increasing incidence of oral squamous cell carcinoma in younger population and male gender, who presented late and came with higher stage of disease in this zone of country. In this study, we aim to assess different histopathological feature, which have prognostic and predictive value, which is routinely used by clinicians for planning of multimodality treatment^{6,7}.

MATERIALS AND METHODS

The present study was a cross-sectional study carried out on total of 400 patients with oral squamous cell carcinoma who attended the surgical oncology unit of Kalyan Singh Super speciality Institute, Lucknow, Uttar Pradesh, India, from November 2020 to November 2021 after considering inclusion and exclusion criteria. The inclusion criteria for our study were histologically confirmed primary squamous cell carcinoma (SCC) without any previous history of chemo or radiotherapy and were eligible for curative radical surgery. Patients who were eligible for palliative surgery with unresectable or metastatic tumors, and incomplete medical records were excluded.

Neck dissection was performed in all patients with clinico-radiologically/ FNAC proved positive nodes as well as in patients with large tumor and clinically negative nodes. Surgery was followed by radiotherapy with or without chemotherapy depending on histopathology report and the national guidelines. Finally patient entered a follow-up program which included clinical and radiological assessments.

All specimens were fixed in 10% neutral buffered formalin and were grossed according to American Joint Committee on Cancers (AJCC) protocol. Tissue was processed in fully automated tissue processor subsequently embalmed in paraffin blocks and cut at 3-5 μ m, H&E stained sections were prepared for histopathological analysis. Reporting was done according to College of American Pathologist (CAP) guideline.

STATISTICAL ANALYSIS

Descriptive statistical analysis has been carried out in the present studied. We used Fischer Exact Probability test and chi-square test analysis in order to identify relations between different histological features, significance was considered when $p < 0.05$. The Statistical software namely SPSS 20.0 version have been used for the analysis of the data and Microsoft word and Excel have been used to generate tables.

AIMS AND OBJECTIVES

1. To assess different histopathological features, which have prognostic and predictive value.
2. To highlight the magnitude of problem in this zone during a 2 year period, by describing selected clinico-pathological data of primary oral squamous cell carcinoma.

RESULTS

A total of 400 cases included in this study, who met the eligibility criteria. Majority of study population (67.16%) were in the age group of less than 50 year. 81.4% of the study subjects comprised of males with a male to female ratio of 4.1:1 (Table-1). The buccal mucosa was the most common site of tumor comprising of 187 cases (46.7% of the cases), followed by tongue 115 cases (28.7% of the cases) (Table-2). Histological differentiation was available in 396 patients which revealed that 69.7% as moderately differentiated SCC followed by well differentiated (16.5%) (Table-3), Lymphovascular invasion was identified in only 7 cases. Out of 400 cases 76 cases (19%) showed perineural invasion. Most common worst pattern of invasion was type 4 (63.2%) followed by type 5 (19.7%). Most of the cases exhibited maximum depth of invasion > 1 cm (199 cases) followed by 0.5-1cm (142 cases) (Table-4). 216 cases (54.2%) reported as tumour with free (> 0.5 cm) margin of clearance followed by close (0.1 to 0.5cm) margin of clearance in 152 cases (38.1%) (Table-5). Ipsilateral/ bilateral neck dissection was done Of these, cases (59.5%) had histologically uninvolved lymph node, while rest of the patients (39.7%) had histologically involved lymph node. Most common reported T staging was pT3 (33.8%) followed by pT2 (27.1%) and pT4a (26.2%). Out of total 159 cases with involved lymph node, 70 cases (44%) were positive for Extra-nodal extension and 89 cases (56.0%) were negative for Extra-nodal extension. (Table -6) Correlation showed that out of 135 cases of pT3 staging 81 cases associated with type 4 worst pattern of invasion and out of 105 cases of pT4a staging 53 cases associated with type 5 worst pattern of invasion (Table-7). Most common type of tumor grading associated with buccal mucosa and tongue tumor was moderately differentiated (Table-8).

Table 01- Distribution of study subject based on age & gender

Variable	Total No. of Cases N (%)
Age	
Up to 30	20 (5.01)
31-40	113 (28.32)
41-50	135 (33.83)
51-60	73 (18.2)
61-70	43 (18.7)
70+	16 (3.7)
Gender	
Female	75 (18.7)
Male	325 (81.4)

Table- 02 Distribution of site of tumor

Site of tumor	Total number of cases N (%)
Buccal mucosa	187 (46.7)
Lip	16 (4)
RMT	27 (6.7)
Lower Alveolus	51 (12.7)
Tongue	115 (28.7)
Hard Palate	4 (1)

Table – 03 Distribution of cases based on histological grade of the tumors

Histological grade	Total No. of Cases	Percentage
Well Differentiated	66	16.5
Moderately	279	69.7
Poorly	51	12.7
NA	4	1
Grand Total	400	100

Table – 04 Distribution of cases based on different mode of invasion status

Variable	Total number of cases N (%)
Lymphovascularinvasion status	
LVI Absent	393 (98.2)
LVI Present	7 (1.75)
Perineural invasionstatus	
PNI Prsent	76 (19)
PNI Absent	324 (81)
Worst pattern ofInvasion	
Type 1	5 (1.2)
Type 2	8 (2)
Type 3	55 (13.7)
Type 4	253 (63.2)
Type5	79 (19.7)
Depth of invasion	
<0.5 CM	52 (13)
0.5-1.0 CM	142 (35.5)
>1.0 CM	199 (49.7)
Could Not be measured	2 (0.5)
NA	5(1.2)

Table– 05 Distribution of status of closest surgical margin in total cases

Status of the closest surgical margin of the tumor	Total number of cases N (%)
Tumour with <0.1cm clearance/ involved margin	40 (10)
Tumour with 0.1–0.5 cm clearance	152 (38.1)
Tumour with >0.5 cm clearance	216 (54.2)
Tumour with dysplasia at excision Margin	8 (2)

Table – 06 Distribution of cases based on metastatic lymph node, t staging and ENE status

Lymph node status	Total number ofcases	Percentage
Lymph node negative formetastasis	238	59.5
Lymph node positive formetastasis	159	39.7
NA	3	0.75
T staging		
pT1	33	8.2
pT2	108	27.1
pT3	135	33.8
pT4a	105	26.4

pT4b	19	4.7
Status of ENE		
ENE -ve	89	56
ENE +ve	70	44

Table-07 Distribution of the worst pattern of invasion according to the T staging

	Type 1	Type2	Type 3	Type 4	Type 5
pT1	1	13	6	11	2
pT2	1	11	23	51	20
pT3	1	4	16	81	33
pT4a	1	17	18	16	53
pT4b	1	8	4	5	1

Table- 08 Distribution of the histological grade of the tumors according to the site

Site	WD	MD	PD	Grand Total
Buccal mucosa	21	158	8	187
Lip	4	8	4	16
RMT	5	16	6	27
Lower Alveolus	16	25	10	51
Tongue	18	74	23	115
Hard Palate	2	2	0	4

DISCUSSION

Squamous cell carcinoma is the most common malignancy of oral cavity, it accounts more than 90% of oral cavity cancers. Epidemiology of oral cancer shows marked variation in different geographical regions hence it must be studied separately for specific geographical region.

In India incidence of oral cancer is higher among females than males. However, the ratio is reversed in our study, male to female ratio was 4.1:1 possibly due to higher incidence of tobacco chewing, alcohol consumption and exposure to high-risk .HPV subtypes in this part of country ^{8,9}.

Majority of the study population (67.16%) were in the age group of less than 50 years. In our study OSCC was more common in younger age group, which is contradictory to the results of previous studies^{10,11}. Other than tobacco and alcohol consumption, frequent risk factors for OSSC in younger age group considered are dietary/ nutritional deficiency, genetic predisposition and exposure to high-risk HPV types.

Buccal mucosa was the most common site of disease, comprising of 187 cases (46.7 % of the cases) followed by tongue 115 cases (28.7 % of the cases). This finding was in harmony with study done by Johnson NW et al and Lee CH et al ^{12,13}. Buccal mucosa is considered as the commonest site of occurrence of primary OSCC in developing countries due to habit of betel and tobacco chewing^{14,15}. In our study most of the cases histologically diagnosed as moderately differentiated SCC (69.7%) followed by well differentiated (16.5%), similar findings were found in the previous studies^{16,17}. Most common type of histological grading associated with buccal mucosa and tongue tumor was moderately differentiated. Genetic, environmental and cultural factors may associate with variation in age gender profile as well

as site of tumor with different countries or different zone from the same country¹⁸.

Studies showed that perineural invasion is an important prognostic histological parameter in oral squamous cell carcinoma and a major predictor for lymph node metastasis, however in our study it was found in only 19% of the cases^{19,20}. In our study most common worst pattern of invasion was type 4 (63.2%) followed by type 5 (19.7%) and majority of cases with metastatic lymph node associated with type 4 and type 5 pattern. pT3 and pT4a stage showed worst pattern of invasion (WPOI) type 4 followed by type 5, pT4b staging showed worst pattern type 5. Different studies showed that WPOI is an important risk factors for lymph node metastasis and associated with a low 5year disease free survival. Hence this is an important prognostic factors and should be reported in histopathological evaluation^{21,22}. A total of 159 cases (39.7%) associated with histologically involved lymph node and most of the cases with involved lymph node reported as pN3b stage. Literature search showed that patient with higher pN stage associated with poor disease-free survival²³. Extra nodal extension is found to be an important prognostic factor. It is associated with higher incidence of contralateral lymph node metastasis, low survival rate and high risk of recurrence^{24,25}. Hence during pathological evaluation capsule of metastatic lymph nodes should be examined and reported in histopathology report. In our study most of the cases exhibited maximum depth of invasion >1cm (199 cases) followed by 0.5-1cm (142cases), this indicate that most of the patient came with large primary tumor and late stage (T3/T4). Depth of invasion is a prognosticator of oral squamous cell carcinoma, it has been found that increased risk of recurrence and lymph node metastasis significantly associated with more than

1cm of Depth of invasion^{26,27}. 152 cases reported as tumour with close (0.1 to 0.5cm) margin of clearance, which that in this zone most patient reached late with large (T3/4) primary tumour. Possibly majority of our patient came from rural areas and have low socioeconomic status and less accessibility to health care center. Beside other histopathological parameters, close/ involved margin of clearance significantly associated with local recurrence^{28,29}.

CONCLUSION

In this study we tried to high light the importance of different histopathological characteristic which has prognostic predictive significance. For resource poor area histopathological parameters are still an effective, standard and low cost method, which can routinely be used for planning of multimodality treatment including radiotherapy and chemotherapy in high risk patients.

In this study we tried to highlight the increasing incidence of oral squamous cell carcinoma in younger population and male gender who presented late and came with higher stage of disease in this zone of country. There is need of initiating more aggressive community awareness programs to educate individuals, especially in the younger age group.

REFERENCES

1. Troiano G, Caponio VCA, Adipietro I, Tepedino M, Santoro R, Laino L, Lo Russo L, Cirillo N, Lo Muzio L. Prognostic significance of CD68+ and CD163+ tumor associated macrophages in head and neck squamous cell carcinoma: A systematic review and meta-analysis. *Oral Oncol.* 2019 Jun;93:66-75. doi: 10.1016/j.oraloncology.2019.04.019. Epub 2019 Apr 28. PMID: 31109698.
2. Nguyen AH, Detty SQ, Agrawal DK. Clinical Implications of High-mobility Group Box-1 (HMGB1) and the Receptor for Advanced Glycation End-products (RAGE) in Cutaneous Malignancy: A Systematic Review. *Anticancer Res.* 2017 Jan;37(1):1-7. doi: 10.21873/anticancer.11282. PMID: 28011467.
3. Panarese I, Aquino G, Ronchi A, Longo F, Montella M, Cozzolino I, Rocuzzo G, Colella G, Caraglia M, Franco R. Oral and Oropharyngeal squamous cell carcinoma: prognostic and predictive parameters in the etiopathogenetic route. *Expert Rev Anticancer Ther.* 2019 Feb;19(2):105-119. doi: 10.1080/14737140.2019.1561288. Epub 2019 Jan 15. PMID: 30582397.
4. Caruntu A, Moraru L, Lupu M, Vasilescu F, Dumitrescu M, Cioplea M, Popp C, Dragusin A, Caruntu C, Zurac S. Prognostic Potential of Tumor-Infiltrating Immune Cells in Resectable Oral Squamous Cell Carcinoma. *Cancers (Basel).* 2021 May 8;13(9):2268. doi: 10.3390/cancers13092268. PMID: 34066837; PMCID: PMC8125877.
5. Wagner S, Wittekindt C, Reuschenbach M, Hennig B, Thevarajah M, Würdemann N, Prigge ES, von Knebel Doeberitz M, Dreyer T, Gattenlöhner S, Klusmann JP. CD56-positive lymphocyte infiltration in relation to human papillomavirus association and prognostic significance in oropharyngeal squamous cell carcinoma. *Int J Cancer.* 2016 May 1;138(9):2263-73. doi: 10.1002/ijc.29962. Epub 2016 Jan 8. PMID: 26662627.
6. Köhler HF, Vartanian JG, Pinto CAL, da Silva Rodrigues IFP, Kowalski LP. The impact of worst pattern of invasion on the extension of surgical margins in oral squamous cell carcinoma. *Head Neck.* 2022 Mar;44(3):691-697. doi: 10.1002/hed.26956. Epub 2021 Dec 14. PMID: 34904762.
7. Shimizu S, Miyazaki A, Sonoda T, Koike K, Ogi K, Kobayashi JI, Kaneko T, Igarashi T, Ueda M, Dehari H, Miyakawa A, Hasegawa T, Hiratsuka H. Tumor budding is an independent prognostic marker in early stage oral squamous cell carcinoma: With special reference to the mode of invasion and worst pattern of invasion. *PLoS One.* 2018 Apr 19;13(4):e0195451. doi: 10.1371/journal.pone.0195451. PMID: 29672550; PMCID: PMC5909609.
8. Andisheh-Tadbir A, Mehrabani D, Heydari ST. Epidemiology of squamous cell carcinoma of the oral cavity in Iran. *J Craniofac Surg.* 2008 Nov;19(6):1699-702. doi: 10.1097/SCS.0b013e31818c04cc. PMID: 19098587.
9. Sarode G, Maniyar N, Sarode SC, Jafer M, Patil S, Awan KH. Epidemiologic aspects of oral cancer. *Dis Mon.* 2020 Dec;66(12):100988. doi: 10.1016/j.disamonth.2020.100988. Epub 2020 Jun 27. PMID: 32605720.
10. Li Y, Jiang B, Huang M, Wang G, Bai Y, Huang W. Retrospective study of the clinicopathological characteristics and prognosis of elderly patients with oropharyngeal squamous cell carcinoma. *J Int Med Res.* 2021 Jun;49(6):3000605211016662. doi: 10.1177/03000605211016662. PMID: 34082601; PMCID: PMC8182203.
11. Sarode G, Maniyar N, Sarode SC, Jafer M, Patil S, Awan KH. Epidemiologic aspects of oral cancer. *Dis Mon.* 2020 Dec;66(12):100988. doi: 10.1016/j.disamonth.2020.100988. Epub 2020 Jun 27. PMID: 32605720.
12. Johnson NW, Jayasekara P, Amarasinghe AA. Squamous cell carcinoma and precursor lesions of the oral cavity: epidemiology and aetiology. *Periodontol* 2000. 2011 Oct;57(1):19-37. doi: 10.1111/j.1600-0757.2011.00401.x. PMID: 21781177.
13. Lee CH, Lee KW, Fang FM, Wu DC, Shieh TY, Huang HL, Chen CH, Chen PH, Chen MK, Kuo SJ, Chang CH, Tsai YS, Chiang SL, Tu HP, Chen BH, Ko YC. The use of tobacco-free betel-quinid in conjunction with alcohol/tobacco impacts early-onset age and carcinoma distribution for upper aerodigestive tract cancer. *J Oral Pathol Med.* 2011 Oct;40(9):684-92. doi: 10.1111/j.1600-0714.2011.01022.x. Epub 2011 Mar 8. PMID: 21385213.
14. Madani AH, Dikshit M, Bhaduri D, Jahromi AS, Aghamolaei T. Relationship between Selected Socio-Demographic Factors and Cancer of Oral Cavity - A Case Control Study. *Cancer Inform.* 2010 Aug 11;9:163-8. doi: 10.4137/cin.s4774. PMID: 20838608; PMCID: PMC2935817.
15. Sharma P, Saxena S, Aggarwal P. Trends in the epidemiology of oral squamous cell carcinoma in Western UP: an institutional study. *Indian J Dent Res.* 2010 Jul-Sep;21(3):316-9. doi: 10.4103/0970-9290.70782. PMID: 20930335.
16. Sethi S, Ohri S, Aggarwal P, Grewal H. Histopathological Factors in Oral Squamous Cell Carcinoma-Should a Clinician Look Beyond Clinical

- Staging? *J Oral Maxillofac Surg.* 2021 Aug;79(8):1694-1705. doi: 10.1016/j.joms.2021.02.014. Epub 2021 Feb 22. PMID: 33744240.
17. Pereira MC, Oliveira DT, Landman G, Kowalski LP. Histologic subtypes of oral squamous cell carcinoma: prognostic relevance. *J Can Dent Assoc.* 2007 May;73(4):339-44. PMID: 17484800.
 18. Losi-Guembarovski R, Menezes RP, Polisel F, Chaves VN, Kuasne H, Leichsenring A, Maciel ME, Guembarovski AL, Oliveira BW, Ramos G, Mizuno LT, Cavalli IJ, Ribeiro EM, Cólus IM. Oral carcinoma epidemiology in Paraná State, Southern Brazil. *Cad Saude Publica.* 2009 Feb;25(2):393-400. doi: 10.1590/s0102-311x2009000200018. PMID: 19219247.
 19. Park J, Megow A, Swalling A, Hodge JC, Foreman A, Boase S, Valentine R, Krishnan S, Ooi EH. Prognosis of oral squamous cell carcinoma with perineural invasion: A comparative study of classification types. *Clin Otolaryngol.* 2020 Jan;45(1):99-105. doi: 10.1111/coa.13472. Epub 2019 Nov 19. PMID: 31677332.
 20. Sahoo A, Panda S, Mohanty N, Jena D, Mishra N, Surabhi, Baisakh MR. Perineural, lymphovascular and depths of invasion in extrapolating nodal metastasis in oral cancer. *Clin Oral Investig.* 2020 Feb;24(2):747-755. doi: 10.1007/s00784-019-02921-0. Epub 2019 May 28. PMID: 31139977.
 21. Chatterjee D, Bansal V, Malik V, Bhagat R, Punia RS, Handa U, Gupta A, Dass A. Tumor Budding and Worse Pattern of Invasion Can Predict Nodal Metastasis in Oral Cancers and Associated With Poor Survival in Early- Stage Tumors. *Ear Nose Throat J.* 2019 Aug;98(7): E112-E119. doi: 10.1177/0145561319848669. Epub 2019 May 9. PMID: 31072197.
 22. Xu B, Salama AM, Valero C, Yuan A, Khimraj A, Saliba M, Zononi DK, Ganly I, Patel SG, 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 Sep;479(3):597-606. doi: 10.1007/s00428-021-03063-z. Epub 2021 Mar 4. PMID: 33661329; PMCID: PMC8417140.
 23. Arun I, Maity N, Hameed S, Jain PV, Manikantan K, Sharan R, Arun P. Lymph node characteristics and their prognostic significance in oral squamous cell carcinoma. *Head Neck.* 2021 Feb;43(2):520-533. doi: 10.1002/hed.26499. Epub 2020 Oct 6. PMID: 33021340.
 24. Hasegawa T, Yanamoto S, Otsuru M, Kakei Y, Okura M, Yamakawa N, Yamada SI, Ota Y, Umeda M, Kirita T, Kurita H, Ueda M, Komori T; Japan Oral Oncology Group (JOOG). Multi-center retrospective study of the prognosis and treatment outcomes of Japanese oral squamous cell carcinoma patients with single lymph node metastasis and extra nodal extension. *J Surg Oncol.* 2018 Jun;117(8):1736-1743. doi: 10.1002/jso.25083. Epub 2018 May 1. PMID: 29714825.
 25. Gao W, Hu Y, Zhu D, Li X, Guo B, Shen Y, Ma C, Du J. Extranodal Extension in Bilateral Cervical Metastases: A predictor of Undesirable Survival Outcomes despite Aggressive Salvage Treatment in Oral Cancer Patients. *J Cancer.* 2021 Aug 3;12(19):5848-5863. doi: 10.7150/jca.60152. PMID: 34475998; PMCID: PMC8408102.
 26. Faisal M, Abu Bakar M, Sarwar A, Adeel M, Batool F, Malik KI, Jamshed A, Hussain R. Depth of invasion (DOI) as a predictor of cervical nodal metastasis and local recurrence in early stage squamous cell carcinoma of oral tongue (ESSCOT). *PLoS One.* 2018 Aug 22;13(8):e0202632. doi: 10.1371/journal.pone.0202632. PMID: 30133515; PMCID: PMC6105019.
 27. Aaboubout Y, van der Toom QM, de Ridder MAJ, De Herdt MJ, van der Steen B, van Lanschoot CGF, Barroso EM, Nunes Soares MR, Ten Hove I, Mast H, Smits RWH, Sewnaik A, Monserez DA, Keereweer S, Caspers PJ, Baatenburg de Jong RJ, Bakker Schut TC, Puppels GJ, Hardillo JA, Koljenović S. Is the Depth of Invasion a Marker for Elective Neck Dissection in Early Oral Squamous Cell Carcinoma? *Front Oncol.* 2021 Mar 12;11:628320. doi: 10.3389/fonc.2021.628320. PMID: 33777774; PMCID: PMC7996205.
 28. Hashmi AA, Iftikhar SN, Haider R, Baig NN, Asif MG, Irfan M. Recurrence and Disease-Free Survival in Head and Neck Squamous Cell Carcinoma After Margin-Free Resection on Frozen Section: An Institutional Perspective. *Cureus.* 2020 Nov 8;12(11):e11385. doi: 10.7759/cureus.11385. PMID: 33312786; PMCID: PMC7725211.
 29. Mitchell DA, Kanatas A, Murphy C, Chengot P, Smith AB, Ong TK. Margins and survival in oral cancer. *Br J Oral Maxillofac Surg.* 2018 Nov;56(9):820-829. doi: 10.1016/j.bjoms.2018.06.021. Epub 2018 Sep 13. PMID: 30220612.