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Original Research Article

A Novel Technique for Intraoperative Assessment of Surgical Margin Using Lugols Iodine and Frozen Section Methods in Oral Squamous Cell Carcinoma

Prajwal K[1*], Amarnath P Upasi[2], Sunil Byadgi[2], Kirthi Kumar Rai[3]

[1]Post Graduate Student, Department of Oral and Maxillofacial Surgery, Bapuji Dental College and Hospital, Davangere, 577004, Karnataka, India
[2]Professor, Department of Oral and Maxillofacial Surgery, Bapuji Dental College and Hospital, Davangere, 577004, Karnataka, India
[3]Professor & Head, Department of Oral and Maxillofacial Surgery, Bapuji Dental College and Hospital, Davangere, 577004, Karnataka, India

***Corresponding Author:** Prajwal K
Post Graduate Student, Department of Oral and Maxillofacial Surgery, Bapuji Dental College and Hospital, Davangere, 577004, Karnataka, India

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Abstract: Surgery is considered to be the primary mode of treatment of head and neck malignancies and the ultimate aim of surgical resection is to obtain a tumor-free margin. No uniform criteria to define a clear surgical margin exist among practicing surgeons. This study intends to find the adequacy of surgical margin marking intraoperatively by Lugol's iodine solution considering the frozen section method for oral squamous cell carcinoma (OSCC). Intraoperative staining with Lugol's iodine and Frozen section analysis of margins of the surgical bed was negative in anterior, superior, and inferior specimens in all the cases, except in one case (4.3%), a posterior margin was found to be positive (unstained) for tumor. The chi-square value of the Lugol's stained margins following resection of the tumor and negative results of frozen section analysis of the same was found to be statistically significant with the P value <0.001. Hence, we conclude that Lugol's iodine helps in obtaining a clear surgical margin intra-operatively where inter-surgeons bias/judgment errors regarding safe margin can be avoided, especially in cases where the frozen section is not feasible.

Keywords: Surgical margins, intra-operative staining, Lugol's iodine, frozen section, oral squamous cell carcinoma.

1. INTRODUCTION

One of the most important but onerous aspects of oncosurgery is corroborating the complete removal of the primary tumor because positive mucosal margins following the resection of oral squamous cell carcinoma (OSCC) have been associated with a high incidence of local recurrence[1]and lowers the 5-year survival by 11–15% [2].

The best chance of surgical cure in oral cancer is complete removal of all malignant cells. Unfortunately, it is not possible to identify the microscopic extent of tumor invasion by inspection and palpation. During resection, it is difficult to identify the region of epithelial dysplasia surrounding the lesion. The width of this margin varies between 1- 2cm depending on the histological type and differentiation. One of the most common reasons to employ a wide range of surgical margins is the difficulty in identifying the clear margin, leaving this unresected will lead to second primary tumors or local recurrences [3].

Vital staining with iodine is one such technique that is used as a diagnostic tool and for various surgical purposes such as detection of sub-mucosal planes during dissection and differentiation of dysplastic cells from normal. Iodine staining of mucosal lesions was first reported by Schiller who used it to identify cervical cancer of the uterus [4]. Thereafter, this method has been employed for the upper gastrointestinal tract [5]. The types of vital staining are, supravital stain where living cells that have been removed from an organism are stained and Intra-vital

stain where the stain is injected or otherwise introduced into the body itself.

Lugol's iodine identifies pre-malignant or malignant epithelium by reacting with glycogen located in the upper and middle parts of the tumor-adjacent oral epithelium, thereby delineating it from unstained potentially malignant or malignant epithelium [6]. It also provides the surgical team with a straightforward method tor precisely identifying the advancing edge of a premalignant condition [7].

Frozen section evaluation is a key technique used by surgeons across specialties for rapid assessment of the presence of tumors in tissues, the most common being the surgical margins. The use of intraoperative frozen section evaluation of surgical margins is an accepted and frequent practice in head and neck oncosurgery [8]. Frozen section analysis during intraoperative consultation is important in ensuring adequate margin clearance. The report given is usually binary and it just indicates the presence or absence of tumor in the sample. Based on this report, the surgeon decides on whether to make additional resections or not [9].

The frozen section technique begins with specimen retrieval from the patient by the surgeon followed by slides preparation, microscopy, and finally rendering the frozen section diagnosis. The frozen section diagnosis is usually communicated verbally to the surgeon by the pathologist via telephone, intercom, video conference, and sometimes in person via an intermediary. Such reports enable the surgeon to decide on whether further resections are needed or not [10].

The present study was conducted to find the adequacy of surgical margin marking intraoperatively by Lugol's iodine solution considering the frozen section method for oral squamous cell carcinoma.

2. MATERIALS AND METHODS

2.1 Source of the Data

The study was conducted in the Department of Oral, Maxillofacial, and Reconstructive Surgery, Bapuji Dental College and Hospital, Davangere, Karnataka.

A total of 23 patients diagnosed with oral squamous cell carcinoma were studied during the period from November 2019 to April 2022.

2.2 Study Design

2.2.1 Inclusion Criteria

- Patients diagnosed with oral squamous cell carcinoma.
- Patients of the age group 20-70years.
- Patient willing for surgical excision of the tumor.

2.2.2 Exclusion Criteria

- Patients with known allergy to iodine solution.
- Recurrence of the primary tumor.

2.2.3 Materials

- Lugol's iodine 3%.
- Carbocystiene syrup.
- Normal saline.
- Two 10 ml irrigation syringes.

2.2.4 METHODOLOGY

Patients fulfilling the inclusion and exclusion were included in the study. A detailed case history was recorded in each case as per the proforma and was followed by a clinical examination. Consent was obtained from all the patients regarding the procedure.

2.3 Surgical Technique

- Patients were operated under general anesthesia with aseptic condition with standard draping.
- The tumor sites were exposed following neck dissection.
- The area to be stained was prepared first by drying the mucosa with sterile gauze, then by applying 10 ml of mucolytic syrup i.e., ambroxol hydrochloride (30mg/5ml), and washed with normal saline.
- 3 % Lugol's iodine was then irrigated over the prepared site and waited for one minute.
- Excess iodine was washed away with normal saline and the surgical margin was established.
- Resection was performed 10mm away from the induration of the cancer and 5mm away from the margin of the Lugol's unstained area.
- Following the completion of the surgical resection of the tumor, representative samples that were equidistant from the tumor margin onto the surgical bed were sent for the frozen section.
- Depending on the frozen section results, closure was achieved if the margins were negative and further resection of 1 to 1.5 cm was performed if the margins were close or positive.

2.4 RESULTS

Collective data was entered in an excel bar and analyzed using 'R software version 3.2.3'.

Results are expressed as

- 'Mean' and 'standard deviation'.
- 'Count' and 'percent'.

2.4.1 Age

In this study, the age distribution was between 35-70 years with Mean ± SD was 51.3 ± 9.9 and range was 37 - 67 years (TABLE 1 AND GRAPH 1).

2.4.2 Sexwise Distribution

Out of 23 patients in the study, 16(69.6%) were male and 7(30.4) were female (TABLE 2 AND GRAPH 2).

2.4.3 Site of OSCC

Sites of OSCC were evaluated and was found that 7(30.4%) cases had SCC of right buccal mucosa, followed by 3(13.0%) cases each of OSCC of left buccal mucosa and OSCC of left GBS (TABLE 3 AND GRAPH 3).

2.4. 4 Frozen Section Results

Frozen section of margins of the surgical bed were evaluated and was found that in all the cases anterior, superior, and inferior specimens were negative, and only in one case(4.3%), a posterior margin was found to be positive for tumor (TABLE 4 AND GRAPH 4).

2.4.5. Intraoperative Staining

Intraoperative staining with Lugol's iodine was evaluated and was found that in all the cases anterior, superior, and inferior margins were stained, and only in one case (4.3%), a posterior margin was found unstained (TABLE 5 AND GRAPH 5).

2.4.6 Histopathological Findings

Histopathology of the margins were sent for the frozen section was assessed and was found that in 23 cases the anterior and superior margins showed normal mucosa. Dysplasia was present in one inferior (4.3%) and one posterior (4.3%) sample (TABLE 6 AND GRAPH 6).

2.4.7 T Stage Distribution

T stage distribution was assessed. Out of 23 cases, 16(69.6%) were in the T2 stage, 5(21.7%) in the T3 stage, and 2(8.7%) in the T4 stage (TABLE 7 AND GRAPH 7).

2.4.8 Gradewise Distribution

Grade-wise distribution was assessed. Out of 23 cases 20(87.0%) were moderately differentiated and 3(13.0%) well-differentiated (TABLE 8 AND GRAPH 8).

3.4.9 TABLES

Table 1: Age distribution

Age (yrs)	No.of cases	%
35-40	4	17.4
41-50	8	34.8
51-60	6	26.1
61-70	5	21.7
Total	**23**	**100.0**
Age (years)	Mean ± SD	51.3 ± 9.9
	Range	37 - 67 yrs

Table 2: Sex-wise distribution

Sex	No.of cases	%
MALE	16	69.6
FEMALE	7	30.4
Total	**23**	**100.0**

Table 3: Sites distribution

SITE	No.of cases	%
OSCC OF RIGHT BUCCAL MUCOSA	7	30.4
OSCC OF LEFT BUCCAL MUCOSA	3	13.0
OSCC OF LEFT GBS	3	13.0
OSCC OF RIGHT LATERAL BORDER OF TONGUE	4	17.4
OSCC OF LEFT LATERAL BORDER OF TONGUE	2	8.7
OSCC OF LEFT RETROMOLAR TRIGONE	1	4.3
OSCC OF RIGHT AND LEFT LABIAL MUCOSA	1	4.3
OSCC OF LEFT LABIAL MUCOSA	1	4.3
OSCC OF RIGHT GBS	1	4.3
Total	**23**	**100**

Table 4: Intra operative findings: frozen section

FROZEN SECTION	Negative		Positive		Total
	NO.	%	NO.	%	
ANTERIOR	23	100.0	0	0.0	23
POSTERIOR	22	95.7	1	4.3	23
SUPERIOR	23	100.0	0	0.0	23
INFERIOR	23	100.0	0	0.0	23
Total	**91**	**98.9**	**1**	**1.1**	**92**

Table 5: Intra operative findings: lugol's iodine staining

STAINING	PRESENT		ABSENT		Total
	NO.	%	NO.	%	
ANTERIOR	23	100.0	0	0.0	23
POSTERIOR	22	95.7	1	4.3	23
SUPERIOR	23	100.0	0	0.0	23

INFERIOR	23	100.0	0	0.0	23
Total	**91**	**98.9**	**1**	**1.1**	**92**

Table 6: Post-operative H P R findings

H P R	NEGATIVE		POSITIVE		Total
	NO.	%	NO.	%	
ANTERIOR	23	100.0	0	0.0	23
POSTERIOR	22	95.7	1	4.3	23
SUPERIOR	23	100.0	0	0.0	23
INFERIOR	22	95.7	1	4.3	23
Total	**90**	**97.8**	**2**	**2.2**	**92**

Table 7: T stage distribution

T Stage	No.of cases	%
T2	16	69.6
T3	5	21.7
T4	2	8.7
Total	**23**	**100.0**

Table 8: Gradewise distribution

Grade	No. of cases	%
MODERATE	20	87.0
WELL	3	13.0
Total	**23**	**100.0**

Table 9: Comparision of vital staining and frozen section

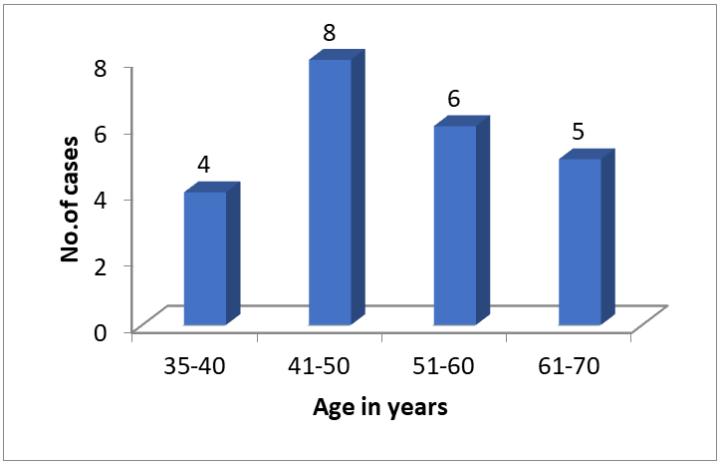
VITAL STAINING	FROZEN SECTION		TOTAL
	POSITIVE	NEGATIVE	
NEGATIVE	1(100%)	0(0%)	1
POSITIVE	0(0%)	91(100%)	91
TOTAL	**1**	**91**	**92**

$X^2 = 92.0$, P<0.001, HS

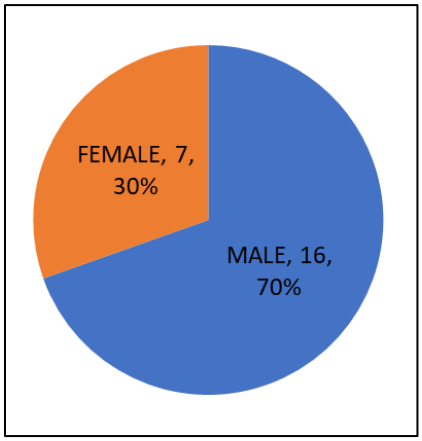
Table 10: Diagnostic value of staining when considered with frozen section

SENSITIVITY	1/1	100%
SPECIFICITY	91/91	100%
PPV	1/1	100%
NPV	91/91	100%
ACCURACY	92/92	100%

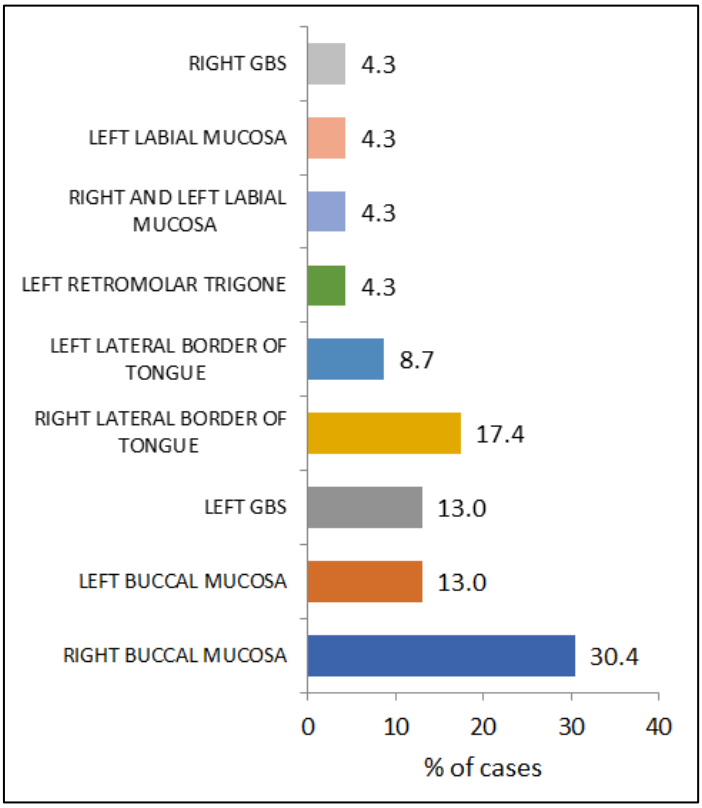
GRAPHS



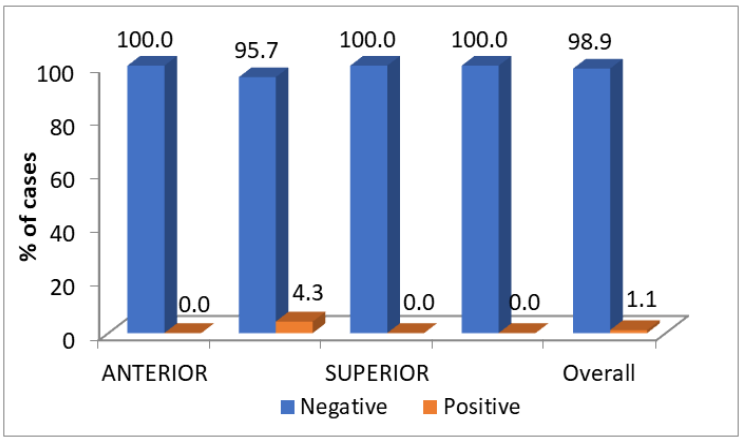
Graph 1: Age distribution



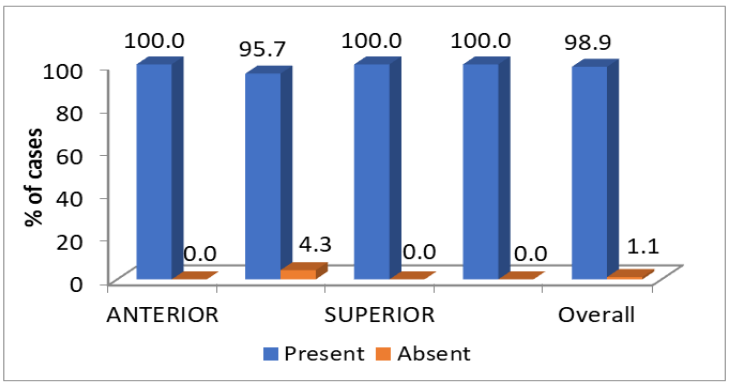
Graph 2: Sex wise distribution



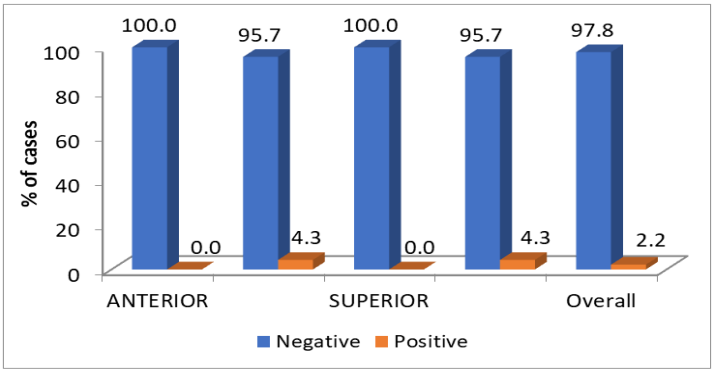
Graph 3: Sites distribution



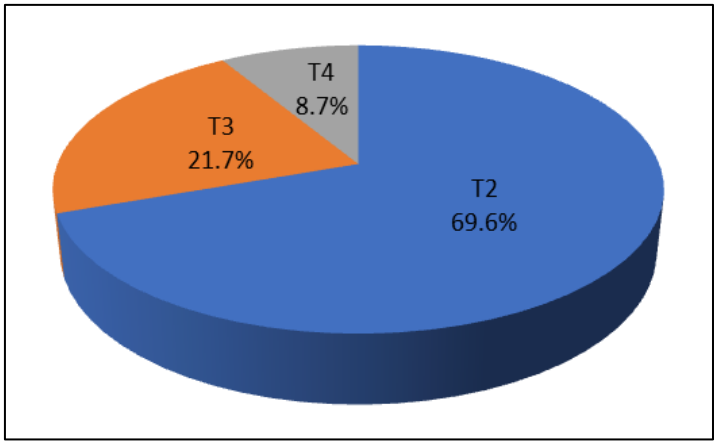
Graph 4: Intra operative findings: frozen section



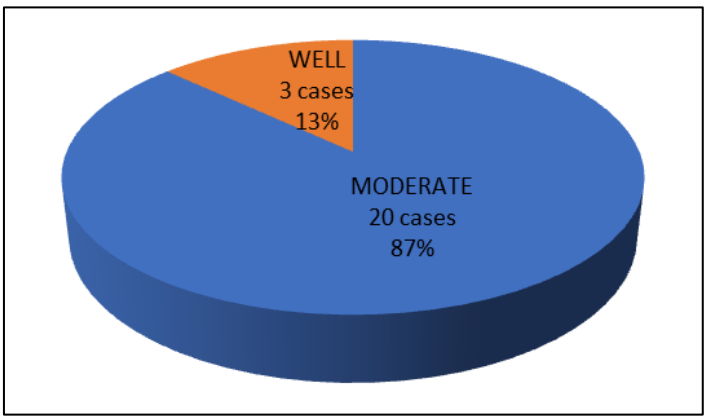
Graph 5: Intra operative findings: lugol's iodine staining



Graph 6: Post-operative h p r findings



Graph 7: T stage distribution



Graph 8: Gradewise distribution

FIGURES

Figure 1: Pre-Operative Picture Showing Lesion at Left Rmt Region

Figure 2: Application of Lugols Iodine

Figure 3: Unstained Lesion in Left Rmt Region

3. DISCUSSION

Radicality is the most important principle in oncologic surgery. The completeness of removal requires a cuff of healthy tissue around the cancerous tissue. In the postoperative follow-up, it is common practice among surgeons, radiotherapists to discuss the appropriate adjuvant therapy for each patient, based on definitive histology and pTNM. Other than staging, the risk factors and co-morbidities of the patient, in most cases, the margin status plays a crucial role in the decision making, since the data in the literature shows documents with worsening of prognosis in patients with positive resection margins [36].

Many factors affect the prognosis of treatment of OSCC such as tumor location, thickness, stage, grade, micrometastasis, cervical metastasis, number and anatomic level of positive nodes, presence, and extent of extracapsular spread, and status of resection margins [37].

Visual distinction between dysplasia and normal mucosa is based on differences between their color and texture. This makes visual clinical examination difficult. It was found that direct inspection of the lesion will always lead to various results among different examiners. Hence, delineation of dysplastic epithelium by direct inspection is not a reliable method [37].

Lugol's iodine has been used to mark the surgical margins intra-operatively considering the frozen section method for oral squamous cell carcinoma in the present study. Lugol's iodine was chosen due to its low-cost, wide availability, and ease of use. Surgical site preparation before application of Lugol's iodine to remove the presence of mucous from the surfaces has been suggested in certain studies. In the present study, we have used mucolytic syrup ambroxol hydrochloride (30mg/5ml) for the same.

Many studies have shown that glycolysis is elevated in cancer cells and such cellular areas with elevated glycolysis are generally seen as unstained lesions during vital iodine staining [12]. Maeda *et al.*, analyzed the lightness values of Lugol's iodine-stained and -unstained mucosa using a colorimeter and discovered significant differences between normal epithelium and epithelium with high-grade dysplasia, and between low-grade dysplasia and high-grade dysplasia. They correlated these differences with PAS-positive cell rates. These results interpreted that iodine staining might be directly related to glycogen content in the oral epithelium [12].

One important observation we made during the vital staining was that Lugol's iodine staining was absent in the deeper areas of the epithelium. We initially suspected that shorter contact time between the vital stain and mucosa may be the reason and hence, increased the staining time from 1 minute to 5 minutes, and still found the same results. This observation led us to conclude that there exists an epithelial barrier

function between epithelial cell layers. Xiao *et al.,* reported this finding in their study that cells neighboring the basal layer have a stronger resistance to cytotoxic iodine than superficial aging cells with loose cell junctions and defective epithelial permeability. The oral epithelium exhibits variable infiltration properties towards various dyes. In the case of vital Lugol's iodine staining, the permeability of oral epithelium and its glycogen content may function together [31].

In the present study, we have chosen Lugol's iodine staining for obtaining "superficial" surgical mucosal margin clearance, which was accurate in 22 of 23 cases. In a study Lugol's staining has been used in evaluating "deep" surgical margins in 15 patients where there was no significant difference in the tumor-margin distance between tissue staining and histopathological assessment in 12 patients. Hence, the authors concluded that the vital tissue staining analysis was useful in controlling deep surgical margins when the tumor tissue was accurately delineated. Also, when we compare Lugol's iodine with Frozen section analysis, vital staining has more advantages such procedure is simpler and more expedient [25]. Also, it helps in the better orientation of the surgical specimens and saves time. In a study by Ribeiro *et al.,* on frozen section analysis, there was a failure to achieve free surgical margins of the invasive tumor in 15% of the cases, despite favorable results on frozen section analysis [42].

In our study, a total of 92 margins from 23 patients were studied. Only one positive margin was noted on frozen section analysis after vital staining, where the staining could not be visualized either due to anatomic limitation as the posterior margin was too posterior to be visualized or may be due to less specificity of Lugol's iodine [44]. Mild dysplasia may not be identified and may get grouped under the normal epithelium. Thus, the staining was effective in 98.9 % of cases (table 4). It is stated that mild dysplasia at the margins does not significantly impair the loco-regional control on the disease-free survival of the patient [45]. According to the study by Brandwein-Gensler *et al.,* the patterns of invasion and lymphocyte response play a significant role in local recurrence more than the status of surgical margins [46].

All resection margins were reported to be free of tumour in the final histopathology report except in one case where, the tumour in the retro-molar trigone region ($T_2N_0M_0$) had unstained lesions (USLs) more than 60mm in size, reaching as far as oro-pharynx. Partial resection was performed as widely as possible. The posterior margin turned out to be positive on frozen section analysis and post-operative histopathological study. The patient developed local recurrence in three months. Similar results were observed in a study where partial glossectomy of T_1 lesion base of the tongue resulted in positive margin and eventually lead to local recurrence within six months [12].

In a study, it was found that it was difficult to obtain disease-free margins in the malignancies affecting the retromolar trigone region and maxilla [16], which was a similar finding in our study in one case. The sites such as the chin, eyelids ears, and nose are considered as high-risk sites because of underlying embryonal fusion planes resulting in skin and subcutaneous tissue having less resistance to tumor invasion and spread [49]. A possible reason for increased margin involvement in these areas is due to a less aggressive approach by the surgeons to avoid functional and aesthetic deficits.

The age of the patients in our study ranged from 37-67 years, which was in accordance with previous studies by Kumar *et al.,* and Bin Ahmed *et al.,* [51, 52]. Overall male to female ratio was 2.3:1, which was similar in studies by Pandey *et al.,* [11] and Bin Ahmed *et al.,* [52].

According to previous literature, nearly 2/3rd of malignancies in India are present in gingivo-buccal sulcus. In our study, buccal mucosa (43.4%) was the most common site involved [53] (Table 3).

8 out of 23 cases included bone in the resected specimen, which accounts for 35% of overall cases, which was a similar observation from the study by Omer *et al.,* where there were 30.4% of involved bony margins [16]. There are numerous modalities to predict mandibular involvement and to improve marginal clearance of bone. They are OPG, CT scan, PET /SPECT, frozen section assessment of cancellous bone.

The chi-square value of the Lugol's stained margins following resection of the tumour and negative results of frozen section analysis of the same was found to be statistically significant with the P value <0.001 (table 9).

The sensitivity and specificity of Lugol's iodine were 100% (1/1) and 100% (91/91) respectively (table10) when considered along with the frozen section in the present study.

The results from the present study showed that the Lugol's iodine helps in obtaining a clear surgical margin intra-operatively where inter-surgeons bias/judgement errors regarding safe margin can be avoided, especially in cases where frozen section is not feasible. The application of Lugol's iodine can be considered for every case and in combination with frozen sections when anatomic subsites (such as posterior margins) cannot be visualized by vital staining.

4. CONCLUSION

Vital staining with Lugol's iodine is a simple, economical, easily available, and time-saving technique that helps the operating surgeons to quickly decide the

safe surgical margin with high accuracy. We could achieve tumor-free surgical margins in 96.6% of cases when this modality was used considering the frozen section analysis. Local recurrence was found only in 4.3% of cases and improved the overall quality of life of patients with oral squamous cell carcinoma. In the centers where frozen section analysis is not feasible, the Lugol's iodine can be used as a substitute. Also, the results of the frozen section were similar to histopathology results in 98.9% of the cases. Hence, the application of Lugol's iodine can be considered for every case and in combination with frozen sections whenever anatomic subsites (such as posterior margins) cannot be visualized by vital staining.

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