



# Clinicopathological study of surgical margins in squamous cell carcinoma of buccal mucosa

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## KEYWORDS

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**Abstract** *Objective:* To evaluate the margins of resected specimen of oral squamous cell carcinoma (SCC) and to document the surgical margin (measured at the time of resection) and margins at the time of pathological examination (after immersion of the specimen in formalin). *Methods:* Patients who were diagnosed and confirmed with squamous carcinoma of buccal mucosa were included in the study. Patients underwent resection of the tumor with a margin of 1 cm. Soon after resection, the distance between outermost visible margin of the tumor and the margin of the specimen was measured and documented. Specimens were fixed in 10% formalin and submitted for gross and histopathological examination. The closest histopathologic margin was compared with the in situ margin (10 mm) to determine and document any shrinkage of the margin and the percentage of discrepancy if any.

*Results:* A total of 52 specimens were collected from patients between January 2014 and December 2014. All specimens were obtained from the oral cavity (n = 52) of which 43 (82.7%) were squamous cell carcinoma and 9 (17.3%) were verrucous variant of squamous cell carcinoma. The average decrease in tumor margins measured after fixation in formalin was

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found to be statistically significant ( $P < 0.05$ ) in 65% of cases.

**Conclusion:** Tumor margin shrinks significantly after formalin fixation by about 25%. The operating surgeon and pathologist should be well aware of such changes while planning for further management thereby ensuring adequate margin of resection and adjuvant treatment wherever required to prevent possible local recurrence of the disease.

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## Introduction

Surgery is the most well-established and accepted mode of initial definitive treatment for a majority of oral cancers for well over a century. Obtaining tumor free surgical margin following resection of localized malignancies is an important requirement for ensuring success in onco surgery.<sup>1</sup> Oral cavity poses several special challenges to adequate resection. Primarily, it possesses a three dimensional complex anatomy which is difficult for insitu measurement of resection margins. Secondly the mucosa and underlying tissues vary widely in their ability to act as barriers to tumor spread. Tumor size is an important prognostic factor in head and neck cancers. Precise measurement of primary oral cavity lesions (both invasive and non invasive), and subsequent margin assessment both intraoperatively and postoperatively are crucial for accurate staging and appropriate management. If there is discrepancy in documenting margins of the tumor by either the surgeon or the pathologist, it can lead to subsequent improper staging of the lesion and inappropriate management.

According to AJCC, the pathological classification of a carcinoma is determined by evidence acquired before treatment which is supplemented and modified by additional evidence acquired during surgery.

The pathological T category is derived from the actual measurement of unfixed tumor in the resected specimen because up to 30% shrinkage of soft tissue may occur after formalin fixation.<sup>2</sup>

The optimal width of the surgical margin for oral cancer has always been an issue of debate. Microscopic tumor at the inked resection margin increases the chance of local recurrence by a factor of 2 or more in most series. The term "positive margin" should be reserved for patients with microscopic tumor at the inked resection margin.<sup>3</sup>

There are 2 explanations for the positive margin phenomenon. The first possible explanation is that microscopic tumor may extend beyond the clinically visible and palpable tumor. Routinely, 1-cm margin of clinically normal tissue around the tumor is resected to achieve at least 5 mm of histopathologically normal tissue; however, this is may not always be sufficient. Extensions or islands of tumor may invade out of the main mass of tumor, resulting in a margin that is closer than anticipated. Alternatively, tissue retraction that occurs after resection and pathologic processing of the specimen may decrease the size of the tissue margin.

The problem of margin shrinkage has been dealt with at other sites, but has not been addressed and quantified adequately in oral cavity cancers.

The aim of this prospective observational study was to evaluate the margins of resected specimen of squamous cell carcinoma (SCC) of buccal mucosa and to document the surgical margin (measured at the time of resection) and margins at the time of pathological examination (after immersion of the specimen in formalin).

## Materials and methods

This prospective observational study was performed in the Department of Otorhinolaryngology and Head and Neck surgery, R.L Jalappa Hospital, Tamaka, Kolar between January 2014 and December 2014 after approval by the Institute's Ethics Committee. This study was performed on resected specimens of 52 patients who satisfied the inclusion criteria.

Inclusion criteria was biopsy proved SCC of buccal mucosa with no previous treatment and good general condition allowing a major surgical procedure. Patients who received neoadjuvant chemotherapy, locoregional recurrence, patients operated or radiated earlier and patients with distant metastases were excluded from the study. All patients were informed regarding the purpose of the study and their written consent was obtained. Basic demographic data including age, gender, tumor location, tumor staging and histological features were documented.

A thorough clinical examination and routine preoperative laboratory tests followed by searching for locoregional and distant metastases were done with computed tomography (CT) and abdominal ultrasonography in all patients.

The studied groups included 52 patients: 38 females (73%) and 14 males (27%), with a female to male ratio 2.7:1. The age of the patients ranged from 45 to 65 (mean 55.15). The borders of the tumor were determined by visual inspection and palpation and were then marked with marking ink. Surgical margin of at least 10 mm was marked circumferentially from the clinically detectable tumor using metric ruler. The distance between visible outermost limit of tumor and the margin of resection was measured using calipers. After approximately 1–2 days of formalin immersion, the measurements were repeated as performed earlier and documentation was done with regard to any change or discrepancy in the measurements. Tumor stage and nodal stage are listed in Table 1. Addictions of the patients in this study are in Table 2.

For regional control, the surgical management included a radical neck dissection (RND), modified radical neck dissection (MRND) or selective neck dissection, depending

**Table 1** Tumor stage and nodal stage.

Staging		n	%
Tumor stage	T <sub>2</sub>	6	12
	T <sub>3</sub>	13	25
	T <sub>4</sub>	33	63
Nodal stage	N <sub>0</sub>	24	46
	N <sub>1</sub>	9	17
	N <sub>2</sub>	19	37

on the primary tumor size and location, stage of clinical presentation and status of cervical lymph nodes. Post-operatively patients having involvement of resected margin, extracapsular extension, involvement of more than 2 cervical lymph nodes, perineural invasion, lymphovascular permeation were scheduled to receive adjuvant radiotherapy or chemotherapy + radiotherapy. The closest margin was compared to the closest in situ margin (10 mm) to determine the percentage discrepancy. Routine examination of all components of the specimen was done using hematoxylin and eosin stained sections after fixation in neutral buffered formalin. The tumor type, tumor grade, degree of keratinization and lymph node status were recorded histologically. Treatment results are in Table 3.

Statistical analysis was performed using the SPSS for Windows program. The study contained binary, categorical, and continuous variables. Binary and categorical variables were represented as frequencies and percentages. Continuous data were represented as mean  $\pm$  SD. An independent t test for testing significance of difference in means was used.  $P \leq 0.05$  was considered to be statistically significant. The difference in reduction for superior and anterior margins was found to be statistically significant.

## Results

Of the 52 patients, 43 patients had ulcerative lesion involving the buccal mucosa consistent with moderately to well differentiated SCC, while 9 patients had ulcero-exophytic type of growth confined to the buccal mucosa which were confirmed as verrucous type of SCC. Of the 52 patients, 13 patients had closest pathological margin less than 5 mm (close margins) while 39 patients had the pathological margin  $\geq 5$  mm. None of the patients had positive margins on histological examination. Average decrease in width of the margin before and after formalin fixation was found to be 0.3 cm (25%). The average decrease in tumor surface area was found to be 0.28 cm<sup>2</sup> (19%).

The mean discrepancy noted between insitu margins and pathological margins was statistically significant ( $P = 0.05$  and  $P = 0.025$  respectively). The difference in reduction of posterior tumor margins was marginally significant ( $P = 0.054$ ), while the inferior tumor margin was not found to be statistically significant ( $P = 0.167$ ). The 13 patients who had close (<5 mm) margins were advised post operative chemoradiation. Among these 13 patients, 9 patients completed post operative chemoradiation, while 4 patients defaulted. Among 9 patients who completed post operative chemoradiation, 2 patients had locoregional recurrence within 6 months of treatment. All the 4 patients who defaulted post operative chemoradiation had early local recurrence within 6 months of treatment.

One patient inspite of completing post operative chemoradiation had local recurrence 11 months following treatment.

All 39 patients who had margins  $>5$  mm did not have locoregional recurrence over a period of 6 months.

Among them 20 patients received post operative radiotherapy. Two patients were advised post operative

**Table 2** Addictions of the patients in this study.

Risk factors in patients		n	%
Addictions of the patients in this study	Areca nut	30	58
	Areca nut + Tobacco (smokeless)	15	29
	Areca nut + Tobacco (smokeless) + Alcohol	3	6
	Areca nut + Tobacco (smokeless) + Alcohol + Tobacco (smoked)	4	7
	Keeping over night quid	34	65

**Table 3** Treatment results.

Histopathology of specimen margins (distance from tumor)	Post operative adjuvant treatment	Compliance to adjuvant treatment	n	%	Locoregional recurrence (<6 months)	
					n	%
Margins <5 mm [n = 13 (25%)]	Chemoradiation	Completed	9	69	2	22
		Defaulted	4	31	4	100
Margins $\geq 5$ mm [n = 39 (75%)]	No post op RT/CTRT	—	17	44	—	—
		Radiotherapy	Completed	20	51	—
	Chemoradiation	Completed	1	2	—	—
		Defaulted	1	2	—	—

chemoradiation in view of extra nodal spread in lymph nodes. One patient complied and 1 patient defaulted the post operative chemoradiation. Both these patients did not recur.

During subsequent follow up of patients, we observed that among 6 patients who had local recurrence, 5 died of disease, while 1 patient is alive with the local recurrence. One patient who had more than 5 mm margin of resection and recurred 11 months after treatment is alive with disease (locoregional).

Four patients died due to other causes after completing 6 months follow up. Two patients were lost to follow up 1 year after treatment. Thirty-nine patients are alive and disease free.

Association between margins and local recurrence (Table 4).

## Discussion

We found a difference between margins measured at the time of surgical resection of SCC of buccal mucosa and the margins measured after pathological review.

Removal of all tumor cells from the site of concern at both macro- and microscopic levels is the ultimate goal of any onco-surgeon treating SCC. One of the main goals of treatment of SCC of buccal mucosa is resection with a minimum of a 5 mm cuff of surrounding histopathologically normal tissue, the violation of which has been consistently correlated with poor prognosis.

Achieving a tumor free margin is a challenge due to the compact and complex anatomy of the oral cavity. In a study of 1522 cases of aerodigestive tract carcinoma, it was discovered that the oral cavity was the most likely site to have margins involved with residual tumor. This study had positive margins in 15% of patients and recommended to perform intraoperative control of margins with frozen section and prompt re-excision wherever required.<sup>4</sup>

It is a common observation that the pathological margin distances are much smaller than the clinical in situ measurements prior to resection.

The resected specimens have been found to shrink after processing (formalin fixation) for histopathological examination. This shrinkage had led to discrepancy between surgical margins and histopathologic margins has resulted in reported rates of clear margins being low ranging from 48% to 70%.<sup>5-7</sup>

In surgical treatment of oral SCC, 1 cm margin of resection is considered adequate. However it may prove to be inadequate if the histopathological margin is reported to be close or positive. There are different explanations for the discrepancy between in situ margins and histopathologic margins. One possibility being that specimens contract significantly after resection and pathologic processing. The other possibility is that tissue under tension decreases in dimension on surgical release from the surrounding tissue.

Another possible explanation is that oral SCC may have microscopic extension beyond the margin visible to the surgeon. Therefore the discrepancy in the clinical and pathological margins is most often due to shrinkage as seen in various studies.

In a study on cutaneous melanoma, 15%–25% shrinkage in the margins has been documented.<sup>8</sup> However in another study on esophageal cancer, the entire specimen was found to shrink by 40% while the tumor shrank only by 10% which was probably due to the replacement of contractile esophageal musculature with non-contractile tumor tissue.<sup>9</sup>

In our study the margins were found to shrink on an average by 25% after fixation with formalin. The discrepancy between surgical margins on table and at the time of histopathology was insignificant for the inferior margins. This could be attributed to the resection of the adjoining mandible (marginal or segmental) since all our patients had tumors staged T<sub>2</sub> or above.

However studies by Boonstra and Chen showed that the shrinkage of margins shrunk by 15% or less after formalin fixation and there was no significant difference among the margins taken in different directions. In morphometric studies they recommended that original dimension of the margin has to be taken into consideration. The shrinkage percentage associated with gender, age, tumor site, tumor size, or histology was not statistically significant.<sup>10,11</sup>

Different studies have shown about 20%–30% shrinkage of buccal mucosa soon after resection and further shrinkage after formalin fixation. However this shrinkage was less in the tumor proper probably due to replacement of muscle by tumor tissue.<sup>12,13</sup> Few studies have reported more shrinkage in early cancers compared to locally advanced ones.<sup>13</sup>

Local recurrence of disease in advanced carcinomas of the head and neck is strongly correlated with the presence of positive or close resection margins after operative treatment.

Cook et al. and Slootweg et al. reported an increased risk of local and nodal recurrence and reduced 5-year survival in patients with positive resection margins while Kreppel et al. reported a 5-year overall survival rate of 45.5% in patients with positive margins. The status of the surgical margin significantly affects the overall survival and is an independent prognostic factor.<sup>14,15</sup>

In a retrospective analysis of upper aerodigestive tract, tumors treated by multimodality therapy, it was observed that adjuvant radiotherapy reduced the local recurrence rate. However recurrence rates were 5 times higher (10.5%) with positive surgical margins, than when surgical margin were clear (2%).<sup>16</sup> In our study, though the objective was only to compare and document the margins before and after formalin fixation, we found that there were early recurrences (within 6 months after treatment) in those patients who had pathological margins reported <5 mm.

**Table 4** Association between margins and local recurrence.

Pathological staging	<5 mm (Pathological margin)	Local recurrence	≥5 mm (Pathological margin)	Local recurrence
T <sub>2</sub>	—	—	6	—
T <sub>3</sub>	2	—	11	1
T <sub>4</sub>	11	6	22	—

The depth of the tumor also affects the prognosis even when surgical margins are adequate as it can involve underlying muscle.<sup>17</sup>

Sieczka et al. concluded that early T stage and negative margins are not adequate predictors of local control and that locally excised T<sub>1</sub> and T<sub>2</sub> tumors with pathologically negative margins had a high rate of local recurrence. They suggested that even patients with early buccal mucosal tumors may benefit from adjuvant therapy to enhance local control.<sup>18</sup>

A recent study by Lubek et al. has reported buccal mucosa cancer to be an aggressive oral cancer requiring adequate margins of resection and adjuvant treatment.<sup>19</sup>

All the patients in this study had carcinoma of the buccal mucosa. However adequate margin of resection and adjuvant treatment showed fairly good local control in our study.

Few authors have reported higher recurrence rate even with early buccal mucosa cancer with clear margins.<sup>19</sup> However in our study, the early recurrences were more common when the margins were close (<5 mm). In our study out of 13 patients who had close margins (<5 mm), 6 had very early local recurrence (within 6 months after treatment) compared to 39 patients with resection margins ≥5 mm among whom only one patient recurred after a period of 11 months following treatment.

This study focused only on comparison of margins at the time of surgical resection and after formalin fixation. A longer follow up may show more recurrences.

## Conclusion

Almost 25% margin shrinkage after resection and processing should be considered at the time of initial resection for all buccal mucosa cancers. The shrinkage of tissues is more for the margins which do not have bony support. Resection margins of >5 mm on histopathological examination after formalin fixation gives better loco regional control and it is mandatory to have at least 1 cm of margin resection all around the tumor and administer appropriate adjuvant treatment for all locally advanced buccal mucosa cancers.

## Conflicts of interest

No potential conflicts of interests were involved in this research.

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