Postoperative pathologic assessment of surgical margins in oral cancer: A contemporary review

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INTRODUCTION

Surgery is the main treatment modality for management of oral malignancies. One of the most critical issues of surgical oncology is achieving complete removal of tumor at primary site. Removal of microscopic or subclinical foci of malignancy is very essential and critical to achieve successful local control of the disease. Resection margin or surgical margin as defined by Hinni et al. is any tissue plane where the surgeon’s knife meets the patient. During surgical removal, the palpable or visible tumor is resected with a fringe of normal tissue to ensure complete removal of tumor. This mainly depends on surgeon’s clinical judgment, interpretations of imaging investigations and preoperative planning of the extent of resection. It has been indicated that failure to achieve a clear surgical margin results in an increased risk of local recurrence and a subsequently reduced chance for survival. Therefore, the importance of obtaining histologically “clear” surgical margins has been a foundation for surgical treatment of oral squamous cell carcinoma.

Resected specimens of tumors are submitted to surgical pathologist, who examines the entire specimen grossly...
and microscopically to comment whether the tumor is completely excised or there is a residual pathology \textit{in situ}. Examination of the resected specimen by surgical pathologist is done intraoperatively as intraoperative consultation and postoperatively as routine pathological examination. Intraoperative microscopic evaluation is done by examination of frozen sections of tissues, which are obtained by cryostat or freezing microtome. This procedure is employed when the operation is underway. Further course of surgery depends on the surgical pathologist's comment. However, frozen section technique has limitations. The main limitation of frozen sections is that due to limited time available, only few margins (generally the margin, which is grossly nearest to the tumor) can be examined. Another limitation of frozen sections is that they are inferior to paraffin sections in terms of microscopic appearance because of inherent artifacts such as ice crystals formation and thawing of sections.\textsuperscript{[11]} Such artifacts make frozen sections difficult to interpret. Notwithstanding the limitations, frozen section examination is an important tool which helps to assess the adequacy of surgical margins intraoperatively. On the other side, status of mucosal margins and pathological stage of the tumor obtained by examination of paraffin sections determine further management, i.e., requirement of adjuvant chemotherapy or radiotherapy or both. Mucosal and soft-tissue margins can be examined during frozen section examination and after routine processing. Intraoperative examination of osseous margins is difficult. The status of osseous margins can be accurately assessed only after decalcification followed by routine processing for paraffin embedding. Thus, postoperative microscopic evaluation of resection specimen and surgical margins after paraffin embedding becomes a very important part of patient management.

The purpose of this review is to summarize the fundamental concepts of pathological assessment of surgical margins in oral squamous cell carcinoma and to recommend a practice standard for it, wherever necessary.

**TYPES OF RESECTION MARGINS**

According to the anatomical structure of tissue, there are three types of resection margins:

1. **Mucosal margins:**
   A mucosal margin is a fringe of mucosa surrounding the tumor, which has been removed along with the tumor to ensure its complete removal [Figures 1 and 2].

2. **Soft-tissue or deep margins:**
   A three-dimensional resection is important to ensure the complete removal of tumor tissue, which obviates the excision of tumor with a cuff of normal or tumor-free soft tissues around and underneath the tumor, i.e., from beyond the deepest invasive focus [Figure 2]. This constitutes the “soft-tissue margin” or a “soft-tissue base” of the resection. Studies have indicated that recurrences more frequently involve the deep resection margins.\textsuperscript{[7]} Deep or soft-tissue margins include all connective tissue components, namely, skeletal muscle, adipose tissue and neurovascular bundles.

3. **Osseous margins:**
   The cut edge of bone removed along with tumor constitutes the osseous margins. The tumor is resected with surrounding bone, in cases where it is thought to involve the jaw bone. Osseous margins are submitted and examined in such resections.

**CONCEPTS OF “POSITIVE” OR “NEGATIVE” RESECTION MARGIN, “ADEQUATE” MARGIN OF RESECTION, “REVISED” OR “SUPPLEMENTAL” MARGIN AND “TRUE” RESECTION MARGIN**

Specimens with no microscopic evidence of tumor or moderate or severe epithelial dysplasia at the surgical margins are considered as completely excised. Resection margins in such cases are known as microscopically “free” of tumor, “clear,” “unremarkable,” “negative” or “adequate.” In contrast to this, specimens which show microscopic tumor at the resection margin are considered incompletely excised, indicating the presence of residual tumor \textit{in situ}. Such resection margins are known as microscopically “involved” or “positive” [Figure 3a-c]. In addition, microscopic evidence of epithelial dysplasia at the mucosal resection margins has also been associated with worse prognosis. Most authors consider that moderate and severe dysplasia at inked resection margin has biological significance akin to that of early invasive carcinoma.\textsuperscript{[3]}
However, the presence of mild dysplasia at the margin is of questionable significance. Thus, surgical margins with moderate or severe dysplasia should also be regarded as “involved” margins. Description of positive margins given by Loree and Strong includes histologically evident potentially malignant changes. They suggested categories of positive margins as under:

1. Lesional tissue within 0.5 mm of the surgical margin (so-called close margins) with the exception of laryngeal lesions
2. Dysplastic epithelium at the margin
3. Carcinoma in situ at the margin
4. Invasive carcinoma at the margin.

Microscopic finding of positive margin during intraoperative pathologic consultation is an indication for the increase of extent of resection or a so-called “revision” of margin. Positive resection margin in final surgical pathology report is an indication for adjuvant radiotherapy and chemotherapy.

There was a great deal of variability in the concept of “adequate surgical margin” in head and neck squamous carcinoma. In an attempt to improve and standardize cancer services worldwide, the UK Royal College of Pathologists in 1998, issued guidelines and a minimum dataset for head and neck carcinomas. According to these guidelines, the surgical margins are divided into “mucosal” and “deep” categories. Each category is further divided into clear, close and involved margin, based on histological distance from invasive front of carcinoma to the inked resection margin. Thus, the surgical margin is considered as “clear” when this distance is >5 mm, “close” when it is 1–5 mm and “involved” when the distance is <1 mm [Figure 4].

Clear but close margin is also considered to contribute to an adverse outcome. Thus, such margins should be quoted as positive or inadequate and generally considered as an indication for adjunctive treatment. The practice of quantifying the microscopic distance of resection margin from tumor is adapted in majority of the hospitals and pathology laboratories worldwide. However, inclusion of such microscopic measurements in surgical pathology report is not a system at many centers. It is strongly recommended that microscopically measured distance of margin from the tumor must accompany the comment of “close margin” in the final pathology report.

The presence of tumor or moderate or severe epithelial dysplasia at the resection margin, i.e., inadequate margin, on frozen section examination, requires increase in the extent of resection to assure complete removal of the tumor. This is done by removal of additional fringes of tissue until “tumor-free” or “negative” margin is achieved. Such additional margins are known as “revised,” “reinforced” or “supplemental” margins. Thus, revised margins are those which are taken from the mucosal site, immediately adjacent to the previous resection margins [Figure 5]. Similarly, sections from the mucosal tissues outside the resection periphery, i.e., sections from the edges of surgical defect left behind the resection constitute true margins [Figure 6]. The status of true margin indicates presence or absence of residual disease in situ. Various noninvasive methods for intraoperative examination of true margins have also been suggested, such as microendoscopy, optical coherence tomography and elastic scattering spectroscopy. However, these methods are far from mature and not used routinely.
The method of margin surveillance today is largely a “specimen driven,” i.e., examination of sections from margins retrieved from resected specimen. Examination of “true” margin constitutes the “defect-driven” approach of margin surveillance. A combined approach for assessment of margins may be necessary for anatomically complex specimens of oral and maxillofacial region, and it is recommended for cases where it is technically difficult to achieve a clear surgical margin. On mucosal surfaces, the tumor is visible. Thus, the surgeon may feel confident about the adequacy of mucosal margins. However, in the deep connective tissue, the tumor is not visible but only palpable. Further, perineural invasion, lymphovascular emboli and infiltrative growth pattern may result in inadequate soft-tissue margin. In such cases, examination of true margins during intraoperative consultation or postoperative margin surveillance can be helpful in planning further course of patient management.

**ORIENTATION OF THE SPECIMEN FOR DETERMINATION OF MARGINS, METHODS FOR SAMPLING OF MARGINS AND MICROSCOPIC IDENTIFICATION OF MARGINS**

The specimen should be accurately oriented for identification of various margins. Ideally, the orientation of the specimen should be done by the operating surgeon. Surgeons frequently indicate the margins by tagging the specimen with sutures. One to two sutures are usually sufficient for the proper orientation of the resection specimen. Usually, sutures in different length or different color indicate different margins and this must be mentioned by the surgeon in the pathology request form, for example, short suture indicates superior margin and long suture indicates lateral margin.

Two methods for retrieval of sections from resection margins are popular.[19]

1. **Radial/perpendicular or right angle method**

This method is usually employed when the margin is near to the tumor so that a peripheral part of tumor can be included in the section to determine the microscopic distance between the inked margin and tumor. With this method, the distance between tumor and margin can be measured in millimeters [Figure 7].

1. **Parallel/en face or shave margin**

This is usually employed when the resection margin is away from the tumor that tumor tissue cannot be included in the section of margin. En face margin assesses greater surface area of the tumor periphery. However, it does not allow the measurement of distance between tumor and margin [Figure 8].
To identify the margin under microscope, the margins are painted with India ink. India ink is an ink, which is used for charcoal painting. It becomes insoluble in water when dried. Thus, it does not wash off when the sections pass through different reagents during tissue processing. Traditionally, black India ink is used [Figure 9]. Different colored waterproof inks are available and they can also be useful to differentiate various margins [Figure 10]. This is especially helpful when multiple margins are involved in a single section.[23]

Various other materials have also been suggested for marking and identification of margins on paraffin-embedded blocks and under microscope, for example, gelatin and few dyes.[21]

**ISSUE OF POSTRESECTION TISSUE SHRINKAGE IN MARGINS**

Tissues removed from human body have inherent tendency to undergo shrinkage. The presence of contractile proteins in connective tissue brings about tissue shrinkage after the tissues are surgically released. Resected specimens show approximately 50% shrinkage in their original height. This “pancake” phenomenon significantly contributes to the inaccuracy of postoperative assessment of surgical margins.[22] Fixation and tissue processing further brings about shrinkage of margins in resection specimens.

Various studies have shown that there is a significant discrepancy in margins measured in situ and margins measured microscopically. Mistry et al. reported significant less shrinkage in margins of greater stage tumors (25.6% in T1/T2 vs. 9.2% in T3/T4 tumors). They suggested a hypothesis that tumors with advanced stage bring more destruction of contractile tissue elements and their replacement by noncontractile tumor tissue, which may be the reason for difference in margin discrepancies in tumors of different stages. However, they did not find significant difference in mean shrinkage of margins in tongue

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**Figure 7:** A diagram explaining the “radial or perpendicular” method for sampling of a resection margin

**Figure 8:** A diagram explaining the “parallel or en face” method for sampling of a resection margin

**Figure 9:** Photomicrograph showing black India ink (black arrows) indicating the margin under the microscope (H&E, ×40)

**Figure 10:** Gross specimen showing superior and upper lip margins painted with pink ink and lower lip, inferior and posterior margins painted with black ink
Failure of complete surgical removal of tumor tissue may result in recurrence. Today, adequacy of the resection is largely based on the histological status of resection margins. Studies have shown that recurrence is seen in many cases where histologically clear margins are obtained during removal of primary tumors. The concept of field cancerization suggests that the so-called histologically clear, i.e., phenotypically normal or tumor-free margins may harbor genetic alterations which may result in development of a recurrence. Such subcellular alterations cannot be identified by conventional protocol of margin examination. This entails the need for a more predictive method for examination of resection margins, which resulted in several researches targeting toward detection of molecular and genetic alterations in histologically clear margins. Brennan et al. examined p53 mutations in histologically negative resection margins and nonmetastatic lymph nodes in resection specimens of head and neck squamous cell carcinoma. They found that 52% of patients harbor p53 mutations in negative resection margins, while 21% patients showed p53 mutation in nonmetastatic lymph nodes. In their study, among the cases showing p53 mutation in histologically clear margins, 38% showed local recurrence.

In addition to the above-mentioned markers, several researches targeting toward detection of submicroscopic genetic alterations.

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and buccal mucosa specimens. Cheng et al. reported the mean value of margin discrepancy in T1/T2 tumors as 51.48% and 75% in T3/T4 tumors. They also observed that resection specimens of tumors arising in buccal mucosa, retromolar area and mandibular alveolus show significant shrinkage of margins than tumors of the maxilla or tongue. Thus, according to their evaluations, intraoral site and stage of tumor influence the amount of tissue shrinkage. El-Fol et al. assessed the extent of discrepancy between the in situ margins and histopathological margins in 61 resection specimens of squamous cell carcinoma of different anatomical locations in oral cavity. They recorded highest discrepancy in margins of buccal mucosa specimens than the other intraoral sites. According to Smith et al., method of resection affects the margin shrinkage in oral cavity tumors. They suggested that use of conventional scalpel for resection results in maximum amount of tissue shrinkage than other methods. They reported minimal tissue shrinkage in tissues resected with cutting diathermy. Thus, from the available literature, it appears that multiple factors are involved in postoperative shrinkage of surgical margins. Site and stage of tumor, age of patient and method of resection play significant role in margin shrinkage. Different protocols for tissue fixation and processing in different laboratories also result in different amounts of tissue shrinkage. It is strongly recommended that the issue of margin shrinkage should be given importance while assessment of margins in resection specimens. Thus, it is advisable to measure the gross distance of margin from the tumor in situ and as soon as the tumor is resected. The amount of tissue shrinkage after fixation and tissue processing can be calculated by using these intraoperative values. Sincere efforts should be made to minimize tissue shrinkage during tissue fixation and processing. Examination of true resection margins may surpass the problem of margin discrepancy and can be a valuable tool for final comment on the margin status. Sarode et al. have suggested the revision in guidelines for histological assessment of resection margins. According to them, the protocols of margin assessment should involve calculation of tissue shrinkage in order to more accurate determination of margin status in resection specimens of oral squamous cell carcinoma.

MOLECULAR ASSESSMENT OF RESECTION MARGINS

Molecular assessment of resection margins may predict higher chances of local recurrence. In the above-mentioned markers, loss of heterozygosity and mitochondrial DNA mutation were also assessed in surgical margins.
There is considerable number of researches based on promising molecular and genetic markers to qualify the histologically clear margins as “clear” on molecular or genetic grounds. However, the concept and practice of molecular assessment of surgical margins have significant limitations today. The laboratory procedures for molecular methods are time-consuming; thus, they cannot be employed intraoperatively. Molecular tissue analysis adds time and expense to the postoperative assessment of margins. The molecular and genetic methods reveal subcellular or genetic alterations in mucosal margins, whereas most inadequate margins and tumor recurrences are encountered in the deep connective tissue.[7] Detection of genetic abnormalities or mutations in resection margins will bring new challenges in the postoperative management strategies of such cases. Whether the treatment plans based on such strategies will be cost-effective for patients is not known. Thus, many questions and conflicts arising from the issue of molecular assessment of resection margins are unresolved.

CONCLUSIONS

The basic concepts regarding margins in oral cancer must be clearly perceived by all those involved in the practice of surgical oral and maxillofacial pathology. Postoperative pathological assessment of resection margins has great impact on management in oral squamous cancer cases. It is advisable to adapt standardized criteria for reporting the margin as adequate or inadequate. Postresection tissue shrinkage should be minimized and must be considered while reporting the margin status. The practice of microscopic measurement of tumor-margin distance should be a protocol and its inclusion is recommended in final pathology report. There has been significant number of researches regarding molecular analysis of mucosal margins. Researches focusing on molecular analysis of soft-tissue margin are the need of the hour. Collaborative efforts of head and neck surgeons and pathologists are strongly needed to improve postoperative margin surveillance in oral cancer worldwide.

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