

Assessing Frozen Section Surgical Margin Status in Oral Squamous Cell Carcinoma

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ABSTRACT

BACKGROUND

A key technique that is used by the pathologists during intraoperative consultation is frozen section evaluation. It is very important for the surgeons to know if the surgical margin is clear of malignant cells and the status of dysplasia during oncosurgery. There should be concordance between the frozen section margin and paraffin-embedded section. The purpose of the study was to evaluate and validate the accuracy of frozen section surgical margin status in oral squamous cell carcinoma (OSCC) among 2 observers.

METHODS

A total of 25 histologically diagnosed OSCC cases from April 2018 to April 2019 were included in the study. A total of 122 surgical margin slides (61 frozen sections and paraffin-embedded slides) were retrieved. The interobserver agreement of frozen section margin status with paraffin-embedded was analyzed.

RESULTS

Interobserver agreement in frozen section margin was 73.80 % with a kappa value of 0.048 (slight) and P-value 0.344. The interobserver agreement for paraffin-embedded margin was 93.40 % with a kappa value of 0.572 (moderate) and P-value 0.005. The concordance between frozen and paraffin section by observer 1 was 85.20 % and by observer 2 was 95.10 %.

CONCLUSIONS

The rate of discordant will get reduced by accurate sampling along with better communication between pathologist and surgeon and at times of uncertainty, a second pathologist's opinion can be considered for re-evaluating the interpretations.

KEY WORDS

Frozen Section Margin Status, Interobserver, Paraffin Embedded Margin Status, OSCC.

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BACKGROUND

Oral cancer is one of the major public health problems accounting for 30 % in India, the sixth most common malignancy worldwide and it ranks among the top three types of cancer in the country.¹⁻² Oral habits like betel quid chewing, smoking, alcohol consumption, genetic mutations are the main etiologies for oral cancer. The three major therapeutic strategies for oral squamous cell carcinoma are surgery, irradiation and chemotherapy. Surgery is the first choice of treatment for oral cancer even though various anticancer agents including molecular target drugs are available.³ The main purpose of surgery is to remove the tumour with oncologically safe surgical margins.

In case of intraoperative diagnosis of newly discovered lesions as well as diagnosis of confirmation diagnosis in the previously biopsied pathological process and to establish the extent of the disease, frozen section evaluation is considered to be the standard procedure which has shown sufficient accuracy. When the patient is on the operating table, a crucial role is being played by the intraoperative frozen section in the surgical management by providing important pathological information of the patient.⁴ This technique decreases the need for reoperation by evaluating the surgical margin, checking the tumour clearance and guiding the surgeon in making an immediate decision on the extent and adequacy of the surgical procedure.⁵ The use of frozen section evaluation in ensuring negative margins for resection of head and neck squamous cell carcinomas has been published as detailed protocols.⁶ At the time of frozen section, apart from surgical margins, the surgeon also has the opportunity to know the precise T size and the tumour thickness as predictors of the occult lymph node metastases⁷ and also allows the surgeons to make a critical decision of neck dissection whenever in dilemma. Frozen section concordance rates with permanent diagnosis average is approximately 98 %.⁸ The accuracy of the frozen section and paraffin-embedded section is said to be 96.7 %.⁹ The concordance rate varies by site, with that of the ovary being approximately 93 %, ¹⁰⁻¹¹ and lower at other sites such as the skin.¹² Less data is available for the head and neck area. Epithelial dysplasia is characterized by atypical microscopic changes in the epithelium that can include but are not limited to prominent nucleoli, hyperchromatic nuclei, nuclear pleomorphism, altered nuclear/cytoplasmic ratios, increased atypical mitotic activity, increased individual cell characterization, basal cell hyperplasia, and basal layer budding. Epithelial dysplasia is graded as mild, moderate, severe dysplasia and carcinoma in situ. Distinctions between the grades are made based on histological evaluation. Knowledge of the degree of dysplasia assists with decision making and helps to predict whether the lesion will progress to cancer or will resolve on its own after removal of the source of the irritant. The fact that the diagnostic grading of epithelial dysplasia has never attained unanimity amongst pathologists is a worrisome picture. The considerable variations in grading epithelial dysplasia might be due to the lack of objectivity in the evaluation of established criteria, the arbitrary division of the grading, lack of calibration of criteria and grading, and lack of sufficient knowledge of which criteria are important for the prediction of malignant

potential. There are hardly any studies analysing the interobserver agreement in frozen sections.

So the intention of the study was to determine the interobserver agreement in frozen section margin status and compare it with the interobserver agreement in paraffin-embedded section margin status. Also to assess and compare the concordance of frozen section margin status with corresponding paraffin-embedded section margin status.

METHODS

We retrospectively reviewed the frozen section cases performed in Oral and Maxillofacial Pathology Laboratory, Saveetha Dental College and Hospital from April 2018 to April 2019. Approval was taken from the Institutional Review board. A total of 25 histologically diagnosed OSCC cases were included in the study where 11 cases were OSCC of tongue, 5 buccal mucosa, 5 gingivobuccal sulcus, 2 maxilla and 2 retromolar trigones. The sample size was calculated based on the previous reference⁹ with the power of study set as 80 %. Surgical margins with intact epithelium were included in the study and the core of the lesion was excluded in the study. A total of 122 surgical margin slides (61 frozen section slides and 61 paraffin-embedded slides) were retrieved. These slides were cross-checked from confirmation.

Patient files in the Pathology department provided data regarding the frozen section case. Frozen section surgical margins and the corresponding paraffin-embedded surgical margins were observed by two oral pathologists for the margin status. Blinding was done to avoid bias.

The interobserver agreements in frozen section margin status, and paraffin-embedded section margin status, concordance of frozen section margin status with paraffin-embedded section margin status were observed and tabulated and statistically analysed. Clearance status and the grading of dysplasia were done by the 2 observers for both frozen section surgical margin slides and paraffin-embedded surgical margin slides. Clearance status was graded as (absence of malignant epithelial cells) clearance adequate and (presence of malignant epithelial cells) clearance inadequate. Dysplasia grading was done based on WHO 2017 classification as mild dysplasia, moderate dysplasia and severe dysplasia.¹³⁻²⁶ Clearance status and grading of dysplasia were tabulated and analysed.

Statistical Analysis

The test results were categorized into concordant and discordant groups. IBM. SPSS statistics software 23.0 version was used to analyse the collected data and interobserver agreement was assessed using Kappa statistics (Cohen's kappa). The P-value < 0.05 was considered statistically significant.

RESULTS

In this study, 61 surgical section slides from both frozen sections and paraffin-embedded sections were analysed. Interobserver agreement in frozen section surgical margin

was 73.80 % with a kappa value of 0.048 (slight) and a P-value of 0.344. The interobserver agreement for paraffin-embedded surgical margin was 93.40 % with a kappa value of 0.572 (moderate) and a P-value of 0.005 (Table 1). The concordance between frozen and paraffin sections by observer 1 was 85.20 % and 95.10 % by observer 2 (Table 2).

	Number of Slides (N)	Agreement Percentage (%)	Disagreement Percentage (%)
Frozen section surgical margin status	61	74 %	26 %
Paraffin embedded surgical margin status	61	93 %	7 %

Table 1. Table Depicting the Interobserver Agreement on Frozen Section Surgical Margin and Paraffin-Embedded Surgical Margin

	Number of Slides	Concordance Percentage (%)	Discrepancy Percentage (%)
Frozen section surgical margin and paraffin-embedded section margin status by observer 1.	61	85 %	15 %
Frozen section surgical margin and paraffin-embedded section margin status by observer 2.	61	95 %	5 %

Table 2. Table Depicting the Concordance of Frozen Section Surgical Margin and Paraffin-Embedded Surgical Margin by Observer 1 and Observer 2.

DISCUSSION

The surgeon’s confidence in frozen section results relies mainly on the diagnostic accuracy of the procedure.¹⁴ Frozen section diagnosis is the intraoperative method which is usually compared to permanent hematoxylin and eosin stained section diagnosis to evaluate the diagnostic accuracy. Evaluating discrepancies, identifying deficiencies and resolving the underlying problems can improve the accuracy of the frozen section.³ Reasons for diagnostic discrepancies generally fall into one of the following categories: technical problems, sampling errors or interpretation errors.¹⁵

The main errors in diagnosis are often in recognition of certain types of lesions, inadequate sampling, technical problems or the lack of communication between the surgeon and the pathologist.^{16,17} Several comparative studies regarding frozen and paraffin sections have been performed to detect the frozen sections in the diagnosis of prostate carcinoma, breast cancer, lung cancer, skin cancer and tumours of the colon, gallbladder, kidney, head and neck.¹⁸

Various studies on the interobserver accuracy of the grades on dysplasia are Abbey et al. interobserver accuracy-slight (K = 0.17), Brothwell DJ et al. - fair agreement (K = 0.37), Kujan O et al. - slight to mild agreement (K = 0.06 to 0.43), Karabulut et al. - 49 % - 69 % agreement- K = 0.27 to 0.45 (fair agreement).¹⁹⁻²² But all of these studies were done for paraffin embedding histopathological slides, not on frozen section slides. That’s why our study aims to determine interobserver accuracy of the grades on dysplasia in frozen section slides. It was observed that the discrepancy between the observer was due to basilar hyperplasia, hyperchromatic nucleus, increased nuclear-cytoplasmic ratio and overlapping of basal cells (sectioning errors). Because of the overlapping of basal cells some sections were graded as mild dysplasia by observer 1, while clear with no dysplasia by observer 2 (indicative of interobserver disagreement) (figure 1).

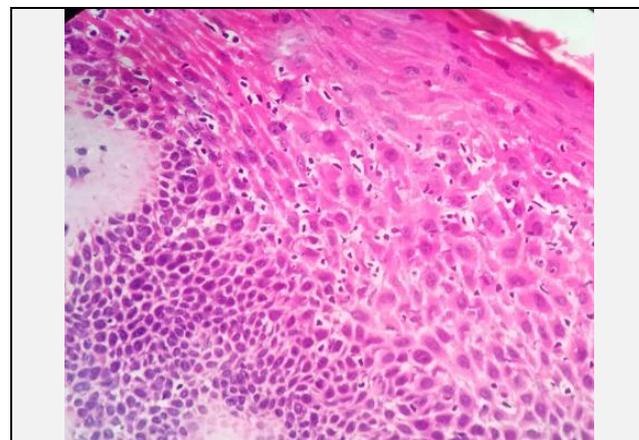


Figure 1. Histological Section of Frozen Section Surgical Margin Showing Features Like Basilar Hyperplasia, Increased Nuclear-Cytoplasmic Ratio and Nuclear Hyperchromatism Where the Observer 1 Graded as Moderate Dysplasia, While Observer 2 Graded as Mild Dysplasia. (Indicative of Interobserver Disagreement)

Black et al. reported the frozen section from the pathologist point of view where he stated inaccuracy in the evaluation of the margins. Pathologists require cooperation with surgeons as some resected specimens are not being anatomically oriented and labelled.²³ There are studies stating that the frozen section is inaccurate for routine investigation of the margins for resected oral cancers other than tongue cancer, as the anatomical structure is complicated and anatomical limits that surgical access to the tumour site is generally poor.²⁴

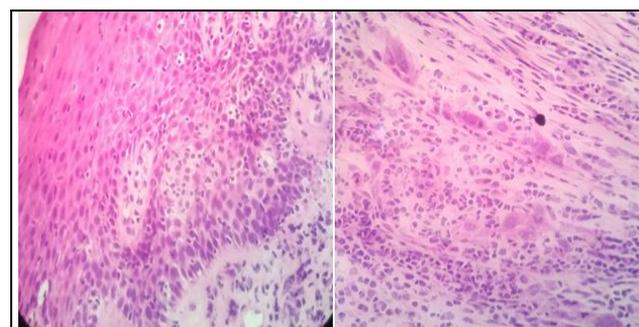


Figure 2. Histological Sections Depicting a Frozen Section of the Same Margin Where the Image on Left Shows Moderate Dysplasia (Observer 1) and the Image on Right Shows Inadequate Clearance for the Same Margin (Observer 2) (Indicative of Interpretation Error)

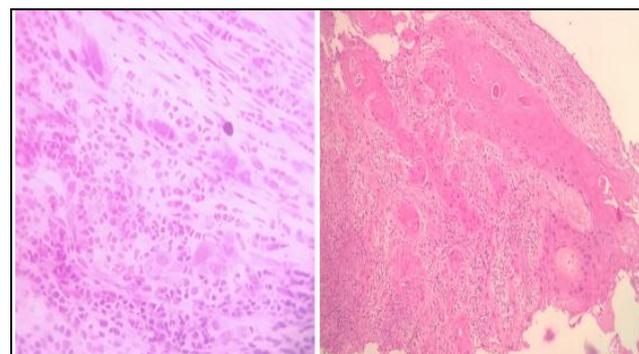


Figure 3. Histological Sections Showing Two Sections Where the Section on Left is Frozen Embedded Section and the Right Image Shows Paraffin-Embedded Section. Both the Sections are from the Same Specimen, the Discrepancy Was Because of the Orientation Error and Sampling Error

The concordance of frozen section margin status and paraffin-embedded section margin status done by Hatami et al. was 97 % but it was observed to be 85.20 % by one observer and 95.10 % by the other observer. The discrepancy was noted due to sampling error (insufficient sampling, deeper levels in permanent sections) (figure 3) and interpretation error (figure 2). In few concordance studies of the frozen section/permanent section diagnosis, interpretive issues have been reported as the major cause for discrepancies. Insufficient experience is a potential cause of interpretive error in interpreting frozen sections.

The discrepancies in the accuracy of frozen section surgical margin and paraffin section surgical margin could be minimised by inking the margins before fixation, Gandour – Edwards et al. (several levels at least 3 of frozen section) and proper discussion with the experienced pathologist.²⁵ In the interpretation of frozen sections, experience plays a major role which helps in lowering the deferred and error rate when specimens are interpreted by more experienced pathologists. Evaluation of the specimens by two observers or even three, when there is uncertainty, reduces the rate of error.¹¹

Identification of genetic and epigenetic changes that could predict the malignant potential of dysplastic lesions. A better understanding and knowledge of the carcinogenesis process would develop newer strategies to identify gross genomic aberrations on a routine basis. Improved studies on chromosomal, genomic and molecular alterations in the potentially malignant lesions would shift the balance from relying on mere morphological alterations for diagnosis to more authentic and predictive factors that bring about these alterations.^{26,27} Establishing a universal image database for each histological criteria would reduce the shadow of subjectivity in grading, though it may not be completely eliminated.

CONCLUSIONS

The present study shows that the frozen section is an accurate and reliable method. Accurate, adequate sampling along with better communication between pathologists and surgeons aid to reduce the rate of discordancy. Re-evaluation of the interpretations by a second pathologist when there is uncertainty helps reduce both discordants.

Limitations

The limitation of the present study is its smaller sample size. Also in the present study, the margin status consisted of only mild dysplasia cases. There were no moderate and severe dysplastic margins noted in the selected cases. So a larger sample size with the inclusion of severe dysplastic margins would give a better rate of concordancy.

Data sharing statement provided by the authors is available with the full text of this article at jemds.com.

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