

CASE SERIES

Accuracy of frozen sections in oral cancer resections, an experience of a tertiary care hospitalSayed Akbar Abbas,¹ Mubasher Ikram,² Muhammad Usman Tariq,³ Ahmed Raheem,⁴ Javeria Saeed⁵**Abstract**

The aim of our study was to determine the accuracy of frozen sections in oral cancer resections. Patients who underwent oral cancer resection at Patel Hospital were included in the study. Tissues for frozen sections were obtained from the mucosal margins and tumour bed. Frozen sections were compared with the paraffin embedded sections which served as the gold standard in our study. Sensitivity, specificity, positive predictive value, negative predictive value, accuracy and kappa statistics were calculated. A total of 299 frozen sections were obtained from 77 patients who underwent oral cancer resection, mean (3.88 ± 0.76) . Frozen sections' sensitivity in relation to permanent sections was 72.7% and specificity of 93.9%. The overall accuracy of the test was 90.9%. Kappa value was calculated to be 0.642, which showed a moderate agreement. Frozen sections are accurate enough to be used in assessing intraoperative margin assessment for tumour clearance.

Keywords: Oral cavity, Squamous cell carcinoma, surgical treatment, intraoperative frozen section, accuracy.

Introduction

Cancer of the oral cavity is the second most frequently reported malignancy in Pakistan in males as well as females. It is frequently encountered in the urbanized southern city of Karachi; primarily owing to its existence to frequently encountered oral habits of pan, chalia and gutka. In males oral cancer is second only to lung cancer and in females it competes with the breast cancer to be placed as the second most frequently encountered malignancy.^{1,2}

Surgical excision of the oral lesion combined with radiation therapy remains the standard of care worldwide. Every effort is made during the surgery for a complete excision of the tumour. In order to maximize the chances

of success and complete removal of tumour, frozen sections are frequently employed intraoperatively.

However, the report of clear margins on frozen section does not guarantee that local recurrence will not occur nor does the report of positive frozen section imply that recurrence is imminent. However, the involved margin of resection does expose the patient to the exponential risk of local recurrence and subsequently to poor prognosis.³⁻⁵

In an attempt to map the extent of tumour resection with healthy margins, technique of frozen section is used in many centers worldwide. It was first used by deREMIER in 1818.⁶ The quality, accuracy and the reduced time to report the results of the frozen section is largely attributed to the use of modern day cryostat. Fresh tissue is frozen in a cryostat machine and thinly sliced with a razor, affixed to a glass slide then hand dipped to fixatives and tissue stains for immediate interpretation. The average thickness of frozen section tissue slice is 5 μm, an improvement from the earliest published accounts of 12 μm, but varies according to the fat content of the tissue and the experience of the technician.⁷ Frozen section technique is designed to address intraoperative observations so as to provide a rapid and reliable answer during surgery. The technique has been employed successfully in various sites in head and neck for diagnosis and to check for tumour clearance.⁸⁻¹² The information thus obtained translates into optimal treatment avoiding aggressive surgery. The accuracy of frozen section diagnosis when compared to the paraffin embedded section is usually reported between 90-96%.¹³⁻¹⁷

However, getting a concordant result on permanent section does not guarantee complete excision of the tumour even when an attempt is made to excise a 1cm healthy margin around the tumour.

Methods

A retrospective case series study was conducted on 77 patients between January 2010 and June 2014 with an aim to determine the intraoperative accuracy of frozen sections in our patients undergoing resections of oral squamous cell carcinoma. The study was approved by the Institutional Ethics Committee (Approval No.40) of Patel Hospital, Karachi. All patients with biopsy proven squamous cell

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carcinoma of oral cavity who were treated at Patel Hospital, Karachi were included. All patients underwent a surgical excision with a curative intent. Exclusion criteria involved all patients with lesions other than oral squamous cell carcinoma. Patients who had prior treatment with radiation, chemotherapy and surgery were excluded from the study.

In all the cases, a margin of 1 cm around the tumour was attempted. Once the entire specimen with tumour was removed, frozen sections were obtained from mucosal and deep margins (tumour bed) from the surrounding tissues to check for microscopic invasion by tumour cells. All specimens sent for evaluation by frozen section were rendered on haematoxylin and eosin stained section of OCT-embedded, snap frozen tissue sectioned at 5-micrometer intervals. Residual tissue was formalin fixed and paraffin embedded, sectioned and later stained with haematoxylin and eosin stains. Permanent sections were reviewed for correlation with frozen section slides. Frozen section was reported by an experienced attending pathologist. Resection margin which showed dysplasia, carcinoma in situ or frankly invasive tumour either at the margin or at a distance of less than 5mm from the margin were labeled as positive margin. In case there was positive report on frozen section by pathologist, further resection of the suspected tissue was carried out till a negative margin was achieved. Patients were followed till the recurrence was noticed, or remained under the follow up.

The sensitivity and the specificity of the frozen sections as compared to the permanent paraffin embedded sections were calculated. Descriptive statistics were calculated in terms of mean and standard deviation. Frequency and percentages

were calculated. 2X2 table was constructed to determine the diagnostic accuracy between frozen sections and permanent sections on final histopathology. Sensitivity, specificity, positive predictive value and negative predictive values were calculated. To observe the agreement between the two categorical variables (frozen and permanent sections) kappa statistics were calculated. The ability of the frozen section to act as an indicator of the complete excision of the tumour was also evaluated. Tumour clearance of more than 5mm on permanent section from the resected specimen was taken as clear margins of excision, while tumour within less than 5mm of clearance, frank tumour at the margins, carcinoma in situ or dysplasia was taken as the positive margin and further resection was done. Data was analyzed by using SPSS 21. Frequencies of the site of the lesion and gender were generated. The sensitivity, specificity and intraoperative accuracy of the frozen section were determined.

Results

A total of 299 frozen sections with an average of 3.88 ± 0.76 specimen per patient were obtained from 77 patients who underwent surgical excision of oral squamous cell carcinoma from Jan 2010 to Jun 2014. There were 45 (58.4%) males and 32 (41.6%) female patients in the study. The mean age of the patients was 48.87 ± 13.78 years (range = 13-80 years). The patients were followed up for a mean of 14.97 ± 19.93 months (range 1-125). Tongue and buccal mucosa squamous cell carcinoma constituted the majority of the cases. Most of the cases were staged as T2 lesion (Table-1).

On receiving a positive report for tumour on frozen section, further resection was performed till healthy

Table-1: Distribution of cases by site of involvement and T-Stage.

Site Involved	No. of Patients	T-stage				P-Value
		T1	T2	T3	T4	
Tongue	29(37.7%)	7(9.1%)	13(7(9.1%)	2(2.6%)	
Cheek	29(37.7%)	4(5.2%)	15(19.5%)	5(6.5%)	5(6.5%)	
Palate	6(7.8%)	2(2.6%)	1(1.3%)	1(1.3%)	2(2.6%)	P=0.622
Others	13(16.9%)	3(3.9%)	5(6.5%)	4(5.2%)	1(1.3%)	
Total	77(10%)	16(20.8%)	34(44.2%)	17(22.1%)	10(13%)	

Table-2: Accuracy of frozen sections against permanent sections.

Results of Permanent section	Results of frozen section		Total
	Positive	Negative	
Positive	8 (10.4%) (TP)	3(3.9%) (FN)	11(15.6%) (TP+FN)
Negative	4(5.2%) (FP)	62(80.5%) (TN)	66(84.4%) (FP+TN)
	12 (TP+FP) Sensitivity=72.7%	65 (FN+TN) Specificity=95.3%	77(100%) (TP+TN+FP+FN) Accuracy= 90.9%

P-value <0.001* Significant.

Kappa Statistics=0.64 or 64%

Substantial agreement.

margin was reached.

Sixty-five patients had negative margins reported on the frozen section out of which 3 patients had false negative results. Twelve patients had positive margins reported on frozen section out of which 4 were false positive when compared with the final paraffin embedded sections (Table-2). The sensitivity of the test was 72.7% (95% CI: 39.3%, 92.7%) and specificity 95.3% (95% CI: 84.4%, 98.0%). Positive predictive value (PPV) was 66.6% and negative predictive value (NPV) was 93.9%. The overall accuracy of the test was 90.9%.

Discussion

The main aim of the oncologic surgeon is to obtain clear margins of resection during surgery. Mostly it depends on the intraoperative judgment of the surgeon to provide clear margins of resection. However, the microscopic extent of the tumour may only be assessed with the help of the intraoperative use of frozen section technique. The importance of obtaining clear margins of resection stems from the fact that those patients who have positive final margins of resection have high rate of local recurrence and subsequently poor prognosis. In this regard, the use of adjuvant treatment has yielded conflicting results. Literature suggests that postoperative radiotherapy in patients with involved margins of resection doesn't alter the prognosis.³

In a study done by Gerber, S., et al.³ overall 5 - year survival was sixty percent in patients with clear margins of resection as opposed to fifty two percent in patients with involved margins of resection in oral cancer. The difference between the two was statistically significant. The incidence of local recurrence in patients with positive margins was twice as compared to negative margins (36% vs 18%). Study also pointed towards the relative ineffectiveness of the adjuvant radiotherapy in patients with involved margins of resection. In addition, Noble AR et al.¹⁸ also confirmed the ineffectiveness of the adjuvant therapy in patients with positive margins of resection. Frozen section evaluation is the commonest and most widely used technique to ensure clear margins of resection during surgery.¹⁹

In literature, the accuracy of frozen section ranges between 96-98% for oral cancer resection.²⁰⁻²² In our study, the accuracy of the frozen section was 90.9%, which is slightly lower but comparable to the published reports. Frozen section was able to pick up 8 patients correctly with positive margins and hence further excision was carried out. Four patients had false positive interpretation on frozen section and lead to extra removal of tissue from the surroundings of the tumour. None of these 4 patients required flap reconstruction as a result of excess removal of tissue. Three patients had false negative reporting. These 3

patients had dysplasia on the final paraffin embedded sections and therefore labeled as positive margins.

The issue of dysplasia demands special attention. General pathologists and head and neck pathologists often differ in the interpretation of dysplasia. In one review, experienced pathologists agreed only 81.5% of the times when distinguishing dysplasia from no dysplasia. Interestingly, when these pathologists were blinded they only agreed 80.3% of the times to their own diagnosis of dysplasia versus no dysplasia.²³ The finding of dysplasia on frozen section is generally believed to increase the chance of local recurrence but this may not hold true on every occasion. In a review of oral dysplasia by Lumerman, only 13.8% of the lesions among 240 patients converted into malignancy. Moreover, 17.5% of the 91 lesions improved or disappeared.²⁴

The problem with the interpretation of the dysplasia may be compounded specially when reporting on frozen section. Our results were significantly influenced by the varied interpretation of dysplasia on frozen section. Two of the four patients who were reported to be having dysplasia on frozen section (false positive) were later confirmed to be having no dysplasia on permanent sections. Three patients who were reported to be having clear margins of resection (false negative) had moderate degree of dysplasia reported on the permanent sections.

Overall, 14 patients had local recurrence after surgical excision of the oral cancer. Patients with clear margins of resection had 11% recurrence rate while patients with close and involved margins of resection had 38% recurrence rate. This is consistent with the reports published in literature where local recurrence rate is 3-35% in patients with negative margins, while for positive margins recurrence rate may approach 100%. In our study, close margins of resection (<5mm) were treated as positive margins and all of them received radiotherapy. There were 23 patients who had close margins of excision out of which 10 (43%) patients developed local recurrence.

Here it is noteworthy that margin shrinkage is often overlooked in the head and neck and especially so in the oral cancer resection. Often, *in vivo* adequate margin of resection transforms into close margin of resection upon removal. Here a case can be made for addition of a 'corrective factor' to overcome the issue of margin shrinkage and avoid getting too many tumours with close margins of resection. Johnson et al.²⁵ reported on the issue of margin shrink in oral cavity of dogs and found that there was 30.7%- 47.3% shrinkage from the time the specimen was removed from the oral cavity to the time it underwent the microscopic examination. They concluded that in order to get clear margins of resection (>5mm) a healthy cuff of

tissue measuring 8-10mm should be removed. However these observations await confirmation on human tissues.

Similarly, permanent sections of the tissue from margins of resection were also not predictors of disease free survival. The explanation to this lies in the fact that accuracy of frozen section in predicting the final margins of clearance was only 36%. Another study on the accuracy of frozen section in head and neck showed that a negative frozen section did not ensure a tumour free margin on the final specimen.²⁶

Conclusion

Frozen section examination is accurate enough to be used routinely in the evaluation of surgical margins in oral cancer resection. It helps obtain clear margins of resection in a significant number of patients where tumour would have been left behind otherwise. However a negative margin on frozen section does not guarantee that final margins of resection would be tumour free all around the specimen, and therefore it does not necessarily exclude the development of local recurrence. The surgeon must understand the limitations of frozen section and continue careful long-term follow-up.

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References

1. Bhurgri Y. Karachi cancer registry data — implications for the national cancer control program of pakistan. *Asian Pac J Cancer Prev.* 2004; 5: 77-82.
2. Bhurgri Y, Bhurgri A, Hassan SH, Zaidi S, Rahim A, Sankaranarayanan R, et al. Cancer incidence in Karachi, Pakistan: first results from Karachi cancer registry. *Inter J Cancer.* 2000; 85: 325-9.
3. Gerber S, Gengler C, Grätz KW, Kruse AL. The impact of frozen sections on final surgical margins in squamous cell carcinoma of the oral cavity and lips: a retrospective analysis over an 11 years period. *Head Neck Oncol.* 2011; 3: 1.
4. Priya S, D Cruz A, Pai P. Cut margins and disease control in oral cancers. *J Cancer Res Therap.* 2012; 8: 74.
5. Mendenhall WM, Hinerman RW, Amdur RJ, Malyapa RS, Lansford CD, Werning JW, et al. Postoperative radiotherapy for squamous cell carcinoma of the head and neck. *Clin Med Res.* 2006; 4: 200-8.
6. Krumbhaar EB, Clio Med 19, Pathology New York: PB Hoeber, Inc. 1937: 171.
7. Black C, Marotti J, Zarovnaya E, Paydarfar J. Critical evaluation of frozen section margins in head and neck cancer resections. *Cancer.* 2006; 107: 2792-800.
8. Wong DSY. Frozen section during parotid surgery revisited: efficacy of its applications and changing trend of indications. *Head Neck.* 2002; 24: 191-7.
9. Gooris PJ, Vermey B, de Visscher JG, Roodenburg JL. Frozen section examination of the margins for resection of squamous cell carcinoma of the lower lip. *J oral maxillofac surg.* 2003; 61: 890-4.
10. Dadas B, Basak T, Özdemir T, Polat N, Turgut S. Reliability of frozen section in determining tumour thickness intraoperatively in laryngeal cancer. *Laryngoscope.* 2000; 110: 2070-3.
11. Tschopp L, Nuyens M, Stauffer E, Krause T, Zbären P. The value of frozen section analysis of the sentinel lymph node in clinically N0 squamous cell carcinoma of the oral cavity and oropharynx. *Otolaryngol Head Neck Surg.* 2005; 132: 99-102.
12. Badoual C, Rousseau A, Heudes D, Carnot F, Danel C, Meatchi T, et al. Evaluation of frozen section diagnosis in 721 parotid gland lesions. *Histopathology.* 2006; 49: 538-40.
13. Sharma S, Prasad B, Pushparaj S, Poojary D. Accuracy of intraoperative frozen section in assessing margins in oral cancer resection. *J Maxillofac Oral Surg.* 2009; 8: 357-61.
14. Ord RA, Aisner S. Accuracy of frozen sections in assessing margins in oral cancer resection. *J Oral Maxillofac Surg.* 1997; 55: 663-9.
15. Junaid M, Choudhary MM, Sobani ZA, Murtaza G, Qadeer S, Ali NS, et al. A comparative analysis of toluidine blue with frozen section in oral squamous cell carcinoma. *World J Surg Oncol.* 2012; 10: 57.
16. Wenig BM. Intraoperative consultation (IOC) in mucosal lesions of the upper aerodigestive tract. *Head Neck Pathol.* 2008; 2: 131-44.
17. Chaturvedi P, Singh B, Nair S, Nair D, Kane SV, D cruz A, et al. Utility of frozen section in assessment of margins and neck node metastases in patients undergoing surgery for carcinoma of the tongue. *J Cancer Res Ther.* 2012; 8: S100-5.
18. Noble AR, Greskovich JF, Han J, Reddy CA, Nwizu TI, Khan MF, et al. Risk Factors Associated with Disease Recurrence in Patients with Stage III/IV Squamous Cell Carcinoma of the Oral Cavity Treated with Surgery and Postoperative Radiotherapy. *Anticancer Res.* 2016; 36: 785-92.
19. Kurita H, Sakai H, Kamata T, Koike T, Kobayashi H, Kurashina K. Accuracy of intraoperative tissue staining in delineating deep surgical margins in oral carcinoma surgery. *Oral Oncol.* 2008; 44: 935-40.
20. Pathak KA, Nason RW, Penner C, Viallet NR, Sutherland D, Kerr PD. Impact of use of frozen section assessment of operative margins on survival in oral cancer. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2009; 107: 235-9.
21. Patel RS, Goldstein DP, Guillemaud J, Bruch GA, Brown D, Gilbert RW, et al. Impact of positive frozen section microscopic tumour cut-through revised to negative on oral carcinoma control and survival rates. *Head Neck.* 2010; 32: 1444-51.
22. Howanitz PJ, Hoffman GG, Zarbo RJ. The accuracy of frozen-section diagnoses in 34 hospitals. *Arch Pathol Lab Med.* 1990; 114: 355-9.
23. Abbey LM, Kaugars GE, Gunsolley JC, Burns JC, Page DG, Svirsky JA, et al. Intraexaminer and interexaminer reliability in the diagnosis of oral epithelial dysplasia. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1995; 80: 188-91.
24. Lumerman H, Freedman P, Kerpel S. Oral epithelial dysplasia and the development of invasive squamous cell carcinoma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1995; 79: 321-9.
25. Johnson RE, Sigman JD, Funk GF, Robinson RA, Hoffman HT. Quantification of surgical margin shrinkage in the oral cavity. *Head Neck.* 1997; 19: 281-6.
26. DiNardo LJ, Lin J, Karageorge LS, Powers CN. Accuracy, utility, and cost of frozen section margins in head and neck cancer surgery. *Laryngoscope.* 2000; 110: 1773-6.