

Intraoperative Surgical Margin Clearance – Correlation of Touch Imprint Cytology, Frozen Section Diagnosis, and Histopathological Diagnosis

Abstract

Introduction: Touch imprint cytology (TIC) and frozen section diagnosis are valuable intraoperative guides for the management of malignancies as they make a prompt therapeutic decision that may prevent surgical re-intervention. The present study emphasizes on the correlation of TIC and frozen section for the evaluation of surgical margins considering histopathological diagnosis as the gold standard. **Aim:** The aim of the study was to assess the accuracy of frozen section diagnosis and TIC in the evaluation of surgical margin clearance. **Materials and Methods:** It is a prospective analytical study of 103 patients carried in the histopathology section of department of pathology for 1 year from July 2017 to July 2018. Specimens were received in the frozen section room, grossed by the standard protocol. Touch imprints of margins were taken, and frozen sections were stained by rapid hematoxylin and eosin stain. The same margins were sent for permanent histopathology sections. **Results:** Of 103 patients, 51 (49.51%) were oral squamous cell carcinoma, 35 (33.98%) carcinoma breast, 9 (8.74%) carcinoma colon, 4 (3.88%) squamous cell carcinoma of skin, 3 (2.91%) basal cell carcinoma, and 1 (0.97%) malignant peripheral nerve sheath tumor. Frozen section diagnosis for margin clearance of the above organs showed that the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were found to be 100%, 98.71%, 100%, 100%, and 99.02%, respectively. The sensitivity, specificity, PPV, NPV, and accuracy of TIC for surgical margin clearance were 46.15%, 100%, 100%, 84.62%, and 86.40%, respectively. **Conclusion:** Frozen section diagnosis is an accurate method for the assessment of surgical margin clearance as compared to TIC. This study evaluated predominantly epithelial malignancies than mesenchymal malignancies, thus emphasizing its utility in it. More research needs to be done for the assessment of the utility of these lesions.

Keywords: Frozen sections, surgical margins, touch imprint cytology

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Introduction

The surgical treatment of any malignancy aims at the removal of all the malignant tissues with a clear surgical margin without malignant cells or dysplasia. Surgical margins can be examined for the absence of malignant cells by various methods such as touch imprint cytology (TIC), frozen section diagnosis, and histopathological diagnosis. Negative surgical margins prevent local recurrences and improve survival. Positive margins which are later identified in permanent sections necessitate repeated surgeries, which cause a delay in starting adjuvant therapy, poor cosmetics due to removal of greater volume of breast tissue when compared with primary surgery, anesthesia risks, increased patient anxiety, increased cost, and greater morbidity.^[1]

Frozen section diagnosis and TIC are valuable for intraoperative diagnosis of surgical margin clearance as they prevent surgical re-interventions. A negative surgical resection margin does not necessarily prevent local recurrences. Larsen *et al.* concluded that frozen section diagnosis for surgical resection margins improved the rate of negative local recurrences and re-excision.^[2] Furthermore, the frozen section includes identification of pathologic process also.

Imprint cytology which was introduced in 1927 by Dudgeon and Patrick is an intraoperative and perioperative tissue assessment technique.^[3] Intraoperative TIC technique does not involve the freezing of tissue. Samples are obtained by touch imprint of the fresh specimen. Strong indications for this technique include lymphoproliferative lesions, central nervous

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system lesions, and thyroid nodule.^[4] It can also be used for minute specimens and to sample tissue that would be difficult to cut with the cryostat. Most noncytological methods assess only 10%–15% of the surface of the lesion. Hence, touch preparation cytology can also be one of the methods to evaluate surgical margins. The principle of imprint cytology is based on lack of cohesion amongst malignant tumors. The cells are stained by rapid staining (both for histopathology and cytology) slides are subsequently viewed under the microscope for diagnosis. The advantage of TIC is that it is simple, quick (2–3) minutes, safe (no loss of diagnostic material), less expensive, and possibly surveys the entire surface area of the lesion.

Accordingly, the current study is designed to assess the accuracy of frozen section and TIC in evaluating surgical margin clearance. Objectives are to evaluate the findings of intraoperative frozen section for surgical margin clearance in cases of malignancies, to evaluate the findings of TIC for surgical margin clearance in cases of malignancies, and to correlate the findings of frozen section diagnosis and TIC in evaluation of surgical margin clearance using conventional hematoxylin and eosin (H and E) as gold standard.

Materials and Methods

The present study evaluated 103 patients. The sample size was determined using Krejcie and Morgan methodology.^[5] It is a prospective analytical study carried out at the Frozen Section Unit and Histopathology Section of the Department of Pathology, Jawaharlal Nehru Medical College and Acharya Vinoba Bhave Rural Hospital, Wardha, for a period of 1 year from July 2017 to June 2018. Patients who were undergoing surgical resection procedures for the diagnosis of oral squamous cell carcinoma (OSCC), carcinoma of the breast, carcinoma of the colon, squamous cell carcinoma of the skin, basal cell carcinoma (BCC), and soft-tissue sarcoma were considered for the study. The specimen was received in the frozen section room of the operating theater complex and was grossed according to the standard protocols with margin resection. Touch imprints covering both the surfaces of the surgical margin were taken and frozen sections were cut from the same. Both these slides were stained by rapid H and E stain.^[6] Later, the same margins were sent to histopathology section of the department of pathology for obtaining permanent histopathology sections. For statistical analysis, these three methods were analyzed, interpreted, and recorded in tabular manner along with suitable graphical representation. Statistical analysis was done using descriptive and inferential statistics using Chi-square test, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy. Software used in the analysis was SPSS 22.0 version and GraphPad Prism 6.0 version (International Business Machine Corporation (IBM), New York, USA). $P < 0.05$ is considered as the level of significance.

Inclusion criteria were patients with diagnosed cases of OSCC, carcinoma of the breast, carcinoma of the colon, carcinomas of the skin, and soft-tissue sarcomas on histopathology; patients undergoing surgical resections for the treatment of the above malignancies; patients of all age groups; both male as well as female patients; and fresh resected specimen.

Exclusion criteria were patients diagnosed with any other organ malignancies; patients who have undergone radiotherapy as a treatment modality for any of the above malignancies initially; and formalin-fixed tissues.

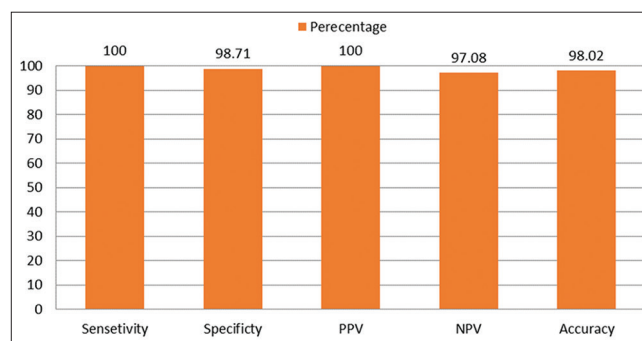
Results

Of the 103 patients, 51 (49.51%) patients were that of OSCC, 35 (33.98%) patients of carcinoma of the breast, 9 (8.74%) patients of carcinoma of the colon, 4 (3.88%) of squamous cell carcinoma of the skin, 3 (2.91%) patients of BCC, and 1 (0.97%) patient of malignant peripheral nerve sheath tumor. Of 49.51% of the cases of OSCC, 73.5% of the patients were male, while 26.5% were female. The sensitivity, specificity, PPV, NPV, and accuracy of frozen section diagnosis for surgical margins were found to be 100%, 98.71%, 100%, 97.08%, and 99.02%, respectively [Graph 1]. The correlation between frozen section diagnosis and histopathological diagnosis was significant with “ P ” value of 0.0001. The correlation between TIC and that of histopathological diagnosis also was found to be significant which was 0.0001. The sensitivity, specificity, PPV, NPV, and accuracy of TIC for surgical margins were found to be 46.51%, 90.76%, 56.56%, 84.62%, and 86.40%, respectively [Graph 2].

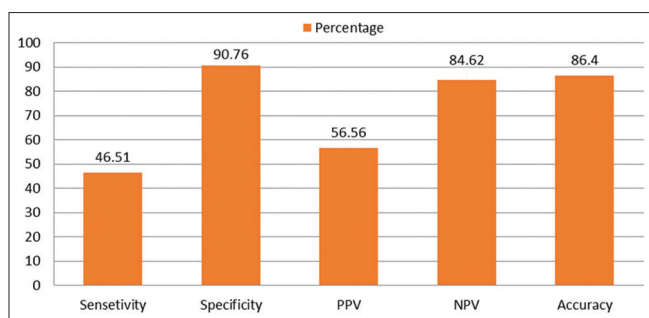
The diagnosis of frozen section and histopathology matched in 101 cases with the histopathological diagnosis, while TIC diagnosis of 89 cases matched with the histopathological diagnosis.

Discussion

The surgical margin is the adjacent mucosal or connective tissue surfaces removed by the surgeon during operative procedures in cases of malignancies. The term “surgical clearance” and “surgical margins”



Graph 1: Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of frozen section diagnosis



Graph 2: Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of touch imprint cytology

are used interchangeably. Margin clearance is important, as positive surgical margins can often lead to local recurrence. The present study highlights the importance of intraoperative diagnostic techniques such as frozen section diagnosis and TIC for surgical margin clearance. The study emphasizes the diagnostic accuracy of these techniques as compared to conventional histopathology as the gold standard for the evaluation of margin clearance status. This proves to be beneficial to the patients as it reduces the chances of re-interventions in cases of positive margins on histopathology. Although TIC for surgical margin clearance is used as diagnostic modality for tumor resection margins, lymphnode metastasis status, its utility for surgical margin clearance still remains unclear as the sensitivity and specificity of TIC were significantly found to be lower than frozen section diagnosis. The correlated results of other studies have been elaborated and discussed herewith.

In a study carried out by Ord and Aisner,^[7] the authors showed concordance with the conventional histopathology sections with an accuracy rate of 99% in cases of OSCC. Ten patients had positive surgical margins on histopathology and three were already diagnosed as positive on frozen sections. The authors found that frozen section diagnosis is an useful technique for the evaluation of surgical margins. The conclusion of this study was similar to that of our study; the present study also found frozen section diagnosis as an extremely useful technique for surgical margin clearance [Figure 1].

Ribeiro *et al.*^[8] studied 82 patients where the concordance between cryostat and paraffin section was found to be 99.5% in cases of OSCC. In the present study, the accuracy rate of the frozen section was found to be 99.5%. In the present study, the accuracy rate of frozen section diagnosis was found to be 99.02%, which is similar to the findings of the above study [Figure 2].

Yadav *et al.*^[9] studied the sensitivity, specificity, PPV, and NPV for margin clearance status in cases of OSCC. The authors found that TIC has an overall accuracy of 83%, sensitivity of 91.1%, specificity of 74.4%, PPV of 79.2%, and an NPV of 88.6%. These findings are not in concordance with the findings of the present study, as in

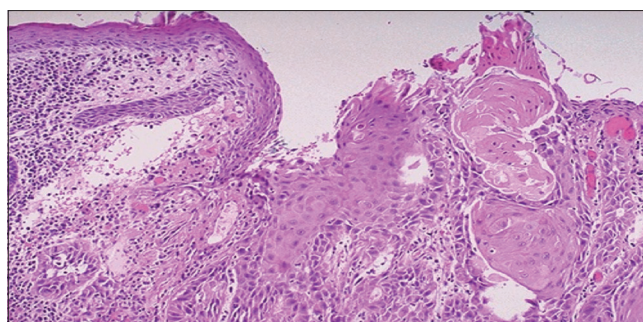


Figure 1: (×10) Positive margin histopathology (conventional H and E stain) oral squamous cell carcinoma

the present study, sensitivity and specificity of TIC were found to be 46.15% and 100%, respectively [Figure 3].

Schulz-Wendtland *et al.*^[10] performed a retrospective analysis of frozen section margin accuracy compared to permanent sections and showed an 84% concordance, with 24% of the patients requiring immediate re-excision intraoperative of the lesion and approximately 20% of patients needing second surgery due to false-negative margins. The findings of the present study showed an accuracy rate of 100% in cases of carcinoma of the breast for surgical margins.

Chakravorty *et al.*^[11] studied 146 patients in 2012 with positive resection margins in 2.7% cases, with a local recurrence of 4.3% cases in carcinoma of the breast patients. In the present study, there were no positive resection margins on frozen sections as well as histopathology.

In a meta-analytical study carried out by Esbona *et al.*,^[12] the authors studied five TIC studies and nine frozen section diagnosis studies to analyze pooled intraoperative sensitivity and specificity. The sensitivity of frozen sections ($83 \pm 13\%$) versus TIC ($72 \pm 38\%$) was not significantly different ($P = 0.53$). Similarly, the specificity of frozen sections ($95 \pm 8\%$) versus TIC ($97 \pm 3\%$) was not significantly different ($P = 0.58$). In the present study, the sensitivity and specificity of frozen section diagnosis and TIC were found to be 100%, respectively, for carcinoma of the breast.

Gomes *et al.*^[13] in their study for intraoperative frozen section diagnosis found sensitivity of 85.17% with a specificity of 100% and NPV of 99.16%. In the present study, only single case of carcinoma evaluation by frozen section diagnosis for surgical margin clearance was reported as negative which came out to be positive on histopathology. While the remaining surgical clearance margins from specimens of carcinoma colon were coherent and matchable with the histopathological diagnosis [Figures 4-6].

Khoury *et al.*^[14] studied 63 patients with rectal carcinoma. Frozen section sensitivity and specificity were 83% and

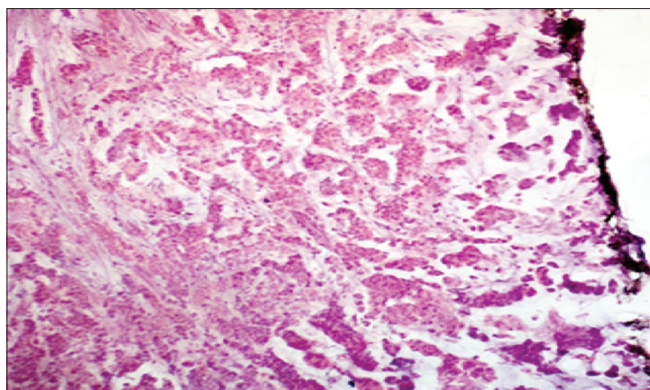


Figure 2: ($\times 10$) Positive skin margin on the frozen section (rapid H and E) oral squamous cell carcinoma

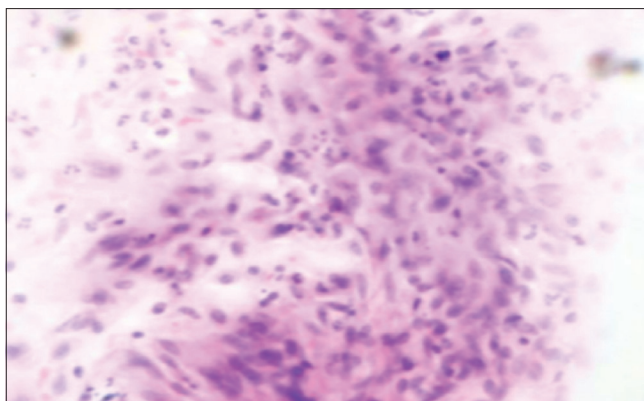


Figure 3: ($\times 40$) Positive margin touch imprint cytology (rapid H and E stain) oral squamous cell carcinoma

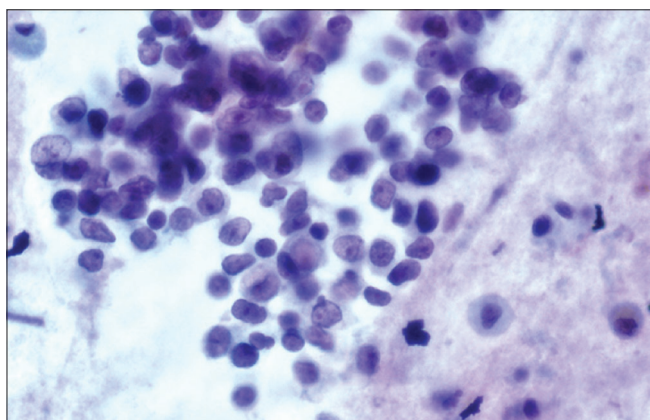


Figure 4: ($\times 40$) Positive margin touch imprint cytology (rapid H and E) carcinoma of the colon

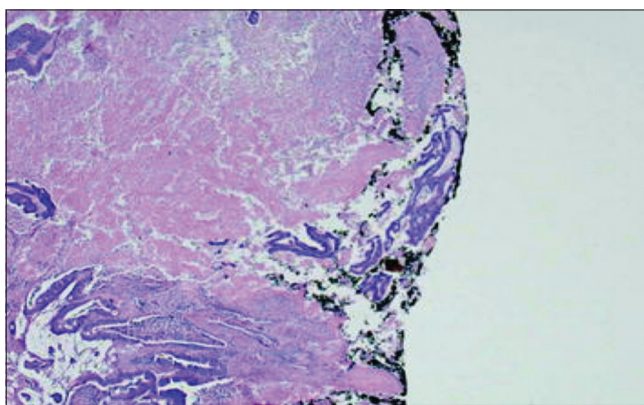


Figure 5: ($\times 10$) Positive margin frozen section (rapid H and E) carcinoma of the colon

98%, respectively. Of total nine cases of carcinoma of the colon, margin clearance status matched in cases of eight specimens (four were reported as positive surgical margins and four were reported as negative surgical margins). One case was reported as negative on frozen section diagnosis and positive on histopathology. The findings of the present study are in concordance with that of the above study, as the findings of frozen section diagnosis correlated in all except one case.

Chambers *et al.*^[15] found that discrepancy between the frozen section and permanent margin was found in 10 of 247 frozen section margin samples and in 8 of the 41 cases by TIC in cases of squamous cell carcinoma of the skin. In the present study, no discrepancy was found between the diagnosis by frozen section margin clearance and permanent histopathology sections.

Salimi *et al.*^[16] in their study on BCC of the skin concluded that due to low sensitivity, specificity, and low kappa value, TIC alone cannot be used for the assessment of margin clearance status in case of BCC. It has to be coupled with frozen section analysis, as this method has higher sensitivity, specificity, and kappa value. In this study, a similar conclusion was derived that TIC can give false-negative results as compared to frozen section diagnosis.

Florell *et al.*^[17] evaluated the utility of TIC and Mohs frozen section in Mohs micrographic surgery for BCC. The sensitivity of TIC for identifying a positive margin was approximately 50% for all BCC types. The specificity was approximately 90% for all BCC types. In the present study, the specificity was found to be 100% and sensitivity was 46.15%. These findings correlated with the findings of the above study.

The limitations of the study included – the individual cell morphology in frozen section is different from that of the conventional histopathology (cells and nucleus appear larger on frozen sections), causing chances of reporting false-positive margins; TIC does not give satisfactory cell yield, thus not proving an adequate intraoperative technique for surgical margin clearance; and false-negative rates of TIC are significant as cell sampling is not satisfactory.

Future perspectives of the current study and methodology explained are – it helps decrease recurrences as clear margins are obtained; alleviates prognosis of the patient as complete resection is obtained; and it gives an efficient, timely diagnosis, minimizing waiting period and reducing the critical time and it proves to be cost-effective for the patient, as re-operative procedure is prevented

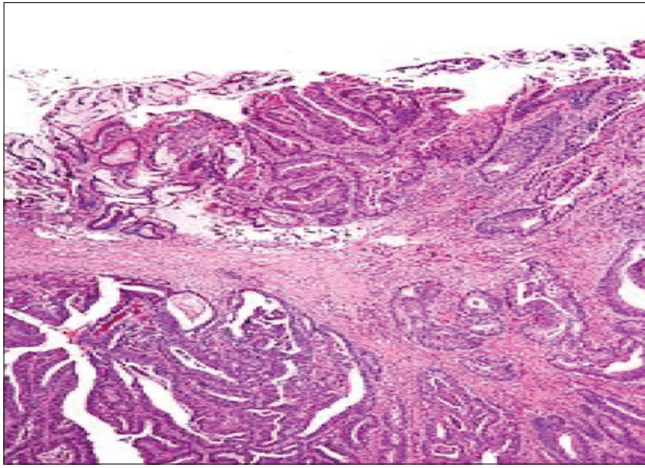


Figure 6: (×10) Positive margin histopathology (conventional H and E) carcinoma of the colon

Conclusion

Intraoperative frozen section diagnosis is an accurate method for the assessment of surgical margin clearance as compared to TIC. The present study evaluated predominantly epithelial malignancies than mesenchymal malignancies, thus emphasizing its utility in epithelial malignancy. More research needs to be done for the assessment of the utility of these modalities in malignant mesenchymal tumors, as in the present study, the sample size for malignant mesenchymal tumor is considerably low.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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