Accuracy of intraoperative frozen-section in assessing margins in oral cancer resection

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Abstract

Objectives To study the accuracy of intraoperative frozen section diagnosis in assessing margins in oral cancer resection and to evaluate the role of frozen section diagnosis as a guide in resection of oral cancer lesions.

Patients and methods The fresh tissue samples from the margins of lesions of patient with preoperative diagnosis of oral squamous cell carcinoma were used. The frozen sections are prepared immediately and examined by oral pathologist for the study. The results of these sections were compared with the results of histologic findings obtained by routine paraffin embedded Haemotoxylin and eosin method.

Results The total of 184 margins, from 47 patients were examined. 178 margins showed concordance with the permanent section of the same tissue sample (seven false negative and one false positive), an accuracy rate of 96.74%.

Conclusion Frozen section is a helpful tool in intraoperative decision making in indicated situation. The results are highly specific but only moderately sensitive. The surgeon and the pathologist must understand the limitations of frozen sections.

Keywords Frozen section · Oral cancer · Tumour resection · Tumour margin

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Introduction

The best chance of surgical cure in oral cancer is complete removal of all malignant cells. Unfortunately, it is not possible to identify the microscopic extent of tumor invasion by inspection and palpation [1].

Intraoperative Frozen Section serves the surgeon by providing diagnosis, tissue recognition and extent of resection necessary and thus helps in the making of therapeutic decisions thereby sparing the patient from possible re-operation and reducing the length and cost of hospitalization [2,3].

At operating table surgeons, are often in a dilemma as to how much they should resect or they may come across an enigmatic tissue in a lesion. To all of these dilemmas, the frozen sections provide an answer [4].

The use of frozen section is varied. They are useful in determining whether a tumour is benign or malignant, so that the extent of

the resection can be decided. It is also helpful in determining the adequacy of the surgical margins. Further, the decision to perform a radical surgery of lymph nodes is entirely dependent on the frozen section. Frozen section help in rapid diagnosis of the lesion and in intraoperative histological evaluation of tumour resection borders. This is done without prolonging the surgery and thus acts as a guide to the surgeon in making a therapeutic decision, while the patient is still on the operating table [9]. Errors in diagnosis are most often due to difficulty in recognition of certain types of lesions, inadequate sampling, technical problems or the lack of communication between the surgeon and pathologist [10].

Many comparative studies regarding frozen and paraffin sections have been done to find out the reliability of frozen sections in the diagnosis of prostate carcinoma, breast cancer, lung cancer, skin cancer and tumors of colon, gallbladder, kidney, head and neck [11].

The current study examined the accuracy of frozen section interpretation in comparison to the histologic findings on permanent sections when assessing surgical margins for oral squamous cell carcinoma. The role of frozen sections in defining margins also examined.

As the diagnosis made by the pathologist from frozen section may have serious consequences for the treatment of the patient, a high degree of accuracy is mandatory and quality control is important. Considering the above factors, a study was planned to evaluate the accuracy of intraoperative frozen section microscopy in assessing the margins in oral cancer [2].

Materials and methods

Present study consists of fresh intraoperative tissue samples from the margins of lesions of patient with preoperative diagnosis of oral squamous

cell carcinoma, were used. The tissue is taken as samples from all margins of the lesion, i.e anterior, posterior, medial, lateral and deep, from patients who are undergoing surgical resection for malignancies.

The frozen sections are prepared immediately and examined by one oral pathologist for this study. The results of these sections are compared with the results of histologic findings obtained by routine paraffin embedded haematoxylin and eosin method. The patients were of varying age groups. Each of the samples obtained were evaluated by both the frozen sections and routine paraffin sections and compared. The reliability of frozen section diagnosis in detecting with accuracy, specificity, sensitivity, false positivity, false negativity, predictive value of positivity and negativity and its efficiency in terms of percentage were estimated.

The present study was carried out by collecting a total number of 184 margins from the 47 biopsies of proven oral cancer patients.

The materials and equipments required for the frozen section study are:

1. Clarke's fluids
2. Formalin
3. Haematoxylin and eosin stains
4. Lithium carbonate
5. Embedding media (OCT, Polyethylene glycol based)
6. DPX (mounting media)

Equipments

1. Cryostat (Leica CM 2800 N)
2. Slides and cover slip
3. Microscope with photomicrograph facility

Reagents used

1. Clark's fluid

Clark's fluid is prepared by mixing 75ml absolute alcohol with 2ml glacial acetic acid in a beaker. This fixative penetrates rapidly and gives good nuclear fixation and reasonably good preservation of cytoplasmic elements.

2. Haematoxylin and Eosin stains

Harris haematoxylin was used in the study and eosin was used as counter stain.

Fig. 1 Cryostat (Leica CM 2800 N)

Fig. 2 Microscope with photomicrograph facility

3. Lithium carbonate

It is obtained commercially as white powder. In the present study saturated solution of lithium carbonates was used for blueing the tissue sections after haematoxylin staining.

4. Embedding media

A tissue freezing medium (supplied by the manufacture) which solidifies in low temperature and binds the tissues to the chuck (tissue holder)

5. DPX (Dibutylphthalate xylene)

This wax soluble mounting media was used in the study.

The cryostat

The cryostat used in the present study is Lieca CM 2800N (digital controls and automatic defrostration facility). The specimen head can be oriented in any direction. The microtome knife used is a high profile disposable blade.

The knife base is fixed and the specimen head can be moved to and fro using the digital advancement facility. The sections of desired thickness as thin as 1µm can be cut. The anti-roll plate prevents curling of the sections and the heat extractor flattens the tissues as it removes the heat from the tissues and block.

Procedure

After the resection of primary lesion, it was placed in saline and sent immediately to the Department of Oral Pathology.

1. The margins were cut using a B.P blade after orienting it in anatomic position. Among the two sections of the margins one was chosen for frozen section study and the other for paraffin section study.

2. The section selected for frozen section study was placed on embedding media which was poured on dry surface of the chucks (tissue holders) the rough concentric or serrated upwards and away from the chuck. The rough concentric or serrated surface of the chucks helps in firm adhesion of tissue to the chuck, through the embedding media.

3. The chuck with tissue and embedding media were kept in the cryostat cabinet. The cryostat cabinet was adjusted to a temperature ranging from -20° C to -25°C. At this temperature solidification of the embedding media occurs. Prior to the solidification of the gel, it was pressed by the heat extractor component of the cryostat.

4. The plane of the tissue was adjusted by screws present on the specimen head of the cryostat. The plane was kept almost parallel to edge of the blade. High profile disposable blades were placed in the blade holder, which is held on a base fixed at 45 degree angulations. These requisites help in obtaining good frozen sections, covering the entire tissue section.

5. The microtome apparatus in the cryostat is a rotary type, where the head part containing the tissue is mobile, while the knife part remains stationary. With the help of anti-roll guide, unfolded frozen sections of 6µ were obtained and the sections were placed on glass slides. The sections get attached to the glass slide due to difference in temperature.

Haematoxylin and eosin staining procedure for frozen sections were done. Similarly, Haematoxyline and Eosin staining procedure for paraffin sections were also done.

The statistical analysis for sensitivity, specificity, false positively, false negativity predictive value of positive test, predictive value of negative test and accuracy have all been determined.

Results

The evaluation and comparison of frozen sections with that of paraffin sections in assessing margins of oral squamous cell carcinoma patients were done. The age and sex of the patient, location of cancer and TNM staging were recorded. The number and location of margins in each case and the infiltration of carcinoma in frozen and in paraffin section, in each of the margins were recorded. The results were analyzed and recorded as true positive, true negative, false negative and false positive in each of the cases. Based on the results recorded in (Table 1), the percentage of sensitivity; specificity, false positively, false negativity, predictive value of positive test and negative test and percentage of efficiency were estimated.

Location of cancer

The primary site of the squamous cell carcinoma was varied; among the forty seven patients, twelve patient had squamous cell carcinoma of buccal mucosa, eleven of lower alveolus, nine are of tongue, five are of lower lip, three are in the retro molar trigone, two were in the floor of the mouth, one each were carcinoma of palate and maxillary tuberosity of the right side of the face, faucial pillars, and submental region.

Assessment of accuracy of frozen section

The comparison of the section from margins i.e. anterior, posterior, medial, lateral and deep, altogether 184 margins from 47 patients showed concordance in 178 margins, i.e. 167 margins were negative in both frozen section and Paraffin method and 11 margins were positive in both frozen section and 16 margins were positive in paraffin method. 12 margins were positive in frozen section. The six margins showed disagreement in frozen section and paraffin embedded Haematoxylin and Eosin method.

Statistical analysis was done, McNemar test was used for 184 samples and a p-value of 0.219 was derived thus showing no significant difference between paraffin and frozen sections.

Graph I Age of the patient: The age of the patients ranged from fourth to ninth decade. Six patients were in fourth decade, 8 in fifth decades, 18 sixth decades, 10 in seventh decades and there are 5 patients more than 70 years of age

Graph II Gender of the patient : Out of the forty seven patients in the study, twenty were females and twenty seven were males

There was one false positive and seven false negative results. The percentage of sensitivity, specificity and accuracy were 72, 94.41, and 96.74 respectively. The percentage values of positive test and negative test and the predictive values of positive test and negative test were 0.59, 28, and 94.74 and 96.

Discussion

The principal indication of performing a frozen section is to obtain a definite immediate diagnosis, on the basis of which surgical decision will be made. Immediate diagnosis allows the surgeon to proceed

with further therapy without any delay. A definite immediate diagnosis is said to be dependent on the reliability of frozen sections. Many authors have carried out frozen section study on soft tissue tumors and margins evaluation in different parts of body, with varying percentages of reliability [6].

In evaluating the significance of surgical margins, there are a number of questions raised by this study and previous publications. How helpful are frozen sections in guiding the surgeon; what is a positive margin, what treatment is indicated for a positive margin; and finally, how confident can the surgeon be that a negative margin ensures that all tumor has been removed.

Frozen sections have been used both in diagnosis and to evaluate margins. In most publications, accuracy for such diagnosis is 96.8% to 98% [18,22,23,24,25].

The reliability of frozen section in diagnosis on lesions in different regions of the body were varied in their accuracy from 91.5% to 98% [10], and in head and neck neoplasm 96% to 99% [6,7].

A large series, 1,947 frozen sections that evaluated margins in head and neck tumors showed an accuracy of 98.3%, with only 1.9% discrepancy [19,20]. Three patients (6%), had positive margins (two positive for tumor one for dysplasia) that were not removed because of involvement of vital structures, compared with 10% of such cases in Byers report [21]. However, 23% of Byers et al.'s patients had positive margins initially that were excised to achieve negative margins. Because this reduced the incidence of recurrence, Byers et al. concluded that frozen sections were beneficial. Our series had only twelve patients (25.53%) with intraoperative frozen sections that were initially positive who have undergone further surgery to give final negative margins. Sixteen patients (34.04%) had final positive margins.

Frozen sections were beneficial in this series as it enabled us to achieve negative margins in eight patients thus preventing reoccurrence. However, there was one false-positive frozen section and four patients who had tumors with positive margins at final diagnosis that were not diagnosed by the intraoperative frozen sections who did not benefit from frozen sections.

35 patients (74.48%) had their tumors adequately excised based only on the surgeon's judgment, which compares to 67% in Byers et al.'s series. The question

Table 1 Details of microscopic evaluation of margins

No. of cases	Number of margins evaluated	Presence of carcinoma in frozen section	Presence of carcinoma in paraffin section	Negative frozen section	Negative paraffin section
47	184	12	16	172	168

Table 2 Tabulation of true positive, true negative, false positive, false negative results

No. of cases	No. of margins examined	True positive	True negative	False positive	False negative
47	184	16	168	1	4

Table 3 Comparative statistical analysis of frozen with paraffin sections

No. of margins	Sensitivity (%)	Specificity (%)	False positive (%)	False negative (%)	Predictive value of positive test (%)	Predictive value of negative test (%)	Efficiency (Accuracy)
184	72.00	99.41	0.59	28	94.74	96.00	96.74

of what further treatment is required when a positive margin is identified on the final specimen is also debatable. Excision of positive frozen section margins intraoperatively has been shown to give the same recurrence and survival rates as cases with initial negative margins [2]. Therefore, immediate excision has been proposed. Leez found a 67%, 5-year survival for patients who underwent a second excision after inadequate primary surgery, compared with 43% for patients given radiation and 21% for those having no further therapy. However, only 50% of the excised specimens were found to contain tumor; either the frozen section sampling had removed all cancer cells or the excision was not in the correct area.

Although Lee recommended immediate re-excision for all patients with positive margins on the final specimen, his results showed that 5-year survival was also improved with postoperative radiation therapy.

According to the literature there is a local recurrence rate of 3% to 35% in patients with negative margins. Recurrence is highest in T4 tumors. In T1 and T2 lesions with negative margins and no adjuvant radiation therapy, recurrence rates are as low as 7% [20].

The predictive value of negative frozen section was said to be useful in clinical practice by providing direct information to the patient, as being free of disease our study has also fulfilled this objective [19].

However, intraoperative molecular analysis of surgical margins may eventually be developed as the 'state of

the art' method for oncologic surgery. Local recurrence is seen with increasing frequency in tumors with negative margins as size increases. Irrespective of the status of the neck nodes, this would appear to validate the use of adjuvant radiation therapy for all T3 and T4 lesions despite negative margins and for T1 and T2 tumors with positive margins where further excision is not feasible.

Conclusion

Frozen section is a helpful tool in intraoperative decision making in indicated situations. The results are highly specific but only moderately sensitive compared with final margins from the resections specimens. The present study is comprised of a total of 184 intraoperative margin specimens from 47 patients of oral squamous cell carcinoma. The results were compared and the statistical analysis for sensitivity (72%), specificity (99.41%), and predictive value of positive test, predictive value of negative test and accuracy of frozen sections were 94.74%, 96.00% and 96.74% respectively.

It is shown that frozen sections were beneficial in this series as, patients who had positive results required revision of margins. The surgeon and pathologist must understand the limitations of frozen section and attempt to minimize cost and sampling error. However, intraoperative molecular analysis of surgical margins may eventually be developed as the 'state of the art' method for cancer surgery.

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