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Reliability of Imprint Cytology and Frozen Sections for Demonstrating Free Surgical Margins in the Periocular Basal Cell Carcinoma

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Abstract

Purpose: The present was conducted to measure the sensitivity and specificity of imprint cytology (IC) and frozen section (FS) in the evaluation of margin involvement of periocular basal cell carcinoma (BCC).

Methods: This cross-sectional study was conducted on 36 patients who underwent an excisional biopsy of periocular BCC. The surgical margins of each excised mass were tested for involvement by tumoral cells using three pathologic methods as follows. Samples were obtained for IC from the marginal territory of the tumor. Then, FS samples were obtained and the tissue was prepared for permanent histopathologic evaluation, the results of which were considered as the gold standard to compare two other methods. Sensitivity, specificity, positive predictive value, and negative predictive value of each method were calculated accordingly.

Results: Thirty-six patients (20 males and 16 females) with the mean age of 73±12 years who had periocular BCC were enrolled and a total of 121 samples were obtained from the margins. The sensitivity and specificity of FS were 94.3 and 97.7% and of IC were 51.4 and 84.9%, respectively. Also, Positive and negative predictive value for FS were 94.3 and 97.7 and for IC were 58.1 and 81.1%, respectively.

Conclusions: Although less expensive and more feasible than FS, IC lacks diagnostic accuracy for demarcating the extent of resection of eyelid BCC.

Keywords: Basal cell carcinoma, Eyelid tumors, Frozen section, Imprint cytology

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Introduction

In approaching periocular skin tumors, defining the exact extension of the neoplastic mass in conjunction with the cosmetic outcome are of utmost importance. Bearing in mind

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that the net diagnosis is usually apparent using various pathological techniques, there is a tendency to less invasive clinical approaches that offer preservation of freer eyelid tissue without compromising their sensitivity and specificity profile.[1]

It is well-established that intraoperative tumor margin evaluation techniques, including the frozen section (FS) and Mohs micrographic surgery, lead to less frequent recurrence compared to simple tumor excision. These so-called "margin control" techniques are helpful in preventing repeated operations if the classic pathologic evaluation is to report margin involvement of the specimen.[2-4] However, both of these techniques are limited by their need for special equipment required for tissue preparation and handling, their time-consuming nature, and their need for subspecialty trained surgeons.

Imprint cytology (IC) as a simple, non-invasive, and rapid method has been applied in the diagnosis of various ocular surface disorders including dry eye syndrome, limbal stem cell deficiency, contact lens-induced conjunctival metaplasia, conjunctival intra-epithelial neoplasia (CIN), and conjunctival melanoma.[5-9] The application of IC needs neither special equipment nor special training and its results are generated fairly rapidly. The aim of the present study is to compare the findings of IC specimens obtained from margins of excised periocular basal cell carcinomas (BCC) with the findings of the FS and compare both techniques (IC and FS) with permanent pathology results as the gold standard.

Materials and Methods

This cross-sectional study was conducted at Farabi Eye Hospital, Tehran, Iran. The study design was approved by the hospital's Ethics Committee for adherence to the declaration of Helsinki regarding research on human subjects (Code Number: IR.TUMS.FARABIH.REC.1398.025). The patient enrollment started in January 2016 and ended in June 2016. The study population included patients with primary periocular BCCs that required biopsy at the discretion of the oculoplastic surgeon. Exclusion criteria were a history of a known malignancy, previous incomplete excision of the lesion, or recurrence of a previously treated lesion. All patients received detailed information about the need for biopsy and its implications and informed consent was obtained accordingly.

In the operating room, the surgeon marked the tumor margin and performed an excisional biopsy. The specimen borders were delineated at superior, inferior, temporal, and/or nasal sides with silk sutures and were sent to the pathology

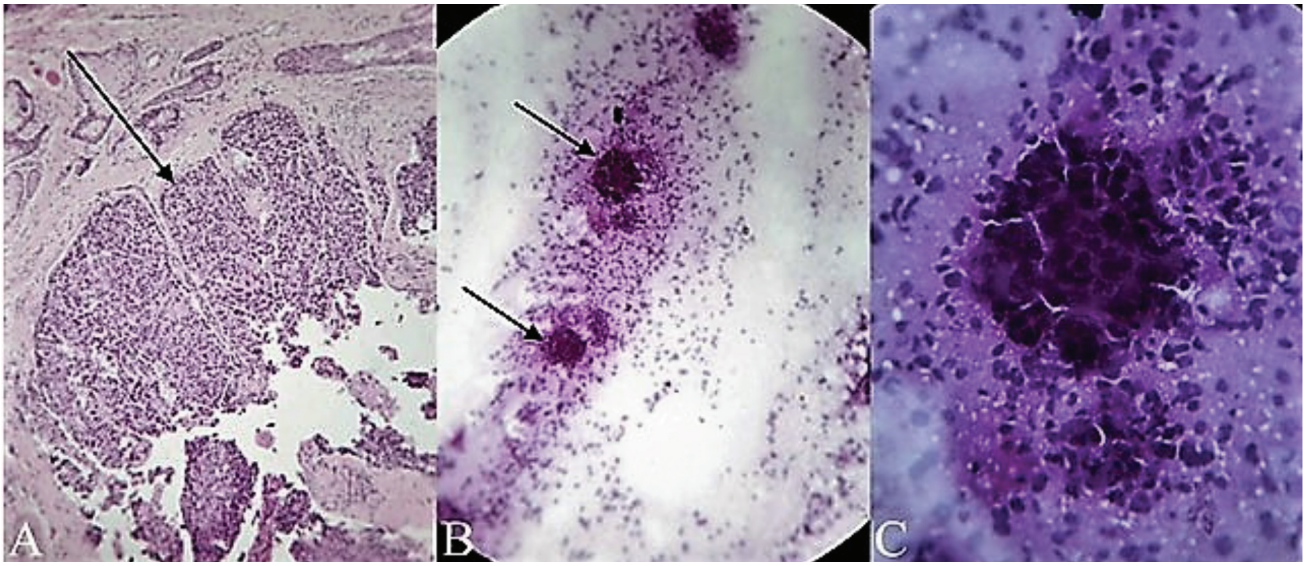

Figure 1. Pathologic examination of a lid tumor from a 48-year-old man with basal cell carcinoma; (A) H & E staining of a lid tumor demonstrating malignant tissue (arrow) (×40 magnification); (B) The IC slide revealed malignant cells (arrows) (×100 magnification); and (C) A nest of malignant cells as in (B) (×400 magnification).

Table 1. Sensitivity, specificity, positive predictive value, and negative predictive value of FS for detection of tumoral involvement of margin specimens (n = 121)

		Permanent pathology result		
		Positive	**Negative**	**Total**
FS Results	**Positive**	**33** Sn = 94.3% (33/35) (CI: 81.39 - 98.42%) PPV = 94.3% (33/35) (CI: 81.39 - 98.42%)	**2**	**35**
	Negative	**2** Sp = 97.7% (84/86) (CI: 92.97 - 99.42%) NPV=97.7% (84/86) (CI: 92.97 - 99.42%)	**84**	**86**
	Total	**35**	**86**	**121**

*FS= Frozen section; Sn= Sensitivity, CI= Confidence interval; PPV= Positive predictive value; NPV= Negative predictive value

laboratory immediately. In the laboratory, first, IC sample from each margin of the tumor was prepared by applying a clean glass slide using the touch imprint method. These smears were air-dried, fixated by cytology spray, and stained with hematoxylin and eosin.

Then, the margins of the specimens were used for the preparation of FSs. The Leica Cryocut 1800 frozen section machine (Leica Microsystems, Bannockburn, IL, USA) was used to produce 5-µm thick sections. These were stained by toluidine blue and examined immediately by the attending pathologist.

After informing the surgeon concerning the result of the FS, 10% buffered formalin was used to fixate frozen specimens and the tissues were incubated in the tissue processor (DS 2080/H, Tehran, Iran). The paraffin blocks were sectioned by the Accu-Cut SRM 200 microtome (Sakura Finetek Europe B.V., Alphen aan den Rijn, Netherlands) and stained with hematoxylin and eosin for permanent pathology evaluation.

The result of permanent pathology was used as the reference for definitive diagnosis (Figure 1A). The imprinting cytology and FS results were finally compared with permanent pathology for determining the accuracy of diagnoses. The touch imprint slides were considered positive if small or large clusters of cells with hyperchromatic nuclei, high nucleo-cytoplasmic ratio, and peripheral palisading were detected using light microscopy (Figures 1B and 1C). Insufficient and/or suspicious smears were discarded. For frozen and permanent sections, BCC is diagnosed when basaloid epithelial tumor arising from the epidermis typically forms a palisade with a cleft forming from the adjacent stroma. Statistical analysis was performed using SPSS software version 18.0 (SPSS, Inc, Chicago, IL).

Results

36 patients, including 20 males (55.6%) and 16 females (44.4%), were enrolled in the study. The average age of the participants was 73±12 years (48 to 95 years). The BCC lesions were localized to the right and left sides of the periocular region in 22 (61.1%) and 14 (38.9%) cases, respectively. The lower lid was the most frequently involved location (in 17 (42.2%) patients), followed by periocular facial region (7 (19.4%) cases), medial canthus (6 (16.7%) cases), and upper lid (6 (16.7%) cases). In our series, no lateral canthal lesions were identified. From this population, 121 marginal samples were obtained. Based on permanent sections results, among 121 sections, 35 positive margins and 86 negative margins were encountered. Frozen and permanent histologic sections results showed a good correlation but IC compared to permanent histopathology showed a marginal result with 13 and 17 false positive and false negative results, respectively.

Sensitivity, specificity, positive predictive value, and negative predictive value of FS and IC for the correct detection of tumoral involvement

Table 2. Sensitivity, specificity, positive predictive value, and negative predictive value of IC for detection of tumoral involvement of margin specimens (n = 121)

		Permanent pathology result		
		Positive	**Negative**	**Total**
IC results	**Positive**	**18** Sn = 51.4% (18/35) (CI: 35.57 - 67.01%) PPV= 58.1% (18/31) (CI: 41.74 - 73.55%)	**13**	**31**
	Negative	**17** Sp = 84.9% (73/86) (CI: 77.76 - 90.33%) NPV=81.1% (73/90) (CI: 73.96 - 86.73%)	**73**	**90**
	Total	**35**	**86**	**121**

*IC= Imprint cytology; Sn= Sensitivity, CI= Confidence interval; PPV= Positive predictive value; NPV= Negative predictive value

of margin specimens (n = 121) are presented in tables 1 and 2, respectively.

Discussion

This study was conducted to find out whether the IC results, in terms of detecting margin involvement, correlate with the findings of FS and permanent pathology in periorbital BCC tumors.

The demographic features of our series demonstrate the tendency for periorbital BCC tumors to occur in the geriatric population, with men more frequently involved, as expected by the current medical concept. To some extent, this result can be explained by the accumulative role of ultraviolet exposure in those who spend outdoors for occupational and recreational activities.[10-12]

We also found that the most common site for periorbital tumors is lower eyelid, followed by facial skin overlying the forehead, cheek, and nose, medial canthus, and upper eyelid. Interestingly, none of our patients had tumor growth involving lateral canthus. Mannor et al. reported the location of eyelid BCC in 841 patients in descending order of frequency in the lower eyelid, medial canthus, upper eyelid, and lateral canthus.[13]

The sensitivity and specificity of IC for specimens obtained from tumor margins were approximately 51% and 85%, respectively. Our finding was similar to the results of Florell et al.[14], who studied the accuracy of the IC and FS in skin BCC margins and demonstrated 50% sensitivity and 90% specificity for IC. According to the results of positive and negative predictive values (approximately 58% and 81%, respectively), we propose that negative results of IC help best in restricting the extent of tumor excision and surgical conclusion. However, positive results of IC do not seem reasonably reliable.

Although IC has been applied in the diagnosis of various ocular surface disorders (including dry eye syndrome, limbal stem cell deficiency, etc.),[5-9] studies regarding the application of IC for periocular tumors are scarce. In the only published study we found, Sen et al. used IC for previously proven malignant ulcerative eyelid lesions.[15] Thirty-two patients were examined and the sensitivity of 84% was reported for the diagnosis of malignant components. While Sen et al. acquired the specimens by applying Millipore papers over the ulcerative lesion,[15] we obtained the specimens after excising the tumor and by applying the glass slides over the margins of the excised mass. Moreover, Sen et al. reported no false positive results but we had false positive results, which account for decreased specificity of our study.[15]

Our study is limited by the absence of a control group and its relatively small sample size.

Although the IC is less expensive and more feasible in comparison to the FS, it characteristically lacks diagnostic accuracy for detection of involvement in margins of periocular

malignancies.

Based on the results of this study, it can be stated that the IC can be applied for demarcating the extent of resection of eyelid BCC tumors. However, randomized clinical studies are lacking and further investigation is needed to determine the exact indications and reliability of this technique.

Conflict of Interest

None declared.

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