Original Article

Sensitivity and specificity of frozen section diagnosis in orbital and adnexal malignancies

Md. Shahid Alam, Andrea Tongbram[1], Subramanian Krishnakumar[2], Jyotirmay Biswas[2], Bipasha Mukherjee[1]

Purpose: To analyze the diagnostic accuracy of frozen section in orbital and adnexal malignancies. **Methods:** A total of 55 cases between January 2006 and December 2011 for which intraoperative frozen section was performed for various orbital and adnexal lesions were included in the study. The frozen section diagnosis was compared with the permanent section diagnosis. Margin clearance was also compared between the two. Data were analysed using SPSS version 14. Odds ratio and cross-tabulation was used to perform the analysis. **Results:** The mean age at presentation was 51.46 ± 20 years. Eyelid was the most common site of involvement. Out of 55 cases, diagnosis was deferred in four cases (7.27%) on frozen section. Among 51 cases, 44 (86%) cases were concordant, whereas 7 (13%) cases were discordant. The sensitivity and specificity of frozen section compared to permanent section for diagnosis of malignancy was found to be 87.2% and 87.5%, respectively. The sensitivity and specificity of frozen section for diagnoses of basal cell carcinoma was found to be 100%, while it was 83.3% and 100% respectively for sebaceous gland carcinoma and 87.5% and 94.9% respectively for squamous cell carcinoma. **Conclusion:** Frozen section had high sensitivity and specificity when compared with permanent section for all three parameters studied. It is an important intraoperative tool that is increasingly being used in histopathological examination of ophthalmic lesions. However, it should not be used as a substitute for the permanent section and critical decisions based on it are best avoided.

Key words: Adnexa, frozen section, orbit, permanent section

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The use of frozen section dates back to 1891 when William H. Welch examined a breast tumor by frozen section technique. The purpose of frozen section is threefold – identification of the tissue of origin, the type of pathological process and assessment of disease-free surgical margins.[1] It allows for intraoperative diagnosis within a short period which is used to guide the surgeon's plan of action. The application of frozen section in ophthalmic pathology began in the 1970s.[2]

The most common indication for ophthalmic frozen section is evaluation of surgical margins in eyelid tumors.[3] The surgeon's confidence depends on the diagnostic accuracy of the frozen section results.[4] To evaluate the diagnostic accuracy of frozen section, it is compared with permanent section which is considered the gold standard.[5] The accuracy rate of frozen section diagnoses is quite high.[6-8] False negative results account for the maximum number of discordant cases between frozen and permanent section diagnosis.[9-11]

The correlation between frozen section and permanent section diagnosis for neoplasms of various internal organs has been evaluated in many studies.[12-20] However, there is no study that evaluates the sensitivity and specificity of frozen section in ophthalmic pathology.

Methods

A retrospective review of cases for which intraoperative frozen section was performed during January 2006-December 2011 in a tertiary eye care centre in India was done. It was a single centre study and cases for which frozen section examination was performed for ocular adnexal, ocular surface and orbital lesions were included in the study. Relevant data were retrieved from patient case record sheets. Institutional review board approval was obtained and the study adhered to tenets of declaration of Helsinki. Informed consent were taken from patients regarding publication of their histopathological diagnosis and details.

The pathologists, who examined the samples, had specialized training in ocular pathology and more than 20 years of experience in dealing with ocular and adnexal specimens.

The specimens submitted were frozen cut with standard frozen section cryostat (Leica model CM1510S), fixed on glass slides and stained with Haematoxylin and Eosin (H and E) stain for examination. The specimens were examined for

Department of Orbit, Oculoplasty, Reconstructive and Aesthetic Services, Aditya Birla Sankara Nethralaya, Kolkata, West Bengal, [1]Department of Orbit, Oculoplasty, Reconstructive and Aesthetic Services, Sankara Nethralaya, [2]Department of Ocular Pathology, Sankara Nethralaya, Medical Research Foundation, Chennai, Tamil Nadu, India

Correspondence to: Dr. Md. Shahid Alam, Department of Orbit, Oculoplasty, Reconstructive and Aesthetic Services, Aditya Birla Sankara Nethralaya, Kolkata - 700 099, West Bengal, India. E-mail: mshahidalam@gmail.com

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the tissue type and probable diagnosis, assessed for surgical margin clearance in cases of suspected malignancy. The remaining tissues were then fixed in 10% formalin, processed and embedded in paraffin and stained with H and E stain and assessed for verification of the tissue type, categorization of neoplasms and evaluation of margins.

The diagnosis by frozen section was correlated with the permanent histopathological diagnoses to assess the diagnostic accuracy of the technique. The results were classified as follows:

1. Concordant when both the frozen and permanent section diagnosis agreed with each other,
2. Discordant when they did not agree or
3. Deferred when frozen section examination could not reach a specific diagnosis.

The sensitivity and specificity of frozen section were analyzed in its ability to diagnose whether a particular tumor was malignant or not, surgical margin clearance, and the three most common adnexal malignancies namely Basal cell carcinoma (BCC), Squamous cell carcinoma (SCC) and Sebaceous gland carcinoma (SGC). Data were analyzed using SPSS version 14 and Odds ratio and cross tabulation was used to perform the analysis.

Results

A total of 55 cases during the study period were included out of which 29 patients were males and 26 were females. The mean age at presentation was 51.46 ± 20 years (range between 3 months and 81 years).

The primary site of involvement was the ocular surface in 27 cases and the eyelid in 23 cases. The lacrimal gland was the primary site in five cases. The orbit was secondarily involved in six cases and intraocular involvement was seen in two [Fig. 1]. There were no cases of primary orbital tumors, apart from lacrimal gland tumor for frozen section.

Different disease pathology as diagnosed on frozen and permanent section is summarized in Table 1.

Out of 55 cases, diagnosis was deferred in 4 cases on frozen section. Of the remaining 51 cases, 44 (86%) were concordant with permanent section diagnoses, whereas 7 (13%) were found to be discordant. Among discordant cases, two cases of pleomorphic adenoma of lacrimal gland were reported as adenoid cystic carcinoma and myoepithelioma on frozen section [Figs. 2 and 3]. Two cases of SCC of the conjunctiva were reported as dysplasia, while one case of conjunctival dysplasia was labelled as SCC on frozen section [Fig. 4a and b]. One case each of keratoacanthoma and SGC of the eyelid was reported as inflammation and SCC respectively [Fig. 5a and b]. Frozen section was not able to arrive at a conclusion regarding the diagnosis in four cases and hence the diagnosis was deferred. The diagnosis of all these four cases on permanent section were conjunctival malignant melanoma, mucoepidermoid carcinoma of the lacrimal sac, round cell tumor of the conjunctiva, and eyelid adnexal tumor most likely SGC. Table 2 illustrates the discordant cases. The cause for discrepancy was sampling error in five (71.4%) cases and misinterpretation in two (28.5%) cases.

The sensitivity and specificity of frozen section compared to permanent section for diagnosis of malignancy was found to be 87.2% and 87.5% respectively with a misclassification rate of 12%. The sensitivity and specificity of frozen section for margin clearance was 100% and 93.1% respectively with a misclassification rate of 4%. The sensitivity and specificity of frozen section in diagnosing three most common malignancies of eyelid and adnexa is summarized in Table 3.

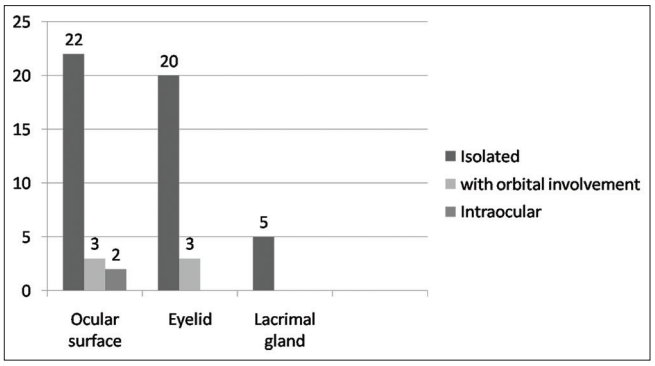

Figure 1: Bar diagram depicting the site of involvement and their frequency

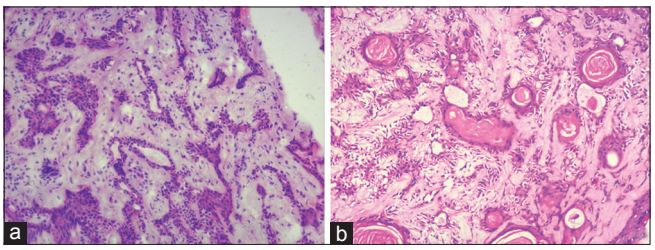

Figure 2: (a) Frozen section microphotograph of a lacrimal gland specimen, depicting basaloid cells lacking ductular structures, leading to the diagnosis of adenoid cystic carcinoma. (b) Permanent section microphotograph of the same specimen after complete excision, depicting tumor composed of myxoid and epithelial component without any evidence of malignancy confirmatory of pleomorphic adenoma

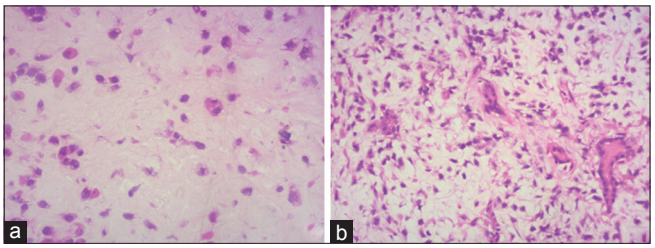

Figure 3: (a) Frozen section microphotograph of a lacrimal gland specimen, depicting plasmacytoid cells in a myxoid stroma suggestive of myoepithelioma. (b) Permanent section of the same specimen after complete excision, depicting nodules of tumor composed of myxoid and epithelial component suggestive of pleomorphic adenoma

Discussion

In our study, frozen section was 87.2% sensitive and 87.5% specific with regard to the presence or absence of malignant cells. The sensitivity for providing the margin clearance accurately was 100% while the specificity for the same was 93.1%. BCC was the most accurately diagnosed malignancy on frozen section with a sensitivity and specificity of 100%.

Frozen section examination in ophthalmic pathology is challenging because the specimens are very small in size and

prone to crush artefacts if handled roughly.[1] It incurs an additional cost for the patient. Multiple studies have been done to evaluate the correlation and accuracy of frozen section and permanent section diagnosis in various organ systems. But to the best of our knowledge, there have been no studies to evaluate the correlation between frozen and permanent section diagnoses in ocular and adnexal diseases. This may partly be due to the expertise involved in handling ophthalmic specimens which measure in millimetres or it may be due to lack of specialities in ophthalmic pathology which makes interpretation difficult.

Frozen section has limitation in diagnosing orbital tumors accurately and they should be used with caution because it is difficult to gauge the extent of involvement as well as orientation of tissues for histopathological examination.[1]

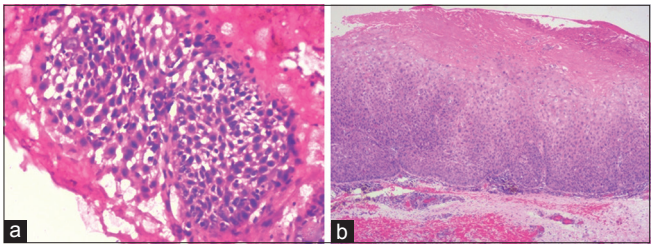

Figure 4: (a) Frozen section of a conjunctival specimen showing dysplastic cells without any evidence of stromal invasion or breach in the basement membrane. A diagnosis of conjunctival dysplasia was made. (b) Permanent section microphotograph of the same specimen showing stromal invasion and breach in the basement membrane suggestive of SCC

Certain tumors like pleomorphic adenoma of lacrimal gland may contain areas which are not representative of the whole lesion. There can be adenoid and adenoid cystic areas in cases of pleomorphic adenoma which can be misleading on frozen section.[21]

Korchak *et al.* described a child with proptosis where frozen section showed features suggestive of rhabdomyosarcoma, whereas in permanent section, it turned out to be a pleomorphic adenoma.[22] We too have a discordant case where pleomorphic adenoma of lacrimal gland was misinterpreted as adenoid cystic carcinoma on frozen section. Chronic inflammation of lacrimal gland with extensive fibrosis may mimic an

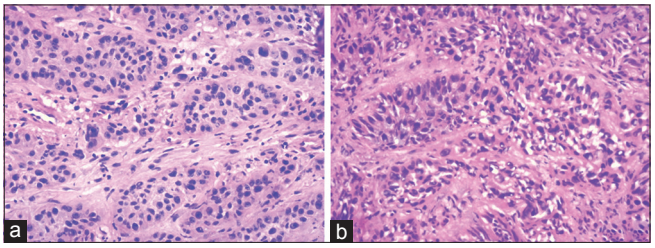

Figure 5: (a) Frozen section microphotograph of an eyelid specimen showing malignant tumor lobules. Tumor cells were lacking in sebaceous differentiation and hence a diagnosis of eyelid SCC was considered. (b) Permanent section microphotograph of the same eyelid specimen showing malignant tumor lobules lacking a clear cut sebaceous differentiation but were positive for oil red O. A diagnosis of poorly differentiated SGC was considered. The misdiagnosis was due to interpretation error because of the tumor being poorly differentiated

Table 1: Table showing different diagnosis by Frozen and Permanent Section

Category	Subtypes	Number of cases	
		Frozen	Permanent
Benign	Keratoacanthoma	1	2
	Myoepithelioma of lacrimal gland	1	0
	Pleomorphic Adenoma Lacrimal gland	0	2
	Capillary hemangioma	1	1
	Dysplasia	3	3
Inflammation	Chronic inflammation		
	Sjogren Syndrome	1	1
	Ocular Cicatricial Pemphigoid	1	1
	Granulomatous	2	3
	Others (Pyogenic granuloma, fibrosis etc)	6	4
Malignancy	SCC	16	15
	SGC	10	11
	BCC	6	6
	Conjunctival Malignant Melanoma	1	2
	Lacrimal Gland Adenocarcinoma	1	1
	Lacrimal Gland Adenocystic Ca	1	0
	Mucoepidermoid Ca of Lacrimal sac	0	1
	Adnexal Tumor	0	1
	Round Cell Tumor	0	1
Deferred	Pleomorphic cells	2	0
	Malignant cells	1	0
	Tumor cells	1	0
Total		55	55

Table 2: Discordant cases between frozen and permanent section

Frozen section diagnosis	Permanent section diagnosis	Cause for discrepancy	Number of cases
Adenoid cystic carcinoma of lacrimal gland	Pleomorphic adenoma	Sampling error	1
Dysplasia	SCC conjunctiva	Sampling error	2
SCC conjunctiva	Dysplasia	Misinterpretation	1
Myoepithelioma of lacrimal gland	Pleomorphic adenoma	Sampling error	1
Inflammation	Keratoacanthoma	Sampling error	1
SCC Eyelid	SGC Eyelid	Misinterpration because of the tumor being poorly differentiated and lacking clear cut sebaceous areas	1

Table 3: Sensitivity and Specificity of Frozen section diagnosis

Category	Sensitivity (95% CI)	Specificity (95% CI)	Kappa value (95% CI)	Misclassification rate	Fischer Exact *P*
Malignancy	87.2%	87.5%	0.70	0.12	0.00
Margin clearance	100%	93.1%	0.89	0.04	0.00
BCC	100%	100%	1	0.00	0.00
SGC	83.3%	100%	0.88	0.03	0.00
SCC	87.50%	94.9%	0.82	0.07	0.00

infiltrative carcinoma of the lacrimal gland on frozen section examination.[1] Melanomas are prone to be misdiagnosed on frozen section.[23] SGC may mimic squamous cell, basal cell, metastatic tumors, melanoma or chalazion on frozen section.[1-3]

The diagnostic accuracy for frozen section is quite high and many studies have shown them to be between 95% and 99%.[5,14-17] In a study by da Silva *et al.*, frozen sections taken from skin were found to be associated with more discordant cases (86.67%) when compared with other organs.[16]

Though many studies have shown that the maximum discordancy is due to false negative results, Onanjin *et al.* reported 17 cases out of 300 as false positive for malignancy in frozen section.[9,10,11,17] We had three false negative and two false positive cases for malignancy. A case of pleomorphic adenoma of the lacrimal gland in our study was misinterpreted as adenoid cystic carcinoma while two cases of SCC of the conjunctiva were labelled as dysplasia on frozen section. This could be because of the sampling and technical difficulties to identify the minimum breach in basement membrane. One case of conjunctival melanoma just showed pleomorphic cells on frozen section and the diagnosis was deferred. Hwang *et al.* in their evaluation of frozen section biopsy in 4434 cases found a higher false negative rate of 1.47% as compared to false positive (0.54%).[24]

Ahmad *et al.* in their study to evaluate the correlation between frozen and permanent section diagnosis found that half of the discordant diagnosis was false positive while remaining half was false negative, with 40% of the discordant cases seen in ovarian tumors.[14] Margin status assessment was discordant in 40% evaluations in melanocytic lesions, where false positive was seen in 6.8%, and false negative in 32.6%.[25] The accuracy of frozen section for margin clearance in skin cancers was found to be about 86%.[26] The concordance rates between frozen and permanent section for margin clearance for malignancy have been generally high ranging between 96% and 99% in various studies,[27-32] which is similar to our results for margin clearance. Mainstein *et al.* suggest that frozen section is not required if tumor is small clinically and can be closed primarily and is performed only for recurrent tumors. In such cases, frozen section was used where a small flap or skin graft was needed for reconstruction to minimize the size of the resected tissue. For cases requiring major reconstruction they preferred to wait for the permanent section reports without doing a frozen section.[25]

Onajin *et al.*[17] in their study in non-melanoma skin cancers found the highest concordance between frozen and permanent section diagnoses in BCC (95%) and the lowest in SCC (23%) which is in agreement to the results of our study where the sensitivity and specificity for diagnosing BCC was maximum.

The deferred rate for diagnosis on frozen section has been reported in a range of 0.04%-6.7%.[5] The deferral rate in our study was slightly higher and was found to be 7.2%.

Frozen section samples are always inferior as compared to the paraffin-based permanent section specimens. The pathologist should be experienced enough to know what to expect and what to look for. The most common causes for discrepancy in the diagnosis between frozen and permanent section are sampling mistake by the surgeon, technical inaccuracies during cutting of the sections, poor staining quality and inexperienced pathologist.[33,34] In our study, sampling error was responsible for discrepancy in the diagnosis in 71.4% cases, while misinterpretation was the cause for 28.5% cases.

Conclusion

Frozen section has high sensitivity and specificity for all three parameters of tumor diagnosis, that is, whether a lesion is malignant or non-malignant, surgical margin clearance and definitive tumor diagnosis. Lacrimal gland lesions, SCC, SGC and melanoma are liable to be misdiagnosed on frozen section.

It should only be used as an intraoperative tool to guide the surgeon. Critical decision making and radical surgeries like exenteration are best avoided based on frozen section diagnosis.

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Conflicts of interest

There are no conflicts of interest.

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