Optic Nerve Tissue Shrinkage During Pathologic Processing After Enucleation for Retinoblastoma

David H. Abramson, MD; Amy C. Schefler, BA; Dena Almeida, BS; Robert Folberg, MD

Objectives: To quantify and analyze the differences between the length of the optic nerve as measured by the ophthalmologist in the operating room after enucleation and the length as measured by the pathologist after fixation.

Methods: The authors performed a retrospective review of patients who underwent either primary or secondary enucleation for retinoblastoma at the Ophthalmic Oncology Center of the New York-Presbyterian Hospital–Cornell campus between November 1979 and August 2001. Intraoperative notes and pathologic reports were reviewed to determine the length of the resected optic nerve as recorded by both the surgeon and pathologist.

Results: Sufficient data for inclusion in the study were available from 100 enucleation specimens belonging to 96 patients. A significant degree of shrinkage of the optic nerve occurred after fixation, with a mean shrinkage of 30.3% from the time of enucleation to the time of measurement by the pathologist. Age at enucleation affected the degree of optic nerve shrinkage; nerves from younger children underwent more shrinkage than nerves from older patients. Sex of the patient and the laterality of disease did not significantly affect optic nerve shrinkage.

Conclusions: A significant degree of shrinkage of the optic nerve occurs in retinoblastoma enucleation specimens after fixation prior to pathologic analysis. This finding must be taken into account when comparing different series and making recommendations for chemoprophylaxis based solely on histopathologic examination.

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SHRINKAGE of tissue in the pathology laboratory before and during fixation and processing is a widely recognized phenomenon. Surgical specimens shrink when exposed to air, and histologic preparations such as formalin fixation, alcohol, and paraffin embedding can cause tissue to retract, resulting in differences between in vivo and excised fixed-tissue dimensions. Although retraction has been observed in previous studies in many different tissue types,1-16 to our knowledge, no previous report has assessed optic nerve shrinkage. Because of scanning electron microscopy of several tissue types, this effect has been attributed to the miniaturization of organelles.17 Tumor clearance margins assessed from fixed tissue can be misleading, particularly if there are differences in the responses of normal and tumor tissues to fixation.18 Such differences as well as increased shrinkage rates in specimens from younger patients have been reported.18,19

Although shrinkage of surgical specimens before and during fixation has been widely reported, shrinkage of optic nerve tissue has never been previously documented. This study was undertaken to determine whether shrinkage of optic nerve tissue occurs in enucleation specimens in a similar manner as it does in other tissue types. The study was also designed to assess whether the degree of shrinkage of the optic nerve has changed with time as surgical approaches to enucleation have improved.

METHODS

We performed a retrospective analysis of all patients with retinoblastoma who underwent enucleation of one or both eyes at the Robert M. Ellsworth Ophthalmic Oncology Center of New York-Presbyterian Hospital–Weill Medical College of Cornell University (New York, NY) between November 1979 and August 2001. Patients whose enucleations were done at other institutions were not included in the analysis, even if pathologic reports or intraoperative dictation were provided. Only reports with exact optic nerve length measurements were included.

In the operating room at the time of enucleation, all measurements were obtained using calipers with the globe placed on a flat surface; no tension was placed on the nerve. All enucleations and specimen measurements were performed by the same surgeon.

For patients with sufficient pathologic data, clinical data on sex, age at enucleation, and laterality were also collected. The statistical significance of the difference between pairs of optic nerve length measurements was determined using the Wilcoxon matched-pairs signed rank test.
Multivariate Cox regression analysis was performed to estimate the effect of the 3 previously mentioned variables on the percentage of optic nerve shrinkage. Statistical analyses were performed using SPSS 8.0 statistical software for Windows (SPSS Inc, Chicago, Ill).

**RESULTS**

We reviewed the medical records of 358 patients who had undergone enucleation between November 1979 and August 2001. For 100 eyes belonging to 96 patients, there were sufficient data for analysis. Patient characteristics are summarized in the Table. The mean length of the optic nerve as measured by the ophthalmologist in the operating room immediately after enucleation was 13.2 mm, whereas the mean length as measured by the pathologist after processing was 9.2 mm. This difference represented a mean shrinkage of 30.3%. These results are statistically significant based on a Wilcoxon matched-pairs signed rank test (z = −7.87; P < .001).

In multivariate Cox regression analysis, patient sex and laterality of enucleation were not significantly associated with the percentage of optic nerve shrinkage. In contrast, age at enucleation was significantly associated with the degree of shrinkage (P = .05). The association was negative: younger patients who underwent enucleation had more shrinkage of the optic nerve than older patients.

Finally, our analysis demonstrated that the mean length of the optic nerve in retinoblastoma enucleation specimens, as measured by the ophthalmologist immediately after resection, has increased during the past 22 years at our institution (Figure). The degree of shrinkage, however, has remained constant across time.

**COMMENT**

This retrospective review included 100 eyes that underwent enucleation as either primary or secondary treatment during the last 22 years at our center. It represents patients operated on by the same surgeon using a standardized approach at 1 institution.

The review demonstrates that the optic nerve in retinoblastoma enucleation specimens undergoes a significant degree of shrinkage after fixation. A mean shrinkage of 30.3% occurred among all specimens. It also indicates that optic nerve specimens from younger children shrink more after fixation than those of older children. These findings are consistent with several previous studies conducted with cutaneous melanoma specimens.18,19

Although tissue shrinkage has previously been reported in neoplastic and nonneoplastic ophthalmic specimens such as uveal melanomas14 and the corneal endothelium,15,16 it has never been studied in the optic nerve. Furthermore, tissue shrinkage effects have been studied in specimens of many human and animal tissue types including the arteries,1,2 pericardium,3 prostate,4 colon and rectum,5,6 neural tissue,7,8 head and neck tissue,9 esophagus,10 smooth muscle,11 and benign and malignant skin lesions.2,13,19 The degree of shrinkage varies widely with tissue type and fixation. The fixative used in this study was 10% buffered neutral formalin, which has reportedly caused up to 50% shrinkage in animal tissue specimens after dehydration in alcohol.20 Several authors report even more shrinkage during exposure to room air immediately after resection than during tissue processing. In one study of colorectal resection specimens, approximately 70% of shrinkage occurred during the first 10 to 20 minutes after removal; the remaining 30% occurred after fixation.5

Many authors have presented methods for counteracting tissue shrinkage effects or calculating volume loss. Two studies, 1 with specimens of bovine pericardium and 1 with rectal sections, reported decreased tissue shrinkage when the specimen was tethered in its original posi-
tion immediately after resection. Some authors report that apparent substantial shrinkage that occurs during tissue processing is largely compensated for by expansion during tissue sectioning and mounting. Other studies have explored methods to reduce artifacts during mounting so that only isometric shrinkage occurs, which can easily be corrected by rescaling the image dimensions. Several articles have explored methods of 3-dimensional reconstruction of tissue images to correct for shrinkage effects. One group has presented a formula that extrapolates in vivo surgical margins from the contracted fixed tissue for cutaneous melanoma specimens. None of these methods have yet been attempted with ophthalmic specimens.

There are many possible factors related to the method of histologic preparation and analysis that we were not able to take into account in our study, which could have affected the degree of observed optic nerve shrinkage. These factors include duration of fixation and duration of specimen exposure to room air. Lack of standardization of these factors may have resulted in an inconsistent degree of optic nerve shrinkage among the patients in our study. Likewise, the lack of standardization of these factors in previous studies may have resulted in a similarly variable effect on the specimens, both within individual studies and when comparing different studies in the past. It is standard teaching in ophthalmic oncology to attempt to get the longest possible stump of optic nerve at the time of enucleation because survival is poor when tumor is present at the cut section of the optic nerve. Many studies have tried to determine the most effective surgical technique to accomplish this goal. For example, Coats et al performed 200 mock enucleations in a human child skull model with both nasal and temporal approaches and varying instruments to determine which technique yielded the longest optic nerve stump. However, our findings in this article dictate that future studies designed to assess the efficacy of various enucleation techniques must have standardized methods of preparing the specimen and a standardized surgical technique. This type of standardization will ensure that the effect of optic nerve shrinkage is consistent for all specimens in the study.

Our findings are consistent with previous studies, indicating that shrinkage can occur in optic nerve specimens in a similar manner to other tissue types. Furthermore, the degree of shrinkage remained consistent across time, even as our enucleation technique improved. However, we were unable to confirm whether the shrinkage of benign optic nerve tissue after fixation and exposure to room air was more pronounced than that of the malignant retinoblastoma tissue. Evidence suggests that such differential shrinkage occurs in skin excision specimens. Future studies should focus on clarifying whether differential shrinkage in benign and malignant tissue occurs in the optic nerve. If this phenomenon does occur in the optic nerve, it could result in a misperception of the presence of tumor at the cut section when there is initially a long stump of tumor-free nerve. In this case, a patient who appears to be at high risk for metastatic retinoblastoma may in fact be at low risk, a scenario with clinical implications for the use of adjuvant therapy. Such misperception of the degree of tumor invasion would also prohibit comparisons among different studies that correlate the degree of tumor invasion with survival.

Several institutions have proposed an international, multi-institutional prospective trial to resolve the current debate about the histopathologic indications for chemoprophylaxis in retinoblastoma. In this kind of study, factors such as fixative type, duration of fixation, and duration of exposure to room air need to be reported and standardized. Such standardization will ensure that the effect of optic nerve tissue shrinkage on the presumed degree of tumor invasion is consistent for all patients.

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Corresponding author and reprints: David H. Abramson, MD, 70 E 66th St, New York, NY 10021 (e-mail: DHAMD@aol.com).

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