Objective: To compare the prevalence of nondiagnostic conjunctival map biopsies in patients with extensive pagetoid sebaceous carcinoma (defined as involvement of 3 or 4 quadrants of the ocular surface) and in patients without extensive pagetoid tumor (defined as involvement of 1 or 2 quadrants of the ocular surface).

Methods: Retrospective medical record and pathologic specimen review of 20 patients treated for sebaceous carcinoma at a tertiary care center. Biopsies with artifactual loss of or damage to the epithelium were categorized as nondiagnostic.

Results: One hundred forty-four map biopsies were reviewed, an average of 7.2 (standard deviation [SD], 4.4) biopsies per patient. Sixteen patients had extensive pagetoid tumor, 4 did not. Fifteen percent of biopsies were nondiagnostic. The frequency of nondiagnostic biopsies in patients with and without extensive pagetoid tumor was 37% and 10%, respectively. The odds ratio of nondiagnostic biopsy in the setting of extensive pagetoid tumor was 5.9 (95% confidence interval, 2.3-15.6; \( P = .004 \)). Six of the sixteen patients (38%) without extensive pagetoid tumor had at least 1 nondiagnostic biopsy, with an average of 1.8 (SD, 1.6) nondiagnostic biopsies per patient (22% of biopsies). Two of the 4 patients (50%) with extensive pagetoid tumor had at least 1 nondiagnostic biopsy, with an average of 5.5 (SD, 3.5) nondiagnostic biopsies per patient (57% of biopsies).

Conclusions: Nondiagnostic, de-epithelialized conjunctival map biopsies are more common in patients with extensive pagetoid tumor than in those with limited or no pagetoid tumor. Artifactual epithelial loss may result from disruption of epithelial adhesion to the basement membrane by infiltrating tumor cells.


SEBACEOUS CARCINOMA ACCOUNTS for 1% to 5% of eyelid malignancies.1 Intraepithelial, or pagetoid, tumor—defined as invasion of the ocular epithelial surface by tumor cells—is estimated to occur in 40% to 80% of cases2,3 and presents unique diagnostic and therapeutic challenges. Because the standard treatment for sebaceous carcinoma is surgical excision,1 extensive pagetoid tumor often requires orbital exenteration.

Accurate staging is essential to determine the extent of resection required to adequately treat pagetoid sebaceous carcinoma, which may span considerable distances along the ocular surface. Staging is based on slitlamp biomicroscopic examination and conjunctival map biopsies. However, some biopsies are found not to contain an epithelium when examined histopathologically because of artifactual epithelial cell loss during specimen acquisition or processing. Biopsy specimens lacking an epithelium cannot be used to determine the presence or absence of pagetoid tumor at their respective harvesting locations.

We hypothesized that intraepithelial tumor results in a greater propensity for artifactual epithelial loss due to disruption of normal epithelial adhesion to the basement membrane by invading poorly cohesive tumor cells. The purpose of this study was to determine the frequency of de-epithelialized, nondiagnostic conjunctival map biopsies in patients with pagetoid sebaceous carcinoma and to test whether the frequency of these biopsies correlates with disease severity.

METHODS

The clinical records and biopsy specimens of 20 patients referred to the University of Michigan for treatment of periocular sebaceous carcinoma between 1991 and 2007 were reviewed. The study was approved by the institutional review board at the University of Michigan. Conjunctival map biopsies were performed in all cases to assess the extent of pagetoid tu-
mor. At least 4 sections along the long axis of each map biopsy specimen were reviewed. If there was absence of the epithelium or if artifacts rendered the epithelium inadequate for diagnosis, the biopsy was categorized as nondiagnostic. If an epithelium adequate for diagnosis was present, the biopsy was categorized as diagnostic. All statistical analyses were performed using GraphPad (GraphPad software, San Diego, California; http://www.graphpad.com/quickcalcs/contingency1.cfm). Data are presented as mean (standard deviation).

RESULTS

The patient population was composed of 8 men and 12 women aged a mean of 69 years (18 years). The mean duration of symptoms was 17 months (21 months). The follow-up period was 26 months (19 months). There were no deaths. Four patients had extensive pagetoid tumor, defined as involvement of 3 or 4 quadrants of the ocular surface. These patients were treated with orbital exenteration.

A total of 144 map biopsies were reviewed; on average, 7.2 biopsies (4.4 biopsies) were sampled from each patient. The histopathologic analysis revealed variable specimen quality (Figure). In diagnostic biopsies, the conjunctival epithelium was well preserved and a diagnosis could be made regarding the presence or absence of pagetoid disease (Figure, A-D). Nondiagnostic biopsies had artificial loss of or damage to the epithelium, particularly crush artifact, that affected the entire specimen (Figure, E-F).

Fifteen percent of the map biopsies were nondiagnostic. The overall frequency of nondiagnostic biopsies in patients with and without extensive pagetoid tumor was 37% and 10%, respectively. Categorical data analysis using 2-tailed Fisher exact test revealed that the frequency of nondiagnostic biopsies correlated with the presence of extensive pagetoid tumor (Table) (P = .008). The odds ratio of nondiagnostic biopsy in the setting of extensive pagetoid tumor was 5.9 (95% confidence interval, 2.3-15.6; P = .004). Diagnostic map biopsies averaged 3.1 mm (2.5 mm) in greatest dimension, while nondiagnostic map biopsies averaged 5.0 mm (3.2 mm) in greatest dimension. Thus, the propensity for loss of the epithelium is not attributable to inadequate specimen size.

Six of the 16 patients (38%) without extensive pagetoid tumor had at least 1 nondiagnostic biopsy. For these 6 patients, the average number of nondiagnostic biopsies per patient was 1.8 (1.6) (22% of biopsies). In 1 patient, a repeat biopsy in the vicinity of 2 previously nondiagnostic biopsies was negative. The remaining 5 patients did not undergo repeat biopsy because of complete absence of pagetoid disease in their other map biopsies. None of the 6 patients developed tumor recurrence during follow-ups averaging 29 months (range, 15-47 months). Two of the 4 patients (50%) with extensive pagetoid tumor had nondiagnostic biopsies. The average number of nondiagnostic biopsies for these 2 patients was 5.5 (3.5) per patient (57% of biopsies). In both cases, subsequent reexcision, including exenteration, revealed that the conjunctiva was positive for tumor in the vicinity of the previous nondiagnostic biopsies. The difference in the frequency of nondiagnostic biopsies between patients with

<table>
<thead>
<tr>
<th>Biopsy Quality</th>
<th>Extent of Ocular Surface Tumor Involvement, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 or 2 Quadrants</td>
</tr>
<tr>
<td>Diagnostic</td>
<td>103 (90)</td>
</tr>
<tr>
<td>Nondiagnostic</td>
<td>11 (10)</td>
</tr>
</tbody>
</table>

*Fisher exact test, P = .008.
and without extensive pagetoid disease was statistically significant (2-tailed Fisher exact test, \( P = .004 \)).

**COMMENT**

We found that nondiagnostic, de-epithelialized conjunctival map biopsies are more common in patients with extensive pagetoid tumor than in those with limited or no pagetoid disease. Fortunately, our patients with extensive disease requiring exenteration were recognized as such owing to either tumor invasion into the conjunctival stroma of the de-epithelialized biopsies or extensive pagetoid involvement of the remaining specimens that warranted exenteration.

Artifactual epithelial loss may result from disruption of epithelial adhesion to the basement membrane by infiltrating, poorly cohesive tumor cells (Figure). Because these specimens are more fragile, clinicians and pathologists should take great care during specimen harvesting and processing to avoid de-epithelialization. In the context of known pagetoid spread from sebaceous carcinoma, total sloughing of the conjunctival epithelium is nondiagnostic but should not be interpreted as a negative biopsy. Instead, the surgeon should be aware of the fact that artifactual sloughing of the conjunctival epithelium in the context of pagetoid spread may indicate involvement of the conjunctiva in this area by a tumor. The threshold for repeat biopsy should be low if the original specimen is nondiagnostic, especially because of the easy accessibility of the ocular surface.

Recent advances in periocular reconstructive procedures have prompted study of tissue-sparing techniques, including cryotherapy\(^4\) and use of topical mitomycin C\(^5\) to treat pagetoid disease. Our results have implications for these modes of treatment, because the extent of involvement is more likely to be underestimated in patients with greater extent of disease.

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