The role of mast cells in vascularized recurrent pterygium

O papel dos mastócitos no pterígio recidivado vascularizado

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ABSTRACT

Objective: To determine and compare the mast cell count in primary and recurrent vascularized pterygium, and in normal bulbar conjunctiva.

Methods: The study included 22 patients with primary pterygium (PP group) and 28 patients with vascularized recurrent pterygium (VRP group) that underwent excision via the limbal conjunctival autograft technique. Normal conjunctiva samples were collected from the superotemporal bulbar conjunctival region, just temporal to the site from which the autograft conjunctival tissue was harvested. The total number of mast cells in the pterygium (primary and recurrent) and control tissue samples was calculated microscopically using 1% toluidine blue stain under 400× magnification.

Results: The mean mast cell count in primary and vascularized recurrent pterygium tissue was 7.45 ± 2.06 mm⁻² and 16.11 ± 4.33 mm⁻², respectively, and the difference was significant (independent samples t-test, P<0.001). The mean mast cell count in pterygium tissue was significantly higher than that in normal conjunctiva tissue in both groups (Student’s t-test, P<0.001).

Conclusion: An increase in the number of mast cells might play a role in the pathogenesis of recurrent pterygium. Determination of a mast cell count cut-off value could be of diagnostic significance for recurrent pterygium.

Keywords: Mast cells; Pterygium/diagnostic; Recurrence; Toluidine chloride; Conjunctiva

INTRODUCTION

Pterygium is one of the most common surface ocular lesions. It is a fibrovascular neoformation characterized by a triangular or wing-shaped overgrowth of abnormal conjunctiva onto the cornea, degenerative and hyperplastic changes, proliferative and inflammatory features, and a rich vasculature. Inflammation induces angiogenic pathways, resulting in neovascularization, which contributes to the development and growth of pterygium. Mast cells are involved in chronic inflammation. Mast cells have also been implicated in both normal and pathological angiogenesis, seen in chronic inflammatory diseases and tumors. The number of mast cells is highly correlated with the extent of both normal and pathological angiogenesis. Mast cells release a number of angiogenic factors, of which vascular endothelial growth factor (VEGF) is considered among the most active. The number of mast cells in a pterygium is higher than that in normal conjunctiva. Ribatti et al. reported that mast cells are involved in the angiogenesis of pterygium, and may play a role in its pathogenesis. Another highly active angiogenic factor released by mast cells is tryptase, a serine protease stored in mast cells. There is an increase in the number of mast cells containing FGF-2 and other angiogenic factors in their secretory granules in pterygium.

The pathogenesis of pterygium is not yet fully known, nor are the differences between primary pterygium and recurrent pterygium. A few studies that differentiated primary and recurrent pterygium reported that the levels of VEGF, basic fibroblast growth factor, and substance P were significantly higher in recurrent disease. Markers for vascular endothelial cells such as CD31+ are increased in pterygium, which supports angiogenesis. A higher number of CD34 cells - a mast cell marker - in recurrent versus primary pterygium has also been reported. The present study aimed to determine and compare the mast cell counts in primary and recurrent vascularized pterygia and in normal bulbar conjunctiva.

METHODS

PATIENTS AND STUDY DESIGN

This prospective case-control study included 22 patients with primary pterygium (PP group) and 28 patients with vascularized recurrent pterygium (VRP group). All patients underwent excision via the limbal conjunctival autograft technique. Normal conjunctival samples were collected from the superotemporal bulbar conjunctival region, just temporal to the site from which the autograft conjunc-
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Med consent was obtained from all the patients.

Punctiva samples in both groups (Student’s t-test, pterygium samples was significantly higher than in the normal conditions, as well as in a series of fundamental functions, including angiogenesis. Mast cells synthesize and release many angiogenic growth factors that are necessary for new vessel development[15]. Ribatti et al.16 reported that mast cells are involved in the angiogenesis of pterygium, which could be one of the reasons for their development[16]. Aspiotis et al. noted that the density of microvessels in pterygium was significantly higher than in normal conjunctiva, and that the overexpression of VEGF was among the most potent angiogenic factors[17]. The number of mast cells in pterygium is higher than that in normal conjunctiva[16]. It was also reported that angiogenesis - measured according to microvessel density - is strongly correlated with the tryptase-positive mast cell count in pterygium tissues[17].

The literature includes few studies on the differences in the pathophysiology of primary and recurrent pterygia. The number of mast cells and microvessels increased in the formation of pterygium. It has been reported that VEGF and neo-vessels play an important role in the pathogenesis of pterygium[15,16], as do mast cells[15,16]; however, to the best of our knowledge, the present study is the first to compare the mast cell count in primary pterygium and vascular recurrent pterygium. In the present study, the mast cell count was two-fold higher in vascularized recurrent pterygium and even in the normal conjunctival regions of the recurrent vascularized pterygium tissues. Moreover, Liang et al.18 reported that there was a strong correlation between the mast cell and microvessel counts, indicating that mast cells promoted neovascularization in pterygia. They also reported that the number of mast cells was correlated with capillary density.

Regarding inflammation, the mast cell count differed significantly between pterygium tissue and normal conjunctiva as was also shown in the current study. Butrus et al. reported that the mast cell count was two-fold higher in pterygium tissue than in normal conjunctiva tissue in age-matched controls[19]. Nakagami et al. also reported a two-fold increase in the mast cell count in pterygium tissue, as compared to normal conjunctiva tissue. Toluidine blue staining has shown that the mean number of mast cells in pterygium tissue specimens was twice that in normal conjunctiva tissue specimens[20]. Zhong et al. compared the mast cell count in 17 primary pterygia, 6 recurrent pterygia, and 6 normal conjunctiva specimens, and reported that the mast cell count was 45.47 mm–2 in the primary pterygium samples and 48.83 mm–2 in the recurrent pterygium samples; both of these findings were significantly higher than in the normal conjunctiva samples[17]. Ermis et al.18 also observed that the mast cell count was higher in pterygium tissue. In all their patients with pterygium in one eye, the mast cell count was higher in pterygium tissue than in the normal conjunctiva of the unaffected eye.

In agreement with previously reported studies, the mast cell count was two-fold higher in primary pterygium tissue than in normal conjunctiva tissue in the present study; however, the mast cell count in the vascularized recurrent pterygium was not two-fold higher than in normal conjunctiva tissue. Nonetheless, the mast cell counts in the recurrent vascularized pterygium tissue and in normal conjunctival tissue were much higher than in the primary pterygium tissue. We think that perhaps a high mast cell count, which is an indicator of increased inflammation, in the overall bulbar conjunctiva tissue - including the normal section - could be the cause of recurrence in patients with vascularized pterygium. It is difficult to determine a definitive mast cell count cut-off value that would be predictive of recurrence of pterygium tissue based on the present findings, but <10 mast cells mm–2 in the excised pterygium tissue could be a predictor of recurrence.

Ratnakar et al.19 examined 30 pterygium specimens microscopically and classified the lesions as angiomaticous, fibrous, and mixed, based on the vascular and collagenous components. They determined that the mast cell count in each type of lesion was strongly correlated with morphological type, as compared to normal conjunctiva. The mast cell count in angiomatous and fibrous types was 15.1 ± 3.1 mm–2 and 9.5 ± 3.2 mm–2, respectively. The researchers posited that an elevated mast cell count could be a predictor for the

**Table. Mean ± SD mast cell counts, according to group and tissue sample**

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal conjunctiva</th>
<th>Pterygium</th>
<th>n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP</td>
<td>3.23 ± 1.54</td>
<td>7.45 ± 2.06</td>
<td>22</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>VRP</td>
<td>10.57 ± 3.16</td>
<td>16.11 ± 4.33</td>
<td>28</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>PP vs. VRP</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.005**</td>
</tr>
</tbody>
</table>

PP= primary pterygium; VRP= vascular recurrent pterygium; *= paired t-test; **= independent t-test.
recurrence of pterygium. In the present study, there was a significant difference in the mast cell count between primary and recurrent vascularized pterygium tissue samples.

Mast cell mediators are involved in numerous aspects of inflammation, including remodeling, tissue repair, and fibrosis. These functions are accomplished via mast cells' direct stimulation of specific connective tissue cell types - in particular, fibroblasts - and via the release or activation of a series of extracellular matrix-degrading enzymes. It has been reported that there is an increase in the proliferation of the fibrovascular layer in recurrent pterygium and the association with an increase in the fibrovascular layer in recurrent pterygium could be due to an increase in the mast cell count. This was also shown in the present study.

A potential limitation of the present study was that vascularized recurrent pterygium tissues were used for enumerating the mast cell count. All the included patients that had vascularized recurrent pterygium had ocular discomfort and were eager to undergo a second surgery, whereas those that had recurrent pterygium with a prominent fibrous component were not eager to have a second surgery. Subsequently, these types of recurrent pterygium tissues were not encountered in this study. Most likely the ocular discomfort in the study patients was a result of an increase in inflammation and the number of mast cells in inflamed areas. Additional studies on recurrent pterygium tissue with a prominent fibrous component that investigated the difference in the mast cell count between the different types of pterygium tissue will be needed to more clearly determine the role of an elevated mast cell count in the pathogenesis of the recurrence of all types of pterygium.

**CONCLUSIONS**

The literature includes only a few studies on the differences between the pathophysiology of primary and recurrent pterygium. Mast cell involvement in the pathogenesis of pterygium has been reported. The present findings show that an increased in the number of mast cells can play a role in the pathogenesis of the recurrence in pterygium. A mast cell count cut-off value in the excised pterygium could be used to screen patients at risk of recurrence. Additionally, new drugs that inhibit mast cell degranulation or currently-used mast cell stabilizers after pterygium excision might yield new methods for the prevention of recurrence of pterygium; this will require additional research.

**REFERENCES**