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# Assessment of Subconjunctival Bevacizumab Injection in Patients with Pinguecula: A Randomized Clinical Study

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## Abstract

**Background:** The purpose of current study was to evaluate the efficacy of subconjunctival bevacizumab (Avastin) injection in reducing pinguecula recurrence following ligation treatment.

**Methods:** This prospective, randomized clinical study was conducted on 20 patients with pinguecula who presented to the ophthalmology department of BU-Ali Sina Hospital, Sari, Iran from September to December 2019. The patients were randomly assigned to two groups including 10 patients with subconjunctival injection of 2.5 *mg*/0.1 *ml* of bevacizumab (Avastin) injection and 10 patients in the control group with any injection. All the patients followed 1 week, 1 month, 3 months, and 6 months after surgery and evaluated through the horizontal size, photophobia, inflammation, and recurrence of pterygium. The variables were assessed using independent t-tests and repeated-measures analysis of variance.

**Results:** Of the 20 patients who participated in the trial, the majority were male (80%). The mean age was 45.1±12.58 and 44.5±10.88 years in the intervention and control groups, respectively (p=0.621). A significant reduction in pterygium size was observed in the intervention group up to 6 months post-operatively (all p=0.037). After surgery, photophobia was significantly reduced in the Avastin injected eyes compared to the control group at 1 (p=0.038) and 4 weeks (p=0.002). Inflammation was also significantly reduced in the intervention group at one and three months post-operatively (both p=0.019). There was only one pinguecula recurrence in the control group within one month of surgery compared to none in the intervention group.

**Conclusion:** Subconjunctival bevacizumab injection shows promise in reducing pinguecula recurrence post-ligation treatment, leading to decreased pterygium size, photophobia, and inflammation. Further studies are required to establish its efficacy and safety as an adjunctive therapy for pinguecula management.

**Keywords:** Bevacizumab (avastin), Inflammation, Pinguecula



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## Introduction

Pinguecula is one of the most common degenerative diseases of the conjunctiva, characterized by the presence of yellowish fat nodules near the limbus (1). Although typically asymptomatic, it results in an unsightly appearance that most researchers believe contributes to pterygium (2). Pterygium is an ocular surface disease that is identified mainly by a triangular-shaped elevated mass extending from the thickened bulbar conjunctiva onto the cornea (3) within the prevalence range of more than 30% in the world (4,5). The critical factor in the development of pterygium is limbal reorganization by the formation of pterygium cells, rather than simple limbal failure. This reorganization is thought to be related to UV-induced damage or genetic susceptibility (6). Environmental irritants such as dust and wind (7), viral agents (8), and inflammatory factors (9) are some other risk factors associated with pterygium. Although often asymptomatic, pterygium can cause photophobia, tearing, or progression towards the visual axis, causing astigmatism and reducing vision. Symptoms include foreign body sensation, irritation, itching, blurred vision, and, as the condition progresses, reduced visual field (10). Thus, early detection and treatment of pinguecula can be crucial in preventing potential complications and maintaining ocular health.

Vascular Endothelial Growth Factor (VEGF) plays a pivotal role in neovascularization and includes subgroups such as VEGF-A, VEGF-B, VEGF-C, and VEGF-D. Elevated VEGF levels are associated with diseases characterized by abnormal blood vessel growth in the eye, such as diabetic retinopathy (11). Several drugs have been developed to counteract the effects of VEGF (12). Among these is bevacizumab (Avastin), an antibody that targets all forms of VEGF-A. Multiple studies have demonstrated the efficacy of bevacizumab in suppressing undesired blood vessel formation in ophthalmology (13-15). Due to the high recurrence rate of surgery and the ocular discomfort that most patients experience after pterygium surgery, it is important to find a method that has the lowest recurrence rate (16). Therefore, this study was designed to evaluate the efficacy of subconjunctival bevacizumab injection in pinguecula as a new method with the lowest recurrence rate after pterygium surgery.

## Materials and Methods

This prospective, randomized clinical study was conducted on patients with pinguecula who presented to the ophthalmology department of BU-Ali Sina Hospital, Sari, Iran between September and December 2019. Based on the eligibility criteria, 20 candidates for pinguecula surgery completed the study and were allocated to 2 intervention arms and followed up to 6 months after surgery. The target sample size of 10 participants per arm was calculated based on 80% power at a 2-tailed significance level of 0.05. All the patients underwent the routine ophthalmic examination including measurement of visual acuity, manifest refraction, keratometry, intraocular pressure, and slit-lamp biomicroscopy examination. Pinguecula was assessed for recurrence, photophobia, and inflammation. Recurrence was evaluated during follow-up examinations and defined as the appearance of new or regrown lesions at the original site, based on measurements of the horizontal size of the pterygium using a slit-lamp biomicroscope equipped with a millimeter graticule.

The grading system included four categories: Grade 1 (<1.5 *mm*), Grade 2 (1.5–3.0 *mm*), Grade 3 (3.0–4.5 *mm*), and Grade 4 (>4.5 *mm*). Photophobia was assessed through patient self-report using a validated Visual Analog Scale (VAS) ranging from 0 (no discomfort) to 10 (severe discomfort) (17). Inflammation was evaluated using slit-lamp biomicroscopy, grading conjunctival redness and swelling on a standardized scale (18).

The study included patients aged 30–70 years who presented with an extraocular nasal appendage and either recurrent inflammation or a self-reported cosmetic concern. Patients with a minimum pinguecula size of 1.5 *mm* were eligible. Patients with a history of ocular surgery, trauma, ocular disease, dry eye, ocular allergy, and pregnancy were excluded. This prospective double-masked clinical trial adhered to the CONSORT statement (CONSORT 2010 Checklist), and it was approved by the institutional review Committee of Mazandaran University of Medical Sciences (Code: IR.MAZUMS.REC.1399.719). The study adhered to the tenets of the Declaration of Helsinki and informed consent was obtained from all the patients prior to surgery and was registered in the Iranian Clinical Trials Registry

(http://www.irct.ir) on 23 July 2024 under registration number IRCT20240702062306N1.

### Randomization

All the patients were randomly allocated to the two intervention arms by an independent investigator (M.S.) using a randomizer list. A 1:1 allocation ratio was used to randomize participants to 1 of the 2 arms. Eligible patients were enrolled sequentially by a blinded research optometrist. Both examiners and the data analyst remained masked to treatment allocation throughout the study.

Patients in the intervention group received subconjunctival injection of bevacizumab (Avastin®, Genentech, San Francisco, CA, USA) during surgery and 1 week after surgery, while patients in the control group received no Avastin injection. Most patients in both groups had comparable daily exposure to light and dust, averaging about 5 *hr*/day.

### Surgical method

All the procedures were performed by the same surgeon (R.J). After local anesthesia, the base of the pinguecula was encircled with a 10-0 nylon suture which was tightened until blanching of the lesion indicated adequate occlusion of feeding vessels. The ligated tissue became ischemic and necrotic and typically sloughs off spontaneously; no excision was performed (17). A subconjunctival injection of 2.5 *mg*/0.1 *ml* of bevacizumab (Avastin) was given around the remaining blebs (19,20). The repeat injection after one week was intended to maintain suppression of VEGF during the early stages of wound healing, when angiogenic activity may resume. After the procedure, all the patients received preservative-free lubricating drops, and no topical corticosteroids or additional anti-angiogenic drops were administered in either group. All the patients were followed up at 1 week, 1 month, 3 months, and 6 months, postoperatively.

### Statistical analysis

All the data were analyzed using SPSS version 24 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as means±standard deviations (SDs), and for categorical data, Chi-square test was used. The independent samples t-test was used to compare the mean horizontal size of the pterygium between the two groups. Repeated measures analysis of variance (ANOVA) followed by Bonferroni post hoc tests was employed to assess within-group changes in the indices. For non-normally distributed data, including photophobia and inflammation, non-parametric tests (Mann–Whitney U and Wilcoxon signed-rank tests) were applied, and the results were reported as mean rank, median, and interquartile range (IQR). A $p<0.05$ was considered statistically significant.

## Results

All 20 screened participants (20 eyes) met the eligibility criteria and completed the trial. In the intervention group, 6 participants (60%) were female, compared with 8 participants (80%) in the control group (p=0.314). Participant characteristics are summarized in table 1.

In both groups, the horizontal surface area of pinguecula was most frequently classified as Grade 2 or Grade 3 (40%), preoperatively. The mean horizontal

**Table 1.** Summary of baseline characteristics of participants

| | Intervention (10 Eyes) | Control (10 Eyes) | p-value |
|---|---|---|---|
| | Mean±SD (Range) | Mean±SD (Range) | |
| Age | 45.10±12.582 31-67 | 44.50±10.88 34-70 | 0.617 |
| Horizontal zone of pinguecula | 2.5±0.849 1.0-4.0 | 2.30±0.823 1.0-4.0 | 0.704 |
| Photophobia | 2.8±0.788 2.00-4.00 | 2.30±0.674 1.0-3.0 | 0.635 |
| Inflammation | 3.4±0.516 3.00-4.00 | 3.0±0.812 2.0-4.0 | 0.480 |

size of the pterygium was significantly reduced up to 6 months postoperatively (all p=0.037) (Table 2). Bonferroni post hoc analysis revealed significant differences between the preoperative measurement and all postoperative time points (p<0.001).

Mean photophobia was decreased up to 6 months after surgery, however, it was significantly different from the control group only at 1 (p=0.038) and 4 weeks (p=0.002), postoperatively (Table 3). There was a mean reduction in inflammation up to six months after surgery (Table 4), which was statistically significant at one month and at three months postoperatively (both p=0.019). Figures 1 and 2 show the sample of eyes in the control and intervention groups. Throughout the study, there was only one case of pinguecula recurrence within one month of the procedure in the

**Table 2.** Horizontal size of pterygium (*mm*) with intendent sample T-test

| Period | Groups | N | Mean±SD | p-value |
|---|---|---|---|---|
| Pre-op | Intervention<br>Control | 10<br>10 | 2.50±0.850<br>2.30±0.823 | 0.704 |
| 1 week post-op | Intervention<br>Control | 10<br>10 | 0.00<br>0.00 | 0.037 |
| 1 month post-op | Intervention<br>Control | 10<br>10 | 0.00<br>0.10±0.316 | 0.037 |
| 3 months post-op | Intervention<br>Control | 10<br>10 | 0.00<br>0.20±0.632 | 0.037 |
| 6 months post-op | Intervention<br>Control | 10<br>10 | 0.00<br>0.20±0.632 | 0.037 |

**Table 3.** The level of photophobia in two groups

| Period | Groups | N | Mean rank | Median | IQR | p-value |
|---|---|---|---|---|---|---|
| Pre-op | Intervention<br>Control | 10<br>10 | 12.10<br>8.9 | 3.0<br>2.0 | 2.0-4.0<br>1.0-3.0 | 0.188 |
| 1 week post-op | Intervention<br>Control | 10<br>10 | 12.90<br>8.10 | 3.0<br>3.0 | 1.0-4.0<br>2.0-3.9 | 0.038 |
| 1 month post-op | Intervention<br>Control | 10<br>10 | 6.75<br>14.25 | 2.0<br>3.0 | 1.0-2.9<br>2.0-4.0 | 0.002 |
| 3 months post-op | Intervention<br>Control | 10<br>10 | 9.95<br>10.05 | 2.0<br>2.0 | 1.0-2.0<br>2.0-3.0 | 0.648 |
| 6 months post-op | Intervention<br>Control | 10<br>10 | 9.35<br>11.65 | 2.0<br>1.5 | 1.0-3.0<br>1.0-2.9 | 0.344 |

**Table 4.** the Severity of Inflammation at two groups

| Period | Groups | N | Mean rank | Median | IQR | p-value |
|---|---|---|---|---|---|---|
| Pre-Op | Intervention<br>Control | 10<br>10 | 11.90<br>9.10 | 3.0<br>3.0 | 3.0-4.0<br>2.0-4.0 | 0.246 |
| 1 week post-op | Intervention<br>Control | 10<br>10 | 11.30<br>9.70 | 3.0<br>3.0 | 2.0-4.0<br>2.0-3.9 | 0.491 |
| 1 month post-op | Intervention<br>Control | 10<br>10 | 7.75<br>13.45 | 1.5<br>2.5 | 1.0-2.9<br>1.0-4.0 | 0.019 |
| 3 months post-op | Intervention<br>Control | 10<br>10 | 7.75<br>13.45 | 1.5<br>2.5 | 1.0-2.9<br>1.0-4.0 | 0.019 |
| 6 months post-op | Intervention<br>Control | 10<br>10 | 8.85<br>12.15 | 2.0<br>2.0 | 1.0-3.8<br>1.0-3.9 | 0.184 |

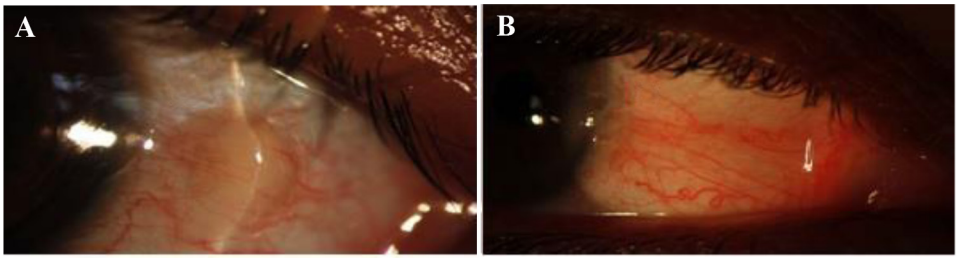



**Figure 1.** The right eye of a 34-year-old man with recurrence in the control group. A) Before treatment: horizontal surface size: 4 (>4.5 *mm*), photophobia: 2 Visual Analog Scale (VAS), inflammation grade: 2 (hyperemia extends up to 2 *mm* from the pinguecula surface). B) After treatment: horizontal surface size: 2 (1.5-3 *mm*), photophobia: 3 VAS, inflammation grade: 4 (hyperemia extends beyond 3 *mm* from the pinguecula, involving surrounding conjunctiva).

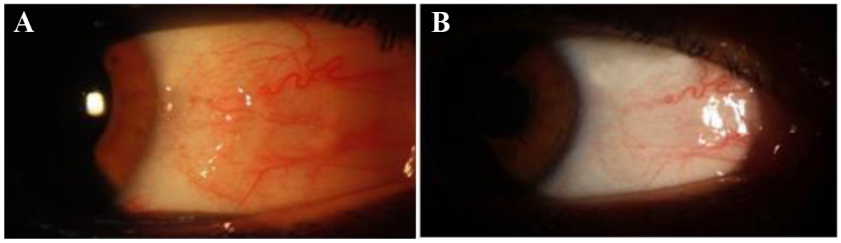



**Figure 2.** The right eye of a 51-year-old woman in the intervention group. A) Before treatment: horizontal surface size: 2 (1.5-3 *mm*), Photophobia: 3 Visual Analog Scale (VAS),Inflammation grade: 4 (hyperemia extends beyond 3 *mm* from the pinguecula, involving surrounding conjunctiva). B) After treatment: horizontal surface size: 0 (less than 1.5 *mm*), Photophobia: 1 VAS), Inflammation grade: 1 (hyperemia is limited to its surface).

control group and none in the intervention group.

## Discussion

Pinguecula is a common degenerative condition of the conjunctiva that can progress to pterygium formation. Factors such as the degenerative process, inflammation, and fibrovascular proliferation play an important role in its development (21). Evaluation of ocular vessel densitometry using optical coherence tomography angiography demonstrated that the vessel density of conjunctivas with pterygium was significantly greater than that of normal conjunctiva or those with pinguecula (22). This means that pterygium development is associated with angiogenesis and neovascularization.

There are several options for the treatment of pterygium. Many studies have shown that VEGF is elevated in the pathogenesis of pterygia. The development of anti-VEGF therapy could induce regression of blood vessels and thus delay the progression of pterygium. Anti-VEGF has been used in conjunction with pterygium surgery using subconjunctival injections and postoperative topical eye drops. Studies suggest that topical bevacizumab may be effective in the treatment of ocular surface neovascularization. There is no conclusive data on the use of subconjunctival bevacizumab for the treatment of pterygia. Subconjunctival bevacizumab injection may reduce the recurrence rate of primary pterygium surgery (23). However, some studies reported that there was no significant difference in the outcome for anti-VEGF bevacizumab as an effective drug in reducing recurrence after pterygium excision (24) even when administered prior to surgery (25). Moreover, some complications such as corneal epithelial defects and erosions have been reported (26).

Recurrence rates with the optimal method of anti-VEGF application remain a challenge. A meta-analysis of the use of bevacizumab in pterygium surgery indicated a reduction in recurrence rates at 12 months follow-up (27), although another meta-analysis found no significant reduction (28). Current adjunctive therapies to pterygium excision include anti-VEGF, which may play a role in reducing pterygium recurrence but may be associated with some complications that make it difficult to recommend for routine primary pterygium excision. Lee *et al* (29) with the evaluation of the complications of cosmetic wide conjunctivectomy and postoperative topical

mitomycin C application with or without bevacizumab injection in 1737 subjects (14% pterygium) reported that this approach had a high rate of complications and reoperations.

In this study, the horizontal surface area of the pinguecula decreased significantly in the Avastin injection groups up to 6 months after surgery. Similarly, Fallah Tafti *et al* (30) observed a reduction in the mean size of the pterygium and corneal involvement in the intervention group (Avastin injection) up to 3 months postoperatively, whereas it increased in the control group. In contrast, some studies reported no significant reduction in horizontal pterygium size after topical 2.5% bevacizumab injection at 3 months (31) and 6 months (32) post-surgery.

It was observed that photophobia tended to decrease up to 6 months after surgery, whereas it tended to increase in the control group at the same time. Consistent with findings of the current study, Enkvetchakul's study (33) showed that intralesional injection of bevacizumab 2 *mg* (1 *mg*/0.04 *ml*) resulted in statistically significant relief of symptoms (including irritation, photophobia, epiphora, redness, discharge, and blurred vision) and signs (inflammation and corneal pterygium area) for at least 6 months in 34 eyes with primary pterygium, without serious ocular or systemic adverse events.

In this study, inflammation was reduced for up to 6 months after surgery in the intervention group compared to the control group. However, Nuzzi *et al* (34) reported a reduction in mean pinguecula vascularity in the intervention group only during the first week. Different outcomes in pterygium size, and ocular disorders after surgery may be attributed to differences in the length of follow-up in different studies and the physicians' approaches to interpreting the results.

In the current study, only one recurrence of pinguecula was observed, which progressed to pterygium one month later in the control group, whereas no recurrence was observed in the intervention group. In contrast, Kocabora *et al* (35) with the assessment of the efficacy of subconjunctival bevacizumab injection in 15 eyes following the surgical treatment of pterygium compared with intraoperative mitomycin-C application found that intraoperative use of subconjunctival bevacizumab is not effective in reducing the rate of pterygium recurrence. However, a single 2.5 *mg/ml* subconjunctival injection of bevacizumab in conjunction with primary pterygium surgery using conjunctival autograft is considered a safe and preventive agent for pterygium recurrence (36). A case study evaluated the impact of bevacizumab (1.25 *mg*/0.05 *ml*) followed by pterygial ligation in 4 eyes and found the combination of sub-pterygial injection of bevacizumab and ligation of the pterygium is a potentially effective procedure for the removal of pterygium (37). Bevacizumab may reduce the recurrence of pinguecula after conjunctival autografting and ligation in patients with long-term follow-up of up to 12 months (38). Although the randomized design strengthens the internal validity of present study, a key limitation of this study is the small sample size, which was affected by the COVID-19 pandemic and reduces the statistical power, particularly for assessing recurrence outcomes. Consequently, these findings should be interpreted with caution. Further randomized trials with longer follow-up periods are required to confirm the clinical significance of bevacizumab in reducing pinguecula recurrence. Nevertheless, adherence to CONSORT guidelines minimized major sources of bias and enhanced the internal validity of this study.

## Conclusion

This study suggests that subconjunctival bevacizumab injection significantly reduces pinguecula size, inflammation, and photophobia in the short term, with no recurrences reported in the intervention group. It may serve as an effective adjunctive therapy in the management of pinguecula and potentially lower the risk of recurrence after ligation. Future developments in anti-angiogenic therapies with improved tissue delivery and enhanced efficacy, while minimizing side effects, hold promise for advancing pinguecula treatment.

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### *Ethics approval*

This study was ethically approved by the Ethics Committee of Mazandaran University of Medical

Sciences (Code: IR.MAZUMS.REC.1399.719) and conducted in accordance with the principles outlined in the Declaration of Helsinki. All the participants provided informed consent to participate in the study.

### *Trial registration*

This trial was retrospectively registered at the Iranian Clinical Trials Registry (http://www.irct.ir) on 23 July 2024 under registration number IRCT20240702062306N1.

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## Conflict of Interest

The authors have no financial interests.

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