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OUT COME OF THE USE OF TOPICAL BEVACIZUMAB IN TREATMENT OF CORNEAL NEOVASCULARIZATION

By

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ABSTRACT

Background: Corneal neovascularization (NV) can occur as a consequence of anterior segment inflammation, injury and ischemia. This pathological response can cause visual impairment or other conditions such as corneal edema, corneal scarring, lipid deposition, increased risk of graft rejection after corneal transplantation, and bleeding during corneal flap preparation in laser in situ keratomileusis (LASIK) surgery. Many studies have shown that anti- vascular endothelial growth factor agents can inhibit corneal neovascularization. One such inhibitor is bevacizumab, a humanized murine monoclonal antibody against all vascular endothelial growth factor isoforms.

Objective: To evaluate the results of using topical bevacizumab (Avastin) as a treatment of corneal neovascularization due to different causes.

Patients and methods: In this study, Bevacizumab was used topically as eye drops with a concentration of 0.5 mg/ml. Total 30 eyes were included in this study. All were diagnosed with corneal neovascularization. Careful history was taken from all patients and they underwent ophthalmological examination including slit lamp biomicroscopy examination , color photos of the anterior segment and intra oculae pressure . All cases were selected from outpatient clinic of the Ophthalmology Department at Al-Azhar University Hospital (Cairo) from October 2018 to October 2020.

Results: The corneal opacity decreased in 13.3% of the studied group. However, the remaining 86.7% didn't show any change at all. The best corrected visual acuity (BCVA) improved in about one third of the studied group (33.3%). However, the remaining 66.7% didn't show any change at all. There was ahighly significant difference between the studied group as regarding effect of Avastin on neovascularization after one and two weeks. It was noticed that moderate response increased from the first to the second week (3.3% versus 56.7% respectively). There were highly statistically significant differences between the different pathologies as regarding Avastin's effect on corneal neovascularization after both one and two weeks.

Conclusion: The use of topical bevacizumab (avastin) eye drops in cases of corneal NV was safe, well tolerated, associated with mild to moderate regression of corneal NV and not associated by any drug related side effects.

Keywords: Bevacizumab (Avastin),Treatment of superficial corneal neovascularization.

INTRODUCTION

The normal cornea is devoid of both blood and lymphatic vessels and actively maintains that avascularity. This so-called corneal—angiogenic privilege is important for corneal transparency and

vision. Furthermore, it contributes to the success of corneal transplantations performed into avascular low-risk recipient beds *(Bock et al., 2012)*.

Koenig et al. (2013) showed that corneal neovascularization (NV) is characterized by the invasion of new blood vessels into the cornea from the limbus. Various inflammatory, infectious, degenerative, or traumatic disorders are associated with corneal neovascularization. This sight- threatening complication occurs when the balance between angiogenic and anti- angiogenic factors is tilted towards angiogenic molecules. Neovascularization is promoted by a complex array of microenvironmental changes that involve a diverse array of cellular and molecular mediators.

Vascular endothelial growth factor (VEGF) is a critical mediator of retinal and iris NV following injury and ischemia, and in diabetic retinopathy too. Corneal epithelial and endothelial cells, endothelial cells of limbal vessels, and fibroblasts and macrophages in scar tissue all excrete VEGF, especially in inflamed and vascularized corneas. The receptors of VEGF (VEGFR1 and VEGFR2) are also found in newly proliferating vascular endothelial cells in inflamed cornea *(Chen et al., 2014)*.

Stevenson et al. (2012) showed that VEGF neutralizing agents have proven invaluable in the treatment of pathologic conditions such as neovascular age-related macular degeneration (AMD) and diabetic retinopathy; furthermore, recent findings suggest that VEGF inhibition may be an effective therapeutic modality for corneal NV.

Bevacizumab (Avastin) is a humanized monoclonal antibody that binds to isoforms of VEGF1. It was initially approved for the treatment of metastatic colorectal cancer; however, it has since been used to treat a variety of ophthalmic conditions, including neovascular age-related macular degeneration, diabetic retinopathy, central retinal vein occlusion, and neovascular glaucoma. The systemic administration of bevacizumab has been associated with several severe and potentially life-threatening complications, including hypertension, impaired wound healing, gastrointestinal perforation, bleeding, arteriolar hemorrhage, and arterial thromboembolic events. The route of administration that provides the best combination of safety, efficacy, and practicality should be pursued; with regard to the cornea, the preferred method of administration is generally ocular surface topical instillation *(Stevenson et al., 2012)*.

The aim of this work was to evaluate the results of using topical bevacizumab (Avastin) as a treatment of corneal neovascularization due to different causes.

PATIENTS AND METHODS

In this study, Bevacizumab was used topically as eye drops with a concentration of 0.5 mg/ml. Total 30 eyes were included in this study. All were diagnosed with corneal neovascularization. All cases were selected from outpatient clinic of the Ophthalmology Department at Al-Azhar University Hospital (Cairo) from October 2018 to October 2020.

Dose: The standard solution (concentration of 0.5 mg/ml bevacizumab) diluted in 0.9% saline.

Method of administration: 1 drop 4 times daily for 2 weeks.

Follow-up: Evaluation was performed pretreatment and in 7and 14 day post treatment.

Inclusion criteria: Patients aged from 15 to 70 years old, acquired corneal neovascularization, and BCVA not less than hand motion.

Exclusion criteria: Patients with no light perception, pregnant females, and corneal dystrophies.

Informed consent was obtained from all patients before administration of Bevacizumab eye drops including their acceptance to use the drops, knew the advantages, disadvantages, risks, possible complications and periodical follow up for 2 weeks.

All patients were subjected to history taking including personal history (name, age, gender, occupation, residence), complaint, present history and past history specially ophthalmic history including history of corneal infection, inflammation, degenerative disorders and traumatic disorders.

All patients were examined as follow:

1. Functional assessment: Visual acuity test using "Landolt's chart".
2. Clinical assessment:
    a. Anterior segment by slit lamp examination, colored photos, and intra ocular pressure.
    b. Posterior segment (fundus) by direct ophthalmoscopy, and indirect ophthalmoscope.

Bevacizumab eye drops were prepared in the hospital pharmacy under complete sterile conditions from the standard solution diluted in 0.9% saline to a concentration of 0.5 mg/ml. The eye drops were stored at 4°C during that time.

The patients were instructed to instill 1 drop 4 times daily for 2 weeks. They were also instructed to maintain punctual occlusion and close their eyes for at least 1 min after drug instillation in order to reduce systemic drug absorption. The patients who were using other ocular drops were instructed to instill the Bevacizumab drops first and wait at least 5 min before instilling other drops (if the patients were already on other medical ophthalmic drops) in order to prevent drug washout.

Follow-up evaluations were performed on days 7 and 14, and they included BCVA as well as slit-lamp examinations. Color photos of the anterior segment were obtained at baseline and at the final visit.

Each patient underwent post-administration ophthalmic examination in the days 7 and 14 as follow:

1. Visual acuity test using "Landolt's chart".
2. Using slit lamp biomicroscopy cornia was examined to detect regression of the vessels either mild which was defined as partial regression of the corneal neovascularization and/or clearing of corneal opacification or moderate, which was defined as clear-cut regression of corneal neovascularization and/or clearing of corneal opacification.
3. Intraocular tension was measured using Goldmann applanation tonometry to detect any changes of IOP.

4. Posterior segment of the eye was examined using indirect ophthalmoscop.

Statistical analysis:

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for the Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Wilk test. Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) was used to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean ± SD (Standard deviation).P value < 0.05 was considered significant.

RESULTS

The age of the studied group ranged between 28 and 70 years with a mean of 52.6 years. More than half of them were males (60%). Half of the patients had pterygium, 16.7% showed pannus. Failed penetrating keratoplasty (PKP), resolved keratitis, and old trauma had the same frequency (10%). However, healed corneal ulcer was only detected in one patient (3.3%) **(Table 1)**.

Table (1): Socio-demographic data and detected pathology of the studied group

Variables	Studied group (n=30)	
Age:		
Mean ± SD	52.6 ± 13.4	
Range	28 – 70	
	No	**%**
Gender:		
Female	12	40
Male	18	60
Pathology:		
Failed PKP	3	10
Resolved keratitis	3	10
Pannus	5	16.7
Pterygium	15	50
Old trauma	3	10
Healed ulcer	1	3.3

The corneal opacity decreased in 13.3% of the studied group. The remaining 86.7% didn't show any change at all. The BCVA improved in about one third of the studied group (33.3%).The remaining 66.7% didn't show any change at all **(Table 2)**.

Table (2): Effect of Avastin on corneal opacity and Avastin on the best corrected visual acuity (BCVA) of the studied group

Variables / Studied group (n=30)	Studied group (n=30)	
	No	**%**
Corneal opacity:		
No change.	26	86.7
Decreased.	4	13.3
BCVA:		
Unchanged.	20	66.7
Improved.	10	33.3

There was a significant difference between the studied group as regards the effect of Avastin on neovascularization after one and two weeks. It was noticed that moderate response increased from the first to the second week (3.3% versus 56.7% respectively) **(Table 3)**.

Table (3): Effect of Avastin on corneal neovascularization after one and two weeks among the studied group

Variables / Time	One week (n=30)		Two weeks (n=30)		P value
	No	**%**	**No**	**%**	
Corneal vascularization:					
No response.	10	33.3	8	24.6	**<0.001**
Mild.	19	63.3	5	16.7	
Moderate.	1	3.3	17	56.7	

There were no statistically significant differences between the different pathologies as regarding avastin's effect on corneal opacity. There were no statistically significant differences between the different pathologies as regarding Avastin's effect on BCVA. Resolved keratitis patients were found to have the highest frequency in decrease in BCVA (66.7%) when compared to other pathologies **(Table 4)**.

Table (4): Comparison of corneal opacity and BCVA among the studied groups.

Groups / Corneal opacity	Failed PKP (n=3)	Resolved keratitis (n=3)	Pannus (n=5)	Pterygium (n=15)	Old trauma (n=3)	Healed ulcer (n=1)	P
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	
No change	2 (66.7)	2 (66.7)	5 (100)	13 (86.7)	3 (100)	1 (100)	**<0.629**
Decrease	1 (33.3)	1 (33.3)	0 (0)	2 (13.3)	0 (0)	0 (0)	
BCVA							
No change	3 (100)	1 (33.3)	4 (80)	9 (60)	2 (66.7)	1 (100)	0.521
Improve	0 (0)	2 (66.7)	1 (20)	6 (40)	1 (33.3)	0 (0)	

There were statistically significant differences between the different pathologies as regarding avastin's effect on corneal neovascularization after both one and two weeks **(Table 5)**.

Table (5): Comparison of corneal neovascularization after one and two weeks among the studied groups

Groups / Corneal vascularization		Failed PKP (n=3)	Resolved keratitis (n=3)	Pannus (n=5)	Pterygium (n=15)	Old trauma (n=3)	Healed ulcer (n=1)	P
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	
After One week	**Mild**	0 (0)	2 (66.7)	2 (40)	14 (93.3)	1 (33.3)	0 (0)	**0.04**
	Moderate	0 (0)	0 (0)	0 (0)	1 (6.7)	0 (0)	0 (0)	
	No response	3 (100)	1 (33.3)	3 (60)	0 (0)	2 (66.7)	1 (100)	
After two week	**Mild**	1 (33.3)	2 (66.7)	2 (40)	0 (0)	0 (0)	0 (0)	**0.001**
	Moderate	0 (0)	0 (0)	1 (20)	15 (100)	1 (33.3)	0 (0)	
	No response	2 (66.7)	1 (33.3)	2 (40)	0 (0)	2 (66.7)	1 (100)	

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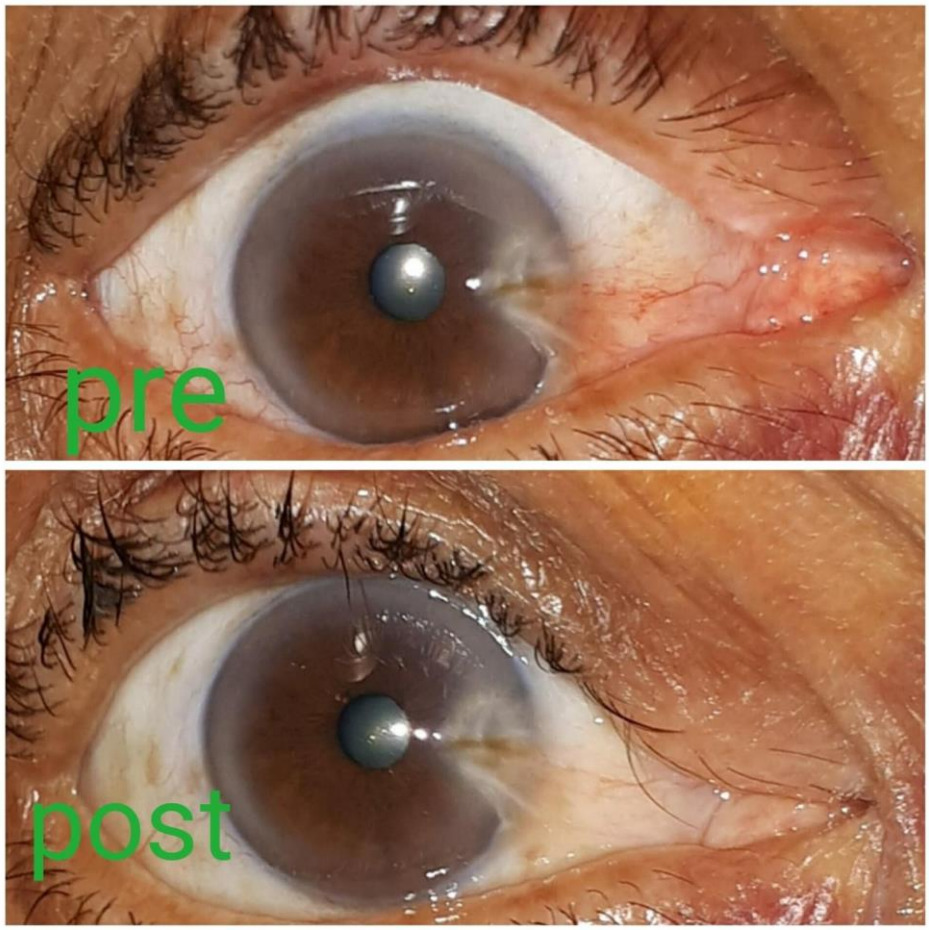

Fig.1: pre and two weeks after treatment of a case of pterygium with moderate regression.

DISCUSSION

Topical application of drug is the preferred method of administration to the cornea, ocular surface, and anterior segment, because achieving a high therapeutic level of medicine in these tissues can often be feasible without imposing systemic side effects. However, topical treatment and periocular injections will only be effective if the drug can penetrate through the ocular barriers *(Dastjerdi et al., 2011).*

Bevacizumab eye drops demonstrated the ability of inhibition the corneal neovascularization and regresed newly formed corneal blood vessels. The effect of the eye drops and the treatment time needed to calm down the angiogenic response were very variable. Reasons for interindividually different effects of bevacizumab eye drops include intensity of angiogenic stimulus, epithelial barrier function (although the eye drops also work with an intact epithelium), time-course of neovascularization (established corneal vessels do not depend on VEGF any more, dosing etc *(Bock et al., 2012).*

Waisbourd et al. (2013) reported that the optimal dosage for treating corneal neovascularization by topical bevacizumab has not yet been established, and various dosage regimens have been recommended.

The aim of this work was to evaluate the efficacy of bevacizumab eye drops 0.5mg/ml on the corneal neovessels. Total 30 eyes of 30 patients participated on this study. All were diagnosed with corneal neovascularization due to different causes including pterygium, failed penetrating keratoplasty, resolved keratitis, healed corneal ulcer, old trauma and pannus, and all the patients received bevacizumab eye drops four times daily for two weeks.

The main follow up parameters included best corrected visual acuity before and after receiving treatment, regression of the newly formed blood vessels, and effect on the corneal opacity.

As regarding the BCVA, in this study, 33.3% showed mild improvement of the BCVA after receiving the treatment and that was consistent with the results of other studies.

Waisbourd et al. (2013) reported improvement of BCVA in 52.9% using topical bevacizumab eye drops with a concentration of 25mg/ml four times daily for two weeks.

Krizova et al. (2014) reported that the improvement of BCVA especially in cases of failed PKP after receiving topical bevacizumab eye drops with a concentration of 2.5mg/ml twice daily for 2 weeks.

Ferrari et al. (2013) reported improvement of the BCVA after receiving topical bevacizumab eye drops with a concentration of 10mg/ml four times daily for 3 weeks.

Regarding the regression of neovessels, in this study there was a significant difference between the studied groups after one and two weeks follow up after receiving treatment. It was noticed that moderate response increased from the first to second week 3.3% versus 17/30 ; 56.7%). Also, other studies reported regression of the corneal neovessels.

Waisbourd et al. (2013) reported that the regression of the neovessels in eleven eyes 65%.

Ferrari et al. (2013) reported regression of the neovascular area in 55.3% of the studied eyes at the end of third week of treatment.

Bock et al. (2012) reported regression of the vascularised corneal area to very variable degrees and a reduction in vascularized area during treatment was ranging from 13% to 75%.

Regarding the corneal opacity, no significant difference was reported before and after treatment in this study. Only 13.3% showed decrease in the corneal opacity. While *Cheng et al. (2012)* documented that the reduction of the opacity was in 20% of studied eyes after receiving topical bevacizumab eye drops with a concentration of 10 mg/ml four times daily for 3 weeks and the different between results due to little number of patients and lesser concentration of topical bevacizumab eye drops in our study.

Ferrari et al. (2013) showed no significant changes in the corneal opacity after treatment. Regarding complications, neither systemic nor local complications were detected in this study, but *Waisbourd et al. (2013)* reported that the local complications in the form of one case each of epitheliopathy, eye lid swelling and chalazion, and these complications were found to be mild and transient and the authors explained them due to the use

of high dose of topical bevacizumab eye drops.

Kim et al. (2012) reported epitheliopathy in six out of ten patients treated with bevcizumab eye drops with a concentration of 12.5 mg/ml. *Koenig et al. (2013)* reported epitheliopathy in 5 out of 27 patients treated for corneal neovascularization with bevacizumab topical eye drops with a concentration of 5 mg/ml and the different brtween results due to the use of high dose of topical bevacizumab eye drops.

Galor and Yoo (2010) reported a case of idiopathic corneal perforation who underwent PKP developed corneal graft melt while using high dose of topical bevacizumab eye drops with a concentration of 25 mg/ml. *Fallah et al. (2010)* and (―Nihms86910.Pdf, n.d.) didn`t mention any local or systemic adverse effects among their patients who received bevacizumab topical eye drops with a concentration of 1-5 mg/ml.

CONCLUSION

The use of topical bevacizumab (avastin) eye drops in cases of corneal NV was safe, well tolerated, associated with mild to moderate regression of corneal NV and not associated by any drug related side effects. This study advices to use small dose topical bevacizumab eye drops for short duration in treatment of corneal NV to achieve the benefit of blood vessels regression with minimal risk of local complications.

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نتائج إستخدام بيفاسيزيوماب الموضعي في علاج الأوعية الدموية بالقرنية

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خلفيـــة البحـــث: يمكـــن أن يحـــدث نموالأوعيـــة الدمويـــة فـــي القرنيـــة نتيجـــة لالتهـــاب الجـــزء الأمـــامي والإصـــابة ونقـــص التروية. يمكـــن أن تتســـبب هـــذه الاســـتجابة المرضـــية فـــي إعاقـــة بصـــرية أو حـــالات أخـــرى مثـــل وذمـــة القرنيـــة وتنـــدب القرنيـــة وترســـب الـــدهون وزيـــادة خطـــر رفـــض الكســـب غيـــر المشـــروع بعـــد زرع القرنيـــة والنزيـــف أثنـــاء تحضـــير ســـديلة القرنيـــة فـــي جراحـــة تصـــحيح تحـــدب القرنيـــة بـــالليزر (الليـــزك). وقـــد أظهـــرت العديـــد مـــن الدراســـات أن عوامـــل النمـــو البطانيـــة المضـــادة للأوعيـــة الدمويـــة يمكـــن أن تمنـــع تكـــوين الأوعيـــة الدمويـــة فـــي القرنيـــة. أحـــد هـــذه المثبطـــات هـــو بيفاســـيزيوماب ، وهـــو جســـم مضـــاد مـــورين وحيـــد النســـيلة متوافـــق مـــع البشـــر ضـــد جميع الأشكال الإسوية لعامل النمو البطاني الوعائي.

الهـــدف مـــن البحـــث: تقيـــيم نتـــائج اســـتخدام بيفاســـيزوماب الموضـــعي (أفاســـتين) كعـــلاج لتكوين الأوعية الدموية في القرنية لأسباب مختلفة.

المرضـــى والطـــرق: فـــي هـــذه الدراســـة ، تـــم إســـتخدام بيفاســـيزوماب موضـــعياً كقطـــرات للعـــين بتركيـــز 0.5 مجـــم / مـــل. تـــم تضـــمين إجمـــالي 30 مريضـــا فـــي هـــذه الدراســـة. تـــم تشـــخيص جميـــع المصـــابين بوجـــود الأوعيـــة الدمويـــة فـــي القرنيـــة. تـــم أخـــذ التـــاريخ الـــدقيق مـــن جميـــع المرضـــى وخضـــعوا لفحـــص طـــب العيـــون بمـــا فـــي ذلـــك عامـــل نمـــو بطانـــة الأوعيـــة الدمويـــة,الفحص المجهـــري الحيـــوي للمصـــباح الشـــقي وصـــور ملونـــة للجـــزء الأمـــامي. تـــم اختيـــار جميـــع الحـــالات مـــن العيـــادة الخارجيـــة بقســـم طـــب وجراحـــة العيون بمستشفى جامعة الأزهر (القاهرة) من أكتوبر 2018 إلى أكتوبر 2020.

نتـــائج البحـــث: إنخفضـــت عتامـــة القرنيـــة فـــي 13.3٪ مـــن المجموعـــة المدروســـة، لكـــن النســـبة المتبقيـــة 86.7٪ لـــم تظهـــر أي تغييـــر علـــى الإطـــلاق. وقـــد تحســـنت حـــدة البصـــر المصـــححة للأفضـــل كمثـــال مـــن 36/6 الـــى24/6 فـــي حـــوالي ثلـــث المجموعـــة

المدروسة (33.3٪)، ومع ذلك فإن النسبة المتبقية 66.7٪ لم تظهر أي تغيير على الإطلاق. كان هناك فرق معنوي كبير بين المجموعة المدروسة فيما يتعلق بتأثير عقار أفاستين على الأوعية الدموية بعد أسبوع أو أسبوعين. لوحظ أن الاستجابة المعتدلة تزداد من الأسبوع الأول إلى الأسبوع الثاني (3.3٪ مقابل 56.7٪ على التوالي). كانت هناك فروق ذات دلالة إحصائية عالية بين الأمراض المختلفة فيما يتعلق بتأثير أفاستين على الأوعية الدموية في القرنية بعد أسبوع أو أسبوعين.

الاستنتاج: إن إستخدام قطرات العين الموضعية بيفاسيزوماب (أفاستين) في حالات القرنية القرنية آمن وجيد التحمل ويرتبط بإنحدار خفيف إلى معتدل للقرنية ولا يرتبط بأي آثار جانبية متعلقة بالأدوية.

الكلمات الدالة: بيفاسيزوماب (أفاستين)، علاج القرنية، نمو الأوعية الدموية.