Original Article

Role of Sub-Conjunctival Bevacizumab in Regression of Corneal Neovascularization

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Purpose: To evaluate therapeutic effect of subconjunctival bevacizumab on corneal neovascularization.

Material and Methods: Thirty two eyes, with corneal neovascularization caused by different ocular surface disorders, were studied. Each eye received 2 injections of 2.5 mg/0.1ml bevacizumab at monthly interval. Morphological changes in corneal neovessels were evaluated using slit lamp biomicroscopy and digital corneal photography.

Results: Out of total 32 patients, 21(65.5%) were males and 11(35.5%) were females. Mean age of all patients was 41.59 ± 17.6 years. Causes of corneal neovascularization included trauma (28.1%), failed corneal graft (21.9%), chemical burn (12.5%), healed corneal ulcer (12.5%), trachoma (3.1%) and unknown cause (21.9%). Mean corneal surface involved by neovessels before injection was 50.56 ± 30.4% which reduced to 35.81 ± 26.94% after sub-conjunctival injection of bevacizumab (p = 0.000) and the extent of neovessels reduced from 7.47 ± 3.83 clock hours to 6.56 ± 3.78 clock hours (p = 0.002). No adverse effect of subconjunctival bevacizumab was noted.

Conclusion: Sub-conjunctival bevacizumab is effective in regressing corneal neovessels partially due to different causes. But for this purpose repeated injections are needed.

Cornea is avascular structure to serve its optical function in a best way. Its neovascularization is always pathologic and represents an important cause of visual morbidity. Corneal neovessels, compromise both the corneal transparency[1] as well as its immune privilege. Patent corneal vessels impair the process by which migrating limbal stem cells differentiate into transparent corneal epithelial cells[2]. Corneal neovascularization is induced by a variety of inflammatory, infectious, degenerative and traumatic (both mechanical and chemical) disorders[3].

To maintain corneal avascularity under basal conditions there are low levels of angiogenic factors and high levels of anti-angiogenic factors in the cornea[4]. Imbalance of this homeostasis may occur in pathogenesis of corneal neovessels[2]. It is shown that there is up-regulation of vascular endothelial growth factor (VEGF) in inflamed and vascularized cornea[5-7]. Thus VEGF promotes angiogenesis in cornea. Anti VEGF antibodies are commonly used to regress retinal and choroidal neovessels in proliferative diabetic retinopathy, wet age related macular degeneration (ARMD) and few other conditions. Bevacizumab, a humanized monoclonal antibody against all types of VEGF[8,9], can be an effective option to regress corneal neovessels. Initially it was used by researchers in animal models and found effective in reducing corneal neovessels. Then it was used in human eyes and its affectivity in regressing corneal neovessels was proved[4]

We planned a study in which effect of sub-conjunctival Bevacizumab (Avastin®) injection on corneal neovessels was assessed. The rationale of the study is that once it is proved by multiple studies that subconjunctival bevacizumab is effective, the regime can be used to regain transparency of cornea in many pathological conditions, where corneal neovessels

develop. Moreover, the regime can also be used to reduce chance of corneal graft rejection in vascularized corneas.

MATERIAL AND METHODS

This quasi-experimental study was conducted at Khyber Teaching Hospital Peshawar, Pakistan from September 2011 to May 2013. Approval was taken from institutional ethical committee of Khyber Medical College Peshawar, Pakistan.

32 Eyes were included in the study. All eyes had moderate to severe corneal neovessels. Eyes with active anterior segment disease like corneal ulcer, active anterior uveitis, etc. were excluded from the study.

Each eye was thoroughly examined on slit lamp, digital photograph taken and extent of corneal neovascularization (CoNV) noted. Moreover corneal clarity in the area involved by neovessels was graded as follows.

Grade I: Iris crypts visible

Grade II: Iris visible but crypts not visible

Grade III: Iris not visible, but slit lamp beam passes into anterior chamber.

Grade IV: Totally opaque cornea

For each patient pre-injection digital corneal photograph was taken. The eye was anaesthetized with 1% topical proparacaine drops. Under operating microscope, sub conjunctival injection of 0.1ml (2.5mg) of bevacizumab was given near the limbus in the area where maximum density of neovessels found in the nearby cornea. Photograph of cornea repeated one week after injection. A second dose of bevacizumab was given one month after the first injection with same protocol. Slit lamp examination for corneal clarity was done and a final corneal photograph was taken one month after the second injection. Pre injection corneal photographs were compared with final photographs in terms of extent of corneal neovascularization in clock hours and percentage of corneal surface involved by neovessels.

The data was analyzed using SPSS version 15. Means with standard deviation were calculated for numerical variables like age and percentage of corneal surface involved by neovessels. Proportions were calculated for string variables like gender, causes of corneal neovessels and clarity of cornea. Paired samples 't' test was used to calculate P- Value and a p-value of 0.005 was considered significant.

RESULTS

Thirty two Eyes were included in the study. Twenty one 65.6% patients were males and 34.4% were females. Mean age of all patients was 41.59 ± 17.6 years. Physical trauma with corneal scarring was the most common cause of corneal neovascularization which was found in 9 (28.1%) patients. This was followed by failed corneal graft in 7 (21.9%), chemical burn in 4 (12.5%), healed corneal ulcer in 4 (12.5%), trachoma in 1 (3.1%) and unknown cause in 7 (21.9%) patients (figure 1). Mean corneal surface involved by neovessels before injection was 50.56 ± 30.4% which reduced to 35.81 ± 26.94% one month after second sub-conjunctival injection of bevacizumab (p=0.000) (Figure 2) and the extent of neovessels reduced from 7.47 ± 3.83 clock hours to 6.56 ± 3.78 clock hours (p = 0.002) (Table 1). No significant change was found in corneal clarity.

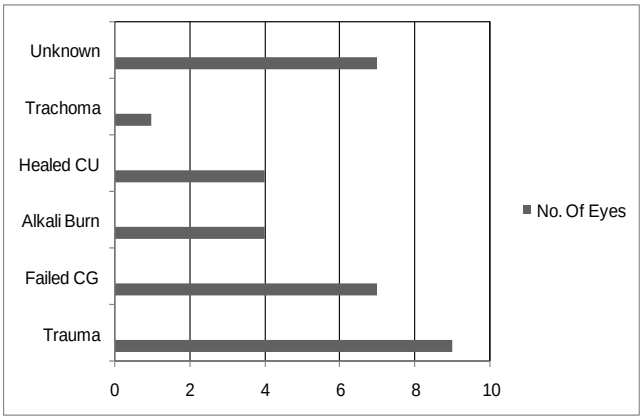

Fig. 1: Causes of corneal neovascularization. (CU = Corneal Ulcer, CG = Corneal Graft)

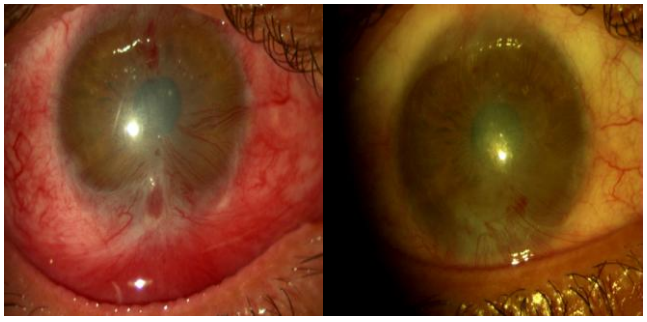

Fig. 2: Corneal neovascularizaion in alkali burn, showing significant reduction in extent of neovessels after subconjunctival injection of bevacizumab.

DISCUSSION

Normally to keep the cornea avascular, there is low level of angiogenic and high level of anti-angiogenic factors in cornea. Imbalance of this homeostasis may occur in inflamed corneas which lead to formation of neovessels in it that primarily sprout from limbal vessels. VEGF-A is produced by a variety of cells including retinal pigment epithelial cells, macrophages, astrocytes, Muller cells, smooth cells and T-cells. VEGF-A up-regulation has been demonstrated in inflammatory diseases associated with neovascularization in human cornea.[10] Role of VEGF in pathophysiology of corneal neovascularization was proved by the researchers in rabbits and other primates[11-14].

Table 1: Corneal neovascularization before and after subconjunctival bevazizumab injection

Vascularization Measure	Before Treatment	After Treatment	P value
Proportion of vascularized cornea	50.56 ± 30.4%	35.81 ± 26.94%	P=0.000
Extent in clock hours	7.47 ± 3.83	6.56 ± 3.78	P=0.002

The anti VEGF role of bevacizumab has been proved in regressing corneal neovessels in animals and human beings[15-21]. Moreover safety of this drug with no harmful effect on corneal cells in vitro was also established[22]. After that this drug was used by many researchers in reducing CoNV in human beings[6,23,24]. No serious side effects of bevacizumab have been reported while used subconjunctival[25-27], intracornreal[28] or topical[29] on ocular surface, to regress CoNV. In our study we did not notice any serious adverse effect of sub-conjunctival bevacizumab (2.5 mg/ 0.1 ml), except mild sub-conjunctival hemorrhage in few eyes which resolved spontaneously.

In our study we observed clinically significant reduction in area of corneal surface involved by CoNV from 50.56 ± 30.4% to 35.81 ± 26.94% or extent of CoNV from 7.47 ± 3.83 clock hours to 6.56 ± 3.78 clock hours. This is consistent with many other international studies. In a study from Canada[25] the extent of CoNV reduced from 6.00 ± 1.2 to 4.6 ± 1.00 clock hours after bevacizumab injection ($p = 0.008$). In this study all eyes received at least two Injections (2.5 mg / o.1 ml). In another study from Egypt by zaki and farid[30], the area of CoNV decreased from 14.00 ± 5.4% to 9.4 ± 3.9% of corneal surface ($p < 0.01$) and extent decreased from 4.3 ± 1.5 clock hours to 2.4 ± 1.1 clock hours ($p < 0.01$) fifteen days after single subconjunctival injection of 2.5 mg /0.1 ml of bevacizumab. In a study from France[23] mean CoNV area decreased from 41.1% to 33.7% at day 45 ($p = 0.000$) and to 33.9% at day 120 ($p = 0.0013$). In this study sample size was 12 eyes and each eye received 2 to 4 subconjunctival bevacizumab (2.5 mg / 0.1 ml) injections. A pilot randomized placebo – controlled double masked trial from Moorfield Eye Hospital, London also proved that mean area of CoNV reduced by -36% in eyes that received 3 sub-conjunctival injections of 2.5mg / 0.1ml bevacizumab at monthly intervals compared with an increase of 90% in eyes that received saline placebo ($p = 0.007$)[31].

In our study we observed that subconjunctival injection regresses newly formed small vessels and not the well-established bigger vessels. This was also observed by Bahar I et al[25] and Petsoglou C et al[31] in their studies. These established vessels are probably not affected by imbalance of VEGF, hence not responding to bevacizumab. Moreover like many other international studies mentioned above[25,30,31]. In our study reduction in CoNV was only partial. This could be due to factors other than VEGF, that can induce CoNV and remain unaffected by bevacizumab[32,33].

CONCLUSION

Subconjunctival bevacizumab is effective in regressing corneal neovessels partially and for this purpose repeated injections are needed. Randomized control trials are needed to prove the results.

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