Original Article

Efficacy of Sub-Conjunctival Bevacizumab in High Risk Corneal Transplantations

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Purpose: To evaluate the efficacy of sub-conjunctival and topical Bevacizumab in high risk corneal transplant survival.

Material and Methods: Eyes with high risk corneal transplantation with corneal neovascularization (NV) were included in this randomized clinical trial. Patients were randomly allocated to 2 groups. Group A and Group B. After penetrating keratoplasty, group A patients received sub-conjunctival bevacizumab (2.5 mg/ 0.1 ml), group B, patients received sham injection. Corneal neovascular invasion area i.e. the fraction of area on transplanted cornea in which vessels are present is measured using mathematical software program MatLab. Primary measurement variable was neovascular invasion area while secondary measurement variable was visual acuity.

Results: Among the 2 groups mean corneal neovascular invasion area was minimum in the sub-conjunctival bevacizumab injection group (group A), with p value < 0.05.

Conclusion: Sub-conjunctival bevacizumab can offer an adjunctive measure to traditional treatment methods of prevention of vascularization on grafted cornea.

Corneal graft rejection is the most important reason that causes corneal graft failure and thus a foremost indication for repeat penetrating keratoplasty[1]. Presence of preexisting blood vessels is an important risk factor for corneal graft rejection[2]. High-risk corneal transplantations such as corneal grafting into the vascularized corneal beds, has greater than 50% immune rejection rate, even with a stringent regimen of topical and systemic immunosuppressive drugs[3].

Many investigators has been using the angiogenesis as the main factor of modification by immune suppressors in order to increase the success of high risk corneal transplantation[4,5]. It is not fully understood what are the factors that disturb the immune privileged state in patients with corneal neovascularization (NV). On the other hand, experimental evidence suggests that certain molecular factors such as the local immunosuppressive cytokines such as transforming growth factor-β, α-melanocyte-stimulating hormone and anterior chamber-associated immune deviation, plays a critical role in maintaining the physiologic serenity in the anterior chanber[6]. Therefore, management of corneal neovascularization after corneal transplantation can be controlled by bounding both sensitization arm as well as rejection arm of the autoimmunity and, consequently, reduce the susceptibility for immune-inflammatory reactions[6].

Vascular endothelial growth factor (VEGF) is the most important mediator of NV[7]. The major role of VEGF in the development of corneal neovascularization was established in experimental models of corneal angiogenesis[7]. VEGF inhibitors, such as Pegaptanib Sodium (Mucagen), Ranibizumab (Lucentis), and Bevacizumab (Avastin), have been used in wet type of age related macular degeneration with successful reasults[8]. In recent times, there has been growing interest in using anti-vascular endothelial growth factor for the treatment of corneal neovascularization when used either topically or subconjunctivaly[9-14]. These studies put forward that anti-vascular endothelial growth factor can be used as

an adjunctive measure to conventional therapies such as corticosteroids to restrain factors that provocate graft rejection in vascularized high-risk corneal transplantation.

For that reason, we wanted to assess the effects of sub-conjunctival bevacizumab injection treatment on corneal graft survival in high-risk (vascularized) corneal transplantation. To make a methodical and complete evaluation of corneal neovascularisation, a quantitative method was used to measure neovessel invasion area. Our results specify that subconjunctival bevacizumab therapy inhibit corneal neovascularization (growth of new vessels on the transplanted cornea) after high-risk corneal transplantation.

MATERIAL AND METHODS

The study was carried out at Isra Postgraduate Institute of Ophthalmology and Yasin eye hospital, Karachi from December 2008 to February 2012 for 38 months. Eyes with high risk corneal transplantation with corneal neovascularization were included in this randomized clinical trial. Ethical approval was taken from the Ethical review committee of Al-Ibrahim eye hospital / Isra Postgraduate institute of Ophthalmology. Informed written consent was taken from every patient included in the study. Indications for corneal transplantation were: vascularized corneal opacity secondary to keratitis or mechanical or chemical trauma, bullous keratopathy, corneal dystrophy, and failed corneal grafts.

Patients were randomly allocated to 2 groups. Group A, and Group B. After penetrating keratoplasty, group A patients were given subconjunctival bevacizumab 2.5 mg/ 0.1 ml in all the quadrants in each patient at the end of corneal transplantation surgery and also on follow ups. In group B, patients were given sham injection in all the quadrants in each patient at the end of corneal transplantation surgery and also on follow ups. Follow-up period was 2 to 8 months (mean 7.1 months). Due to poor compliance in our community longer follow-ups were not possible.

Primary measurement variable was neovascular invasion area while secondary measurement variable was visual acuity. A method we have used was that the objective quantification of cornea. For objective quantification, we first captured a sequence of slit-lamp images of transplanted corneas. Graphics editing software (Photoshop CS2) was used to outline the blood vessels in the transplanted corneal image. The corneal neovascular invasion area i.e. the fraction of area on transplanted cornea in which vessels were present was measured using mathematical software program MatLab (Fig. 1). (Matlab is highly specialized analytical software, which has a feature in it that it calculates exact area on a specialized grid pattern giving percentages of the specified area as well, when the picture from photoshop is opened in it will calculate its percentage coverage using its analytical grids). Percentage of corneal neovascular invasion area was calculated from the transplanted cornea. Visual acuity was measured using snellens acuity chart. Statistical analyses was done using SPSS version 20.0. Student's *t*-tests were applied. $P < 0.05$ was considered significant. Complications occurred were noted as well.

RESULTS

Eighty two patients were included in the study, of them 41 were in the group A (subconjunctival bevacizumab injection), 41 in Group B (subconjunctival sham injection). Out of these 82 patients, males were 61 (74.4%) while females were 21 (25.6%). They were between 47 years of age to 59 years of age with mean age of 51.22 years.

Among the 2 groups mean corneal neovascular invasion area was minimum in the subconjunctival bevacizumab injection group (group A), with p value < 0.05. (Fig. 2). Maximum no of patients (36) attained visual acuity of 6/36 or better in the sub-conjunctival bevacizumab group compared to 17 in sham group. ($p < 0.05$) (Fig. 3).

DISCUSSION

Corneal neovascularization has been renowned as an important risk factor for transplant rejection after keratoplasty. The results of our study suggest that bevacizumab can lessen the sternness of corneal neovascularization when used subconjunctivally. It results in a noteworthy falling off of neovascular invasion area. In addition, bevacizumab when used subconjunctivaly promotes graft survival considerably in the increased peril corneal transplantations.

Even though our results were noteworthy but the regression of corneal neovascularization was not complete. The main reasons for this was that, the quantity and extent of treatment were insufficient to completely antagonize vascular endothelial growth factor. Many apprehensions reported regarding the side effects of bevacizumab for the treatment of

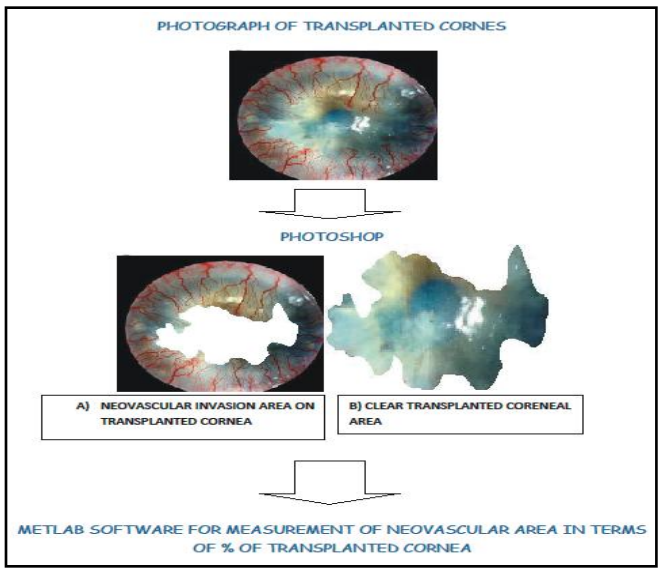

Fig. 1: Method and softwares used for measurement of neovascular invasion area on transplanted cornea

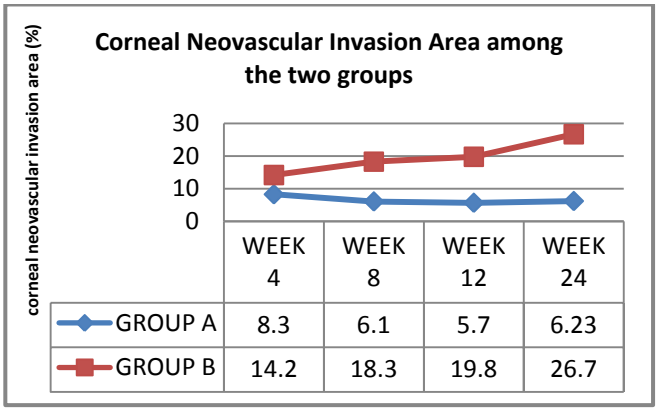

Fig. 2: Corneal neovascular invasion area among the two groups

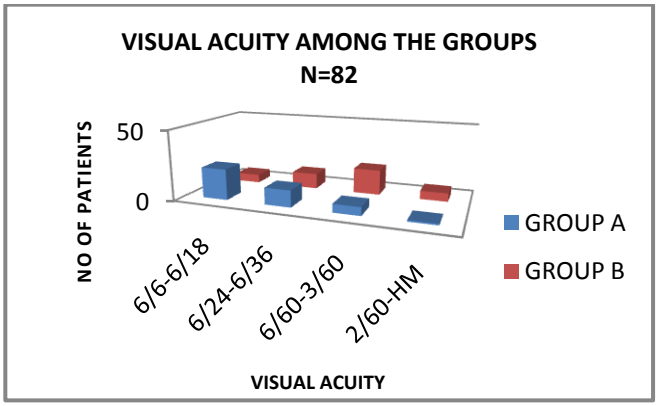

Fig. 3: Visual acuity among the groups n=82

corneal neovascularization,[12] we limited our treatment of bevacizumab to the 8 weeks only after the keratoplasty. Pre-existing corneal vessels in the recipient bed of may not be as susceptible as neovessels to anti-vascular endothelial growth factor treatment[18]. Another important factor of incomplete regression of corneal neovascularization that other relevant proangiogenic factors like fibroblast growth factor, interleukin-1, tumour necrotic factor-α, and IFN-γ are also up regulated and involved in corneal neovascularization[6]. Lastly, bevacizumab is a specific antibody for a subtype of VEGF called VEGF-A only; other types are not covered by it.

Corneal neovescularization is consistently related with increased corneal graft rejection rates, and level of vascularization at the time of corneal transplantation was considered, correlated with corneal graft endurance[19]. One study by Frederick[20] showed that corneal graft rejection occurred in 3.5% of cases with no neovascularization due to endothelial causes, 13.3% of mildly vascular cases, 28% of moderately vascular cases, and 65% of heavily vascular cases. In the present study, significant and marked regression of corneal neovascular invasion area occurred with sub-conjunctival treatments. The only possible complications observed were sub conjunctival hemorrhage which resolved spontaneously after few days.

CONCLUSION

Sub-conjunctival injection of Bevacizumab (Avastin) is very effective in treating corneal neovascularization. It not only reduces neovascularization growth chances but also increases the corneal graft survival. Subconjunctival bevacizumab can offer an adjunctive measure to conservative therapies such as steroids in preventing corneal graft rejection caused by neovascularisation in vascularized (high - risk) corneal transplantations. More work / research is needed to identify the exact amount and frequency of administration to achieve the best clinical results.

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