IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 16, Issue 7 Ver. X (July. 2017), PP 50-54
www.iosrjournals.org

A Randomized Controlled Prospective Study To Assess The Role of Subconjunctival And/or Intrastromal Bevacizumab Preoperatively In Preventing Corneal Graft Rejection In Corneal Scars With Vascularization More Than 2 Quadrants

Dr. Seema Yadav[1], Dr. Ishwar Singh[2], Dr. Sachin Walia[3], Dr. David Aggarwal[4]
[1]*Junior Resident, Department of Ophthalmology, Rajindra Hospital, Patiala, Punjab, India*
[2]*Assistant Professor, Department of Ophthalmology, Rajindra Hospital, Patiala, Punjab, India*
[3]*Professor and Head, Department of Ophthalmology, Rajindra Hospital, Patiala, Punjab, India*
[4]*Junior Resident, Department of Ophthalmology, Rajindra Hospital, Patiala, Punjab, India*

Abstract
Purpose: *To evaluate the safety and efficacy of subconjunctival and/or intrastromal bevacizumab preoperatively in preventing corneal graft rejection in corneal scars with vascularization more than 2 quadrants.*
Methods: *This randomized, placebo controlled clinical trial was conducted on 20 eyes of 20 patients attending the ophthalmology OPD, Rajindra Hospital Patiala, randomized to Group 1 (bevacizumab) 10 patients and Group 2(Balanced salt solution), 10 patients. Group 1 received a cycle of three subconjunctival and/or intrastromal injections of 5mg/0.2ml Bevacizumab. After last injection, all patients underwent keratoplasty. An adjunctive injection was performed on half of the patients intraoperatively at the end of surgery. Group 2 received balanced salt solution in same manner. Each patient was submitted to a complete eye examination. The improvement in BCVA, absence of immune rejection signs in the graft at examination was considered as main outcome measure.*
Results: *There were stastically significant difference between the groups in variables postoperative BCVA, postoperative corneal thickness and intra-operative bevacizumab in preventing corneal rejection.*
Conclusion: *Subconjunctival and/or intrastromal Bevacizumab had significant effect in preventing the corneal graft rejection in corneal scars with vascularization more than 2 quadrants and was not associated with any adverse effects.*
Keywords: *Bevacizumab, Corneal graft rejection, Corneal scars, Vascularization, Keratoplasty*

I. Introduction

Although described for more than 100 years, corneal transplantation has become increasingly common since the 1960s. In 2014, 46,173 corneal transplantations were performed in United States.[1] It has evolved from its primitive form to the present day surgery due to improved eye banking procedures, technical improvement in the form of good suturing material, higher quality microscopes, and better drugs to suppress postoperative inflammation. Graft rejection is one of the leading causes of corneal graft failure. With the introduction of topical steroids, and other immunosuppressive drugs, the graft survival rate has remarkably improved.[2]

Definition: First described by Paufique et al in 1948,[3] later by Maumenee (1951),[4] and later elaborated by Khodadoust and Silverstein in 1969,[5] corneal graft rejection is a specific process in which a graft that has been clear for at least 2 weeks suddenly succumbs to graft edema in conjunction with anterior segment inflammatory signs.[6-7]

Corneal graft rejection is defined as a complex immune mediated process resulting in decompensation of the transplanted cornea. It is characterized by one of the following:[8-9]

- Development of epithelial and or endothelial rejection line and stromal rejection band.
- Recent unilateral anterior chamber reaction with keratic precipitate.
- Increase in corneal thickness (edema) in a previously clear compact graft with visible aqueous cells.

Incidence: About 60,000 corneal grafts are performed worldwide, of which up to 30% of eyes with penetrating keratoplasty experience at least one episode of rejection and about 5-7% lead to eventual graft failure.[10-12] Reported incidence of corneal graft rejection varies from 2.3% to 68%.[13] The study by Alldrege and Krachmer in 1981 reveals the corneal graft rejection occurs in up to 50% of the recipients and is the single most important reason of graft failure [14] Patients with corneal stromal vascularization have a high risk of immunological failure. The degree of vascularization (i.e., number of vessels and number of quadrants involved) is associated with both the risk of rejection and the time interval between penetrating keratoplasty and onset of

rejection. Once corneal rejection occurs, the difficulty of reversal also depends on the degree of corneal vascularization. Most authors consider stromal vascularization of two quadrants or more to be a high risk factor [15-19]

Corneal neovascularization(CN) is associated with high levels of inflammatory cells and mediators within the graft, which could provoke graft rejection.[20] Neovascularization is a sequel of an altered balance between angiogenic and antiangiogenic factors and an insult to the cornea may enhance the production of angiogenic stimulators, such as vascular endothelial growth factor(VEGF).[21]

Until now, the Literature has reported the topical, subconjunctival, intracameral and intrastromal application of Bevacizumab [21], as VEGF inhibitor, to reduce CN; but results were variable and partial. Furthermore, VEGF has an effect on vascular endothelium permeability, recruitment of inflammatory cells, inflammatory mediators release and chemotaxis. [22-24]

II. Methods

This randomized, placebo- controlled clinical trial was conducted on the patients attending the ophthalmology OPD of Rajindra hospital Patiala from July 2016 to June 2017 with corneal scars with vascularization more than 2 quadrants. Written informed consent was obtained from all patients and they were explained about the possible consequences. We interviewed the patient before hand to obtain information like personal data, contact number, any medical or ocular history, any history of drug allergy.

Inclusion Criteria

1. Patients more than 18 years of age
2. High risk transplantation due to corneal neovascularization more than 2 quadrants like patients of post herpetic leucomas and repeated corneal transplant rejections.

Exclusion Criteria

1. Patients with Non-high risk keratoplasty
2. Patients with Glaucoma
3. Patients with any other systemic pathology
4. Patients with allergy to Bevacizumab

All the patients underwent slit lamp examination to see anterior segment. Best corrected visual acuity (BCVA), manifest refraction, IOP (measured by schiotz indentation tonometry), Corneal thickness (measured by Pentacam), and detailed slit lamp examination. Routine investigations like blood pressure, random blood sugar was done to all patients. Topical antibiotic was started prior to day of surgery. We randomized the patients into two groups: A group (10 eyes of 10 patients) and B group (10 eyes of 10 patients).

Surgical Technique

In Group A, eyes were anaesthetized with topical paracaine eye drops and povidone iodine 5% solution was used to disinfect the periocular skin and ocular surface. Subconjunctival and/or intrastromal 5mg/0.2ml Bevacizumab (Avastin;Genentech, Inc., South Francisco, CA, USA) was injected at the site of corneal neovascularization by means of 26G needle. The entire dose was equally distributed in all the quadrants, adjacent to limbus. Instromal and subconjunctival injections were both performed in the presence of main stromal vessel: we injected half dose into the stroma at the origin of major feeder vessel and other half dose into the adjacent conjunctiva. Group B was injected subconjunctival balanced salt solution in the same manner. In Group A patients, a cycle of three Bevacizumab injections was performed monthly by the same surgeon prior to the surgery. After last injection, all patients underwent penetrating keratoplasty. Half of the patients received an adjunctive subconjunctival injection of 5mg/0.2ml Bevacizumab at the end of the keratoplasty procedure.

Group B after receiving balanced salt solution injections monthly underwent penetrating keratoplasty and half of the patients received an adjunctive subconjunctival injection of balanced salt solution at the end of keratoplasty. After surgery, topical (prednisolone eyedrops in a tapering dose for 6 months) and systemic corticosteroids (prednisolone 30mg/day, tapered in a month) with topical antibiotic (1% moxifloxacin eyedrops four times a day for 2 weeks) were administered equally in both groups. The entire population study was submitted to slit lamp examination before keratoplasty and during follow up to detect signs of corneal graft rejection. Follow up visits were performed at 1 day, 7 days, 1 month, 3, 6 months postoperatively. The clinical absence of immune rejection signs in the graft was considered as main outcome measure in Bevacizumab treated cases. In postoperative visits, following factors are evaluated refraction, IOP, corneal thickness, slit lamp examination and signs of graft rejection (development of endothelial and epithelial rejection line and stromal rejection band, recent anterior chamber reaction with keratic precipitate, increase in corneal thickness in a previously clear compact graft with visible aqueous cells).

II. Results

The study was conducted on 20 eyes of 20 patients with 10 eyes in each group. All patients completed the postoperative visits, except two who were lost to follow-up at month three and another subject at month six. There was no stastically significant difference between the study groups in terms of demographic data, operated eye, preoperative BCVA, corneal vascularization, preoperative corneal thickness and IOP (Table 1).

There is stastically significant improvement in postoperative BCVA (p=0.041) in between the study groups and postoperative corneal thickness in Group A (540.30±25.81) from Group B (595.70±68.854). However there is no stastically significant difference between the study groups in terms of postoperative epithelial rejection line, endothelial rejection line, stromal rejection band, anterior chamber reaction and IOP (Table 2).

As shown in Table 3 half of the patients in Group A received intra-operative bevacizumab (p=0.010) along with the pre-operative bevacizumab and revealed stastically significant difference (Table 3).

Table 1. Demographics and ocular characteristics of the study patients

	Group A	Group B	X^2	p value
Number	10	10		
Gender (Female/Male)	3/7	4/6	0.220	0.639
Eye (Right/Left)	6/4	4/6	0.800	0.371
Age (Years)	64.50±5.968	63.70±5.736	0.306	0.763
Corneal vascularization (3/4Quadrants)	7/3	7/3	1	1
Preoperative BCVA			1.024	0.906
6/60	1	1		
FC 1M	1	2		
FC 2M	3	2		
HMNF	4	3		
PL	1	2		
Preoperative Corneal thickness (µm)	525.00±14.174	534.60±7.706	1.882	0.076
Preoperative IOP (mmHg)	16.90±1.729	16.90±2.132	0.000	1.000

BCVA, best corrected visual acuity; IOP, intraocular pressure FC, finger counting, HMNF, hand movement near face, PL, perception of light, Values are presented in mean ± standard deviations

Table 2. Postoperative variables

	Group A	Group B	X^2	p value
Postoperative BCVA			10.080	0.041
6/24	4	0		
6/36	4	2		
6/60	1	4		
HMNF	1	4		
Postoperative epithelial rejection line (-/+)	10/0	9/1	1.053	0.305
Postoperative endothelial rejection line (-/+)	9/1	8/2	0.392	0.531
Postoperative stromal rejection band (-/+)	8/2	6/4	0.952	0.329
Anterior chamber reaction			3.619	0.306
-	8	6		
1+	1	0		
2+	1	2		
3+	0	2		
Postoperative corneal thickness (µm)	540.30±25.281	595.70±68.854	2.388	0.028
Postoperative IOP (mmHg)	17.50±1.841	17.80±1.317	0.419	0.680

Table 3. Intra-operative Bevacizumab

	Group A	Group B	X^2	p value
Intraoperative bevacizumab (-/+)	5/5	10/0	6.667	0.010

III. Discussion

Corneal neovascularization is considered one of the major risk factor for graft failure after keratoplasty. Various substances have been previously reported as potential vessel inhibitors, such as corticosteroids, non-steroidal anti-inflammatory drugs, methotrexate [25], cyclosporine A [26], thalidomide [27]. However, all these drugs may cause several side effects. The present study was conducted to evaluate the safety and efficacy of subconjunctival and/or intrastromal bevacizumab preoperatively in preventing corneal graft rejection in corneal

scars with vascularization more than quadrants. This is compatible with some other studies reporting beneficial effect from bevacizumab administration on preventing corneal graft rejection in corneal scars with vascularization. In the present study, all the patients were followed for at least 6 months. Corneal graft rejection was defined as development of epithelial and or endothelial rejection line and stromal rejection band, recent unilateral anterior chamber reaction with keratic precipitate, increase in corneal thickness (edema) in a previously clear compact graft with visible aqueous cells. The rejection rate in group A is less than in group B and no serious ocular effect was observed.

Bevacizumab, as anti-VEGF has been shown in experimental models [21] and in humans [28-30]. It also induced more effective regression of CN compared to Ranizumab. [31] Another study, the effect of subconjunctival Bevacizumab as adjunctive treatment to prevent graft rejection in high risk corneal transplantation was supported in murine model. The parameter analyzed was the mean percentage reduction of neovascular area after drug injections. [32] Our findings could be explained with the Bevacizumab inhibition of the vascular endothelium permeability, inflammatory mediators release and chemo tactic effect of VEGF. [21] As suggested by Cursiefen et al. [33], VEGF-A recruitment of inflammatory cells, particularly monocytes and macrophages, plays a critical role in inducing inflammatory neovascularization by supplying and amplifying signals essential for pathological hem angiogenesis and lymph angiogenesis.

IV. Conclusion

In conclusion, this study found that subconjunctival and/or intrastromal Bevacizumab shows significant reduction in corneal graft rejection in corneal scars with vascularization more than quadrants and was also not associated with local adverse effects. The limited number of patients, lack of routine follow-up were the shortcomings of the study.

References

[1]. Eye Bank Association of America 2014 Eye Banking Stastical Report. Available at http://www.restoresight.org/wp-content/uploads/2015/03/2014_Stastical_FINAL.pdf. Accessed: June 16, 2015
[2]. Banerjee S, Dick AD: Recent developments in the pharmacological treatment and prevention of corneal graft rejection. Expert Opin Investig Drugs 12:29-37, 2003
[3]. Shapiro MB, Mandel Mr., Krachmer H: Rejection, in Bright B (ed): Corneal Surgery, Theory, Technique and Tissues. St LOUIS, cv Mosby Company, ed 2 1997, pp. 254-68
[4]. Maumenee AE: Clinical aspects of the corneal homograft reaction. Invest Ophthalmol 1:244-52, 1962
[5]. Khodadoust AA, Silverstein AM: Transplantation and rejection of individual cell layers of cornea. Invest Ophthalmol 8:180-95, 1969
[6]. Koay PY, Lee WH, Figueiredo FC, et al: Opinions on risk factors and management of corneal graft rejection in the United Kingdom. Cornea 24:2992-6,2005
[7]. Miedziak AI, Tambasco FP, Lucas-Glass TC, et al: Evaluation of triggers for corneal graft rejection. Ophthalmic Surg Lasers 30:133-9,1999
[8]. Maumenee AE: Clinical aspects of corneal homograft reaction. Invest Ophthalmol 1244-52, 1962
[9]. Polak FM: Histopathological and histochemical alterations in the early stage of graft rejection. J Exp Med 116:709-13, 1962
[10]. [10]. _: The Australian Corneal Graft Registry. 1990 to 1992 report. Aust NZ J Ophthalmol 21(Suppl):1-
[11]. 48, 1993
[12]. DES Marchais B, Bazin R, Boisjoly HM, et al: Role of presensitization and donor-recipient cross matching in corneal graft outcome. Cornea 17:141-5, 1998
[13]. Pleyer U, Milani JK, Dukes A, et al: Effect of topically applied anti- CD4 monoclonal antibodies on orthotopic corneal allografts in ra model. Invest Ophthalmol Vis Sci 36:52-61, 1995
[14]. Shapiro Mb, Mandel MR, Krachmer JH: Rejection, in Bright B (ed): Corneal Surgery, Theory, Technique and tissues. St Louis, CV Mosby Company, ed 2 1997, pp. 254-68
[15]. Polak FM: Corneal transplantations. Invest Ophthalmol Vis Sci 12:85-93, 1973
[16]. Hill JC, Immunosupression in corneal transplantation. Eye 1995:9:247-53.
[17]. The Collaborative Corneal Transplantation Studies (CCTS) Research Group. Effectiveness of Histocompatibility matching in high-risk corneal transplantation. Arch Ophthalmol 1992:110:1392-93
[18]. The Collaborative Corneal Transplantation Studies Research Group. Designs and methods of the Collaborative corneal transplantation studies. Cornea 1993; 12:93-103.
[19]. Maguire MG, Stark WJ, Gottsch JD, Stulting RD, Sugar A, Fink NE, et al. Risk factors for corneal graft and rejection in the Collaborative Corneal Transplantation Studies. Ophthalmology 1994; 101:156-47
[20]. Volker-Dieben HJ, D' Amaro J, Kok-Van Alphen CC. Hierarchy of prognostic factors for corneal allograft survival. Aust N Z J Ophthalmol 1987; 15:11-18.
[21]. Awadein A (2007) Subconjunctival bevacizumzab for vascularized rejected corneal grafts. J Cataract Refract Surg 33:1991-1993
[22]. Chang Jin-Hong, Garg Nitin K, Lunde Elisa et al (2012) Corneal neovascularization: an anti-VEGF therapy review. Surv Ophthalmol 57(5):415-429
[23]. Amano S, Rohan R, Kuroki M et al (1998) Requirement for vascular endothelial growth factor in wound and inflammation related corneal neovascularization. Invest Ophthalmol Vis Sci 39:18-22
[24]. Niederkorn JY (2007) Immune mechanisms of corneal allograft rejection. Curr Eye Res 32:1005-1016
[25]. Pleyer U, Schlickeiser S (2009) the taming of the shrew? The immunology of corneal transplantation. Acta Ophthalmol 7:488-497
[26]. Joussen AM, Kruse FE, Volcker HE et al (1999) Topical application of methotrexate for inhibition of corneal angiogenesis. Graefes Arch Clin Exp Ophthalmol 237:920-927
[27]. Lipman RM, Epstein RJ, Hendricks RL (1992) Suppression of corneal neovascularization with cyclosporine. Arch Ophthalmol 110:405-407

[28]. D'Amato RJ,Loughnan MS, Flynn E et al (1994) Thalidomide is an inhibitor of angiogenesis. Proc Natl Acad Sci USA 91:4082-4085
[29]. Benayoun Y, Adenis JP, Casse G et (2012) Effects of subconjunctival bevacizumab on corneal neovascularization: results of a prospective study. Cornea 31 (8):934-944
[30]. Symes RJ, Poole TRG (2010) Corneal graft surgery combined with subconjunctival bevacizumab (Avastin). Cornea 29:691-693
[31]. Acar BT, Halili E, Acar S (2013) The effect of different doses of subconjunctival bevacizumab injection on corneal neovascularization. Int Ophthalmol 33(5):507-513
[32]. Stevenson W, Cheng SF, Dastjerdi MH et al (2012) Corneal neovascularization and the utility of topical VEGF inhibitor: ranizumab (Lucentis) vs bevacizumab (Avastin). Ocul Surf 10(2):67-83
[33]. Dastjerdi NH, Saban DN, Okanobo A et al (2010) Effects of topical and subconjunctival bevacizumab in high risk corneal transplant survival. Invest Ophthalmol Vis Sci 51(5):2411-2417
[34]. Cursiefen C, Chen Lu, Borges LP et al (2004) VEGF-A stimulates lymphangiogenesis and hemangiogenesis in inflammatory neovascularization via macrophages recruitment. J Clin Invest 113:1040-1050

Dr. Seema Yadav. "A Randomized Controlled Prospective Study To Assess The Role of Subconjunctival And/Or Intrastromal Bevacizumab Preoperatively In Preventing Corneal Graft Rejection In Corneal Scars With Vascularization More Than 2 Quadrants." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) 16.7 (2017): 50-54.