Original Research Article

Clinico-pathological correlation of patients undergoing keratoplasty for suspected HSV keratitis in tertiary care centre

Swati Tomar[1], Anant Sharma[1*], Yogesh Shukla[1], Veena Saxena[2]

[1]Department of Ophthalmology, [2]Department of Pathology
Nims Medical College and Hospital, Nims University, Jaipur, Rajasthan, India
[*]Corresponding author email: **doc.anant.sharma@gmail.com**

International Archives of Integrated Medicine, Vol. 2, Issue 7, July, 2015.

Available online at http://iaimjournal.com/
ISSN: 2394-0026 (P) **ISSN: 2394-0034 (O)**
Received on: 23-06-2015 **Accepted on:** 30-06-2015
Source of support: Nil **Conflict of interest:** None declared.

Abstract

Aim: To identify histopathologic features of HSV keratitis and correlate them to their clinical findings following penetrating keratoplasty for suspected HSV keratitis and predict the graft outcome on oral Acyclovir treatment.

Material and methods: A clinical study of 20 patients who presented to us with clinical picture suspected of HSV keratitis that underwent penetrating keratoplasty at a teaching hospital situated in rural area of India. Out of 20 patients 18 were primary grafts and in 2 patients repeat penetrating keratoplasty was done following graft failure due to rejection. A detailed review of the histopathology of the excised corneal button was performed to identify associations between clinical data (disease activity, vascularity, graft outcome) and histopathologic data (inflammation, neovascularization, gross). Patients with features of HSV keratitis on histopathology were started on oral prophylactic acyclovir therapy post penetrating keratoplasty.

Results: All patients had clinically quiescent disease for at least 6 months before surgery. The visual outcome was better in 14 patients on oral acyclovir post penetrating keratoplasty in suspected HSV keratitis positive on histopathology including 2 repeat keratoplasty which were not earlier started on oral acyclovir. Histopathology of 12 cases including 2 repeat Penetrating keratoplasty revealed active corneal inflammation with epithelial irregularities along with patchy loss of Bowman's membrane, infiltration of anterior stroma by lymphocytes, leukocytes and plasma cells. Diffuse fibrosis and neovascularisation of stroma was present which was correlated clinically. No recurrence was seen in these patients on follow up of 1 year. Of the 6 patients without any histopathologic inflammation in their corneas, only 1 experienced an allograft rejection.

Conclusion: Histopathological inflammation is a marker for HSV status and its correlation helps in judicious use of acyclovir and reduces the risk of recurrence of HSV keratitis. Oral acyclovir therapy

post penetrating keratoplasty acts as adjunct to improve the visual outcome in patients with positive histopathological changes.

Key words

Histopathology, Keratoplasty, HSV, Acyclovir.

Introduction

Patients undergoing penetrating keratoplasty (PKP) for sequelae of herpes simplex virus (HSV) keratitis are at higher risk for adverse corneal allograft outcomes. The post-operative course can be complicated by high rates of HSV recurrence, graft rejection, and graft failure. Prior histopathologic evaluation for HSV in host corneal tissue removed at the time of Penetrating keratoplasty can lead to better graft outcome. So in this study we aimed to identify histopathologic features of HSV keratitis and correlate them to their clinical findings following penetrating keratoplasty for suspected HSV keratitis and predict the graft outcome on oral acyclovir treatment.

Material and methods

This study included retrospective analysis of 20 cases of corneal opacities that underwent keratoplasty for suspected HSV keratitis. The Study was conducted between the periods of June 2012 to September 2013. Reviews of the patients' clinical findings and the histopathologic slides of their excised corneal buttons were performed to identify association between clinical and histopathological findings. Out of 20 patients, 18 were primary grafts and in 2 patients repeat penetrating keratoplasty was done following graft failure due to rejection. Approval from ethical committee of our institute was taken for the study.

Results

Host corneal tissue was evaluated for the presence of histopathological changes like Epithelial irregularities, Pathy loss of bowman's membrane or bowman's membrane detachment, infiltration of anterior stroma by lymphocytes, plasma cells, neutrophils, Diffuse fibrosis, Neovascularisation of stroma (**Figure - 1B, 2B, 3B, 4B**). Each of the 20 specimens were rendered a pathological diagnosis and graded for the presence of inflammation (none visible, present) and for the presence of neovascularization (none visible, present). Out of total 20 cases, 16 were males and 4 were females (**Graph - 1**). Mean age at presentation was 56 (range 7-80 years), disease presentation range from 5 to 70 years. Average follow up was from 6 months to 1 year. 14 patients out of total 20 showed corneal histopathological changes and were started on oral acyclovir 400 mg twice daily postoperatively (**Table - 1**). Corneal neovascularization was clinically present preoperatively in 16 (80%) patients (**Figure - 1A, 2A**) and was seen histopathologically in 14 (70%) of the specimens excised during penetrating keratoplasty (**Figure - 1B, 2B**). Neovascularisation was seen in 14 patients including 2 repeat Penetrating keratoplasty (**Figure - 3B**). Corneal infiltration was seen in 12 patients including 2 repeat penetrating keratoplasty (**Figure - 3B**). In the remaining 6 patients no inflammation was seen and Out of these 6, only 1 experienced allograft rejection (**Table - 2**). Patients with clinical evidence of neovascularization preoperatively were found to have histopathologic evidence of inflammation postoperatively. (**Figure – 3A, 4A**)

Discussion

Study on Herpes simplex virus keratitis for histopathology and corneal allograft outcomes depicted the histopathologic evidence of neovascularization or inflammation in tissue removed at the time of corneal transplantation predicts patients who are at risk for allograft rejection or failure. Such patients are prime candidates for close monitoring and intensive therapy [1]. Histopathologic presence of

neovascularization for allograft failure in herpes simplex virus (HSV) keratitis revealed 31% of corneal specimens with neovascularisation and predicted subsequent allograft failure out of 62 corneas in study [2-5].

Figure - 1A: Clinical picture of patient eye illustrating diffuse corneal opacity with vascularisation.

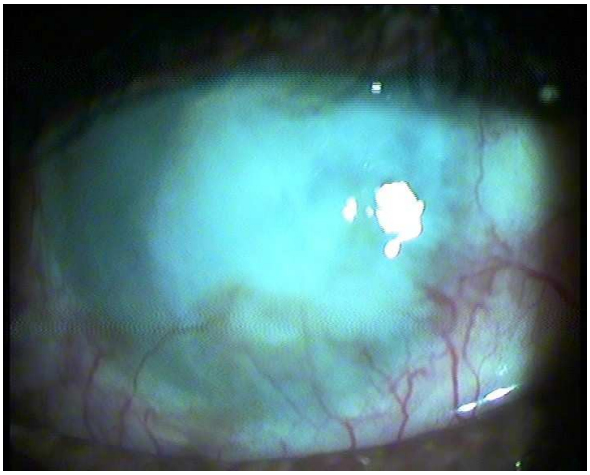
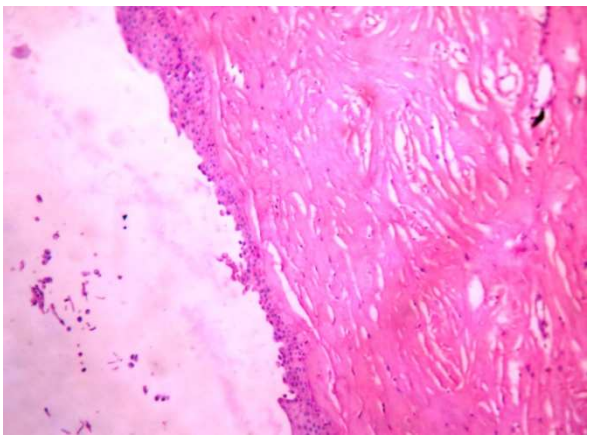


Figure – 1B: Histopathological picture showing patchy loss of bowman's membrane with stromal infiltration and neovascularization and fibrosis.



Numerous prior studies have shown that the preoperative clinical presence of neovascularization is a risk factor for corneal allograft failure [6-8]. Further, allograft rejection episodes have been shown to be more difficult to treat in patients with preoperative clinical neovascularization [9]. Very little prior research has been done evaluating the histopathologic features of corneal tissue removed at the time of corneal transplantation.

Figure - 2A: Clinical picture of patient eye depicting full thickness corneal opacity with 360^0 superficial vascularisation.

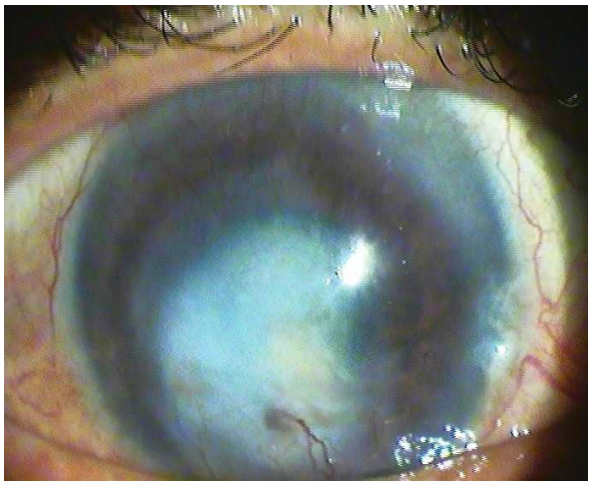


Figure – 2B: Histopathological picture depicting fibrosis and prominent dilated blood vessels with RBC.

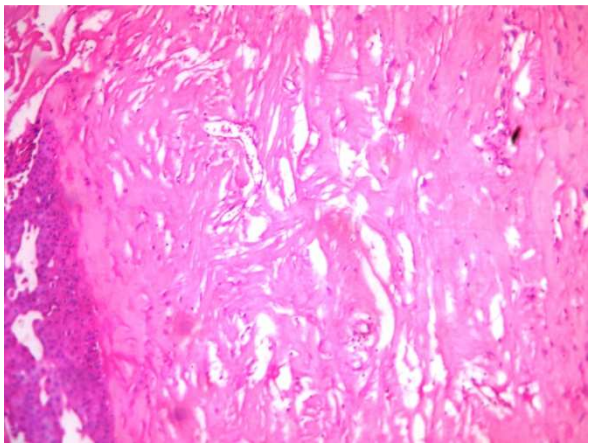


Figure – 3A: Clinical picture of patient eye post penetrating keratoplasty graft failure.

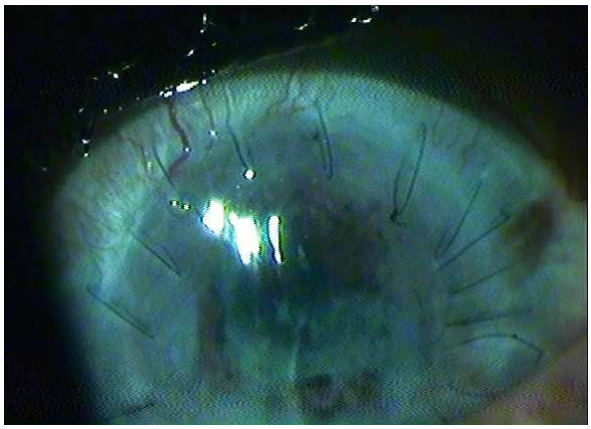


Figure – 3B: Histopathological picture with stromal infiltration and neovascularization.

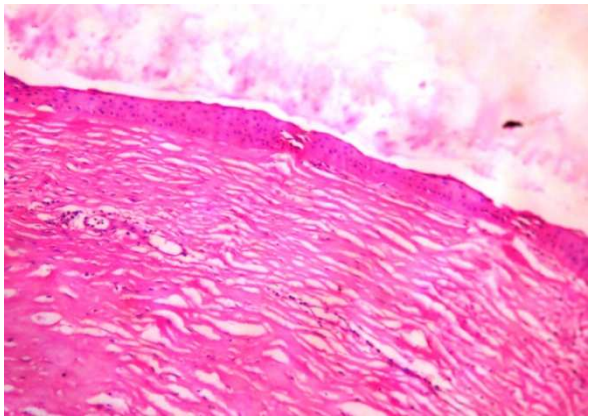


Figure – 4A: Central and paracentral corneal scar post herpes stromal keratitis.

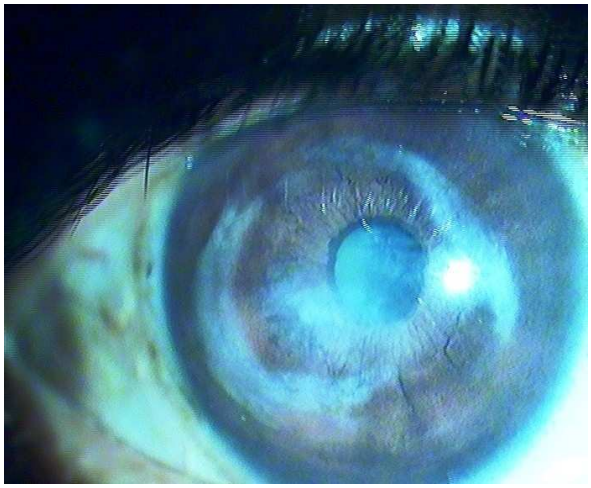
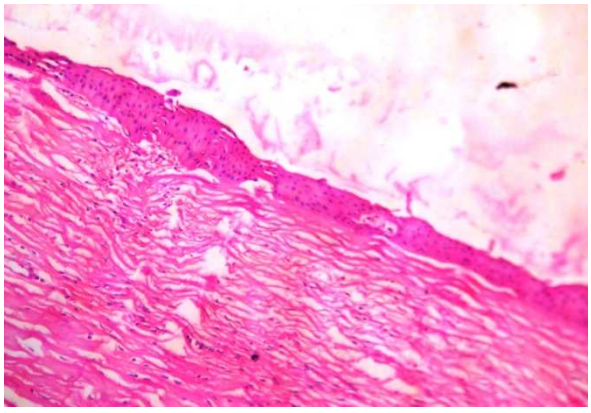


Figure – 4B: Stromal infiltration in corneal on histopathology.



One study published in 2004 by Branco and associates [10], looked at the records of all corneal tissue submitted from 1972 to 2001 to the pathology laboratory at the University of California at San Francisco. There were 4,207 grafts performed, 76 (1.8%) of which were for HSV keratitis. They reported on the pathological findings in corneas with a clinical diagnosis of HSV keratitis including neovascularization in 59%. The authors did not comment on what effect the presence of this finding had on subsequent allograft failure or HSV recurrence. We found that the histopathologic presence of neovascularization in the corneal tissue is an important predictor of graft failure. Its presence within the central corneal tissue that is removed at the time of PKP probably indicates that these patients had severe stromal disease placing them at higher risk for subsequent failure. The histopathologic presence of blood vessels in the corneal tissue indicates that the neovascularization extends to the edge of the host corneal bed and is therefore present at the graft margin at the time of transplantation. Identifying these features in patients and their excised tissues may be helpful in identifying the patients who are at highest risk for adverse allograft outcomes. These findings are of practical significance to the clinician in care of patients after PKP. Corneal neovascularization was present preoperatively in 80% of patients on clinical examination. However, it was found histopathologically in 70% of the specimens excised during PKP. This discrepancy may have resulted from clinical examinations which documented peripheral preoperative corneal neovascularization in tissue not removed during PKP. Less likely is that histopathologic sectioning did not include an axis involved with neovascularization as the specimens were examined under a dissecting microscope and sectioned along the axis of greatest neovascularization as described above. There was a statistically significant correspondence between the presence of preoperative clinical corneal neovascularization and the histopathologic presence of inflammation in the same corneal specimens after removal at PKP ($P = 0.01$). This suggests that corneal neovascularization is an important preoperative clinical factor indicative of actual inflammation in the diseased cornea. This association between preoperative clinical corneal neovascularization

and the histopathologic presence of inflammation in the excised tissue has important pathogenetic and clinical implications. Neovascularization may be essential to the delivery of cellular and serum components of inflammation and host immune responses, setting the stage for graft rejection and failure [11, 12].

Table - 1: Graft outcome of patients with histopathological changes started on oral acyclovir.

Case		Graft clarity			Visual outcome			Recurrence
	Pre-op VA	Post-op day			Post-op day (BCVA)			At 1 year
		1ST day	6 mon	1 year	1ST day	6 mon	1 year	
1	HMCF	CLEAR	SE	CLEAR	6/36	6/60	6/24	NONE
2	HMCF	SE	CLEAR	CLEAR	6/60	6/24	6/12	NONE
3	FCCF	CLEAR	CLEAR	CLEAR	6/36	6/18	6/9	NONE
4	HMCF	SE	SE	CLEAR	6/36	6/36	6/18	NONE
5	FC 1M	CLEAR	SE	CLEAR	6/36	6/36	6/12	NONE
6	FC 2M	SE	SE	SE	6/36	6/24	6/24	NONE
7	HMCF	SE	CLEAR	CLEAR	6/36	6/18	6/9	NONE
8	HMCF	SE	CLEAR	CLEAR	6/36	6/18	6/12	NONE
9	FC 2M	CLEAR	SE	SE	6/24	6/36	6/24	NONE
10	FC 3M	CLEAR	CLEAR	CLEAR	6/24	6/12	6/9	NONE
11	HMCF	SE	CLEAR	CLEAR	6/60	6/24	6/12	NONE
12	FCCF	CLEAR	SE	SE	6/36	6/36	6/24	NONE
13	HMCF	CLEAR	SE	CLEAR	6/24	6/24	6/12	NONE
14	FC 1M	CLEAR	CLEAR	CLEAR	6/24	6/18	6/9	NONE

Table - 2: Graft outcome of patients without histopathological changes not started on oral acyclovir.

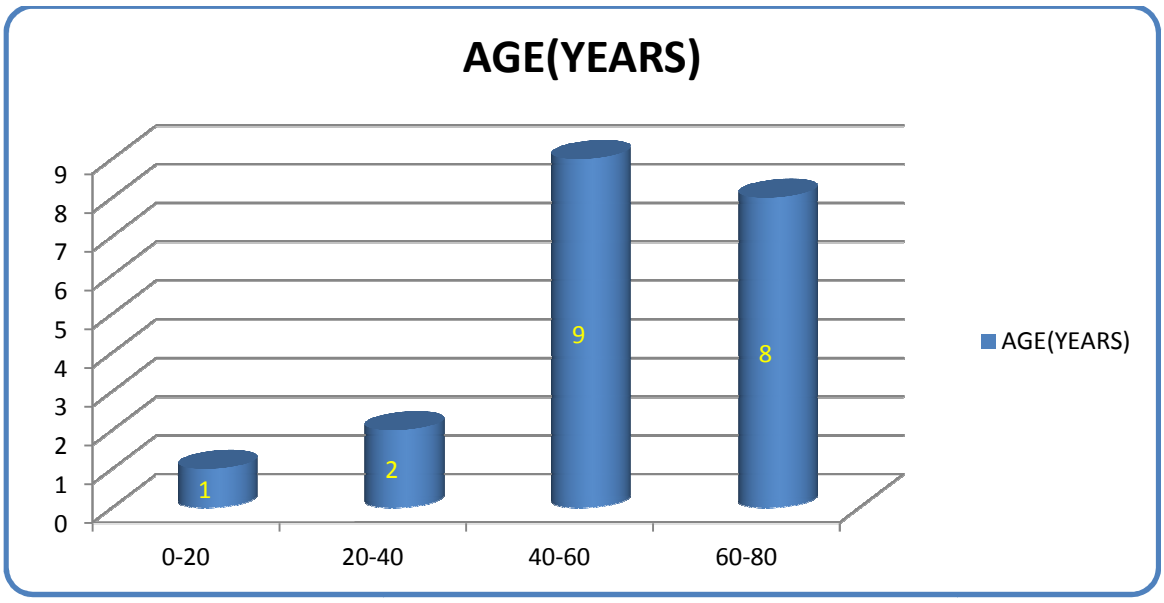
Case		Graft clarity			Visual outcome			Recurrence
	Pre-op VA	Post-op day			Post-op day (BCVA)			At 1 year
		1ST day	6 mon	1 year	1ST day	6 mon	1 year	
1	HMCF	CLEAR	CLEAR	CLEAR	6/36	6/24	6/9	NONE
2	FCCF	CLEAR	SE	CLEAR	6/36	6/24	6/12	NONE
3	FC 1M	SE	CLEAR	CLEAR	6/60	6/24	6/18	NONE
4	HMCF	SE	SE	SE	6/60	HMCF	HMCF	REJECTION
5	FC 1M	SE	CLEAR	CLEAR	6/60	6/36	6/24	NONE
6	HMCF	SE	CLEAR	SE	6/60	6/24	6/18	NONE

(S.E. - stromal edema, FCCF - finger counting close to face, HMCF - hand movements close to face, BCVA - best corrected visual acuity)

Despite the retrospective nature of this study, the histopathologic features of neovascularization in the removed host corneal tissue were well defined, as were the clinical endpoints of allograft failure and HSV recurrence. This lends confidence that the conclusions drawn from the study are likely to be true and clinically relevant. Moreover, as the histopathologic findings were determined in tissue grossed and processed in the usual fashion for corneal surgical pathology specimens, the observations made are relevant to routine clinical practice. This establishes a role

for the pathologist in assisting the clinician in their choice of postoperative patient management. Graft failure in individuals with HSK is most commonly attributable to allograft rejection and herpetic disease recurrence [13], and our favorable results may reflect the instigation of combined antiviral and local immunosuppressive therapy immediately after transplantation, as recommended by Ficker [14]. The functional outcome of corneal grafts in herpetic eyes has also been reported to be markedly improved by the systemic administration of acyclovir [15].

Graph - 1: Age distribution of cases.



Acyclovir penetrates the eye well and, when administered at a dosage of 400 mg five times per day, furnishes the aqueous humour with levels that are likely to reduce corneal pools of the virus. Despite its widespread use it is extremely rare for clinical isolates to exhibit resistance. Since viral replication is known to be effectively inhibited by acyclovir [15], attempts have been made to combine its topical delivery with topical steroid therapy. Success with this combined treatment was first achieved by the Herpetic Eye Disease Study. The strategy being found to hinder the progression of disease and curtail its duration without increasing the risk of HSV recurrence. Many authors have reported on the existence of a positive correlation between graft failure rate and corneal vascularization [14].

Conclusion

Males were more commonly affected than females. Average age of presentation was 56 years. The most common indication for surgery was corneal opacification leading to diminision of vision (80%). The range of visual acuity pre-operatively varied from HM to FC and that of post-operative varied from 6/60 to 6/9 (table 1 and table 2). Histopathologic evaluation post penetrating keratoplasty improves our understanding of the underlying disease process in HSV keratitis and helps to customize the postoperative treatment plan. Histopathological inflammation and corneal neovascularization is a marker for HSV status and its correlation helps in judicious use of acyclovir and reduces the risk of recurrence of HSV keratitis. Oral acyclovir therapy post keratoplasty acts as a adjunct to improve the visual outcome in patients with considerate histopathological changes.

References

1. Roni M Shtein, Victor M Elner. Herpes simplex virus keratitis: Histopathology

and corneal allograft outcomes. Expert Rev Ophthalmol., 2010; 5(2): 129–134.
2. Denise D. Garcia, Roni M. Shtein, david c. Musch, Victor M. Elner. HSV keratitis: Histopathologic neovascularization and corneal allograft failure. Cornea, 2009; 28(9): 963–965.
3. Garcia Dd, Farjo Q, Musch Dc, Sugar A. Effect of prophylactic oral acyclovir after penetrating keratoplasty for herpes simplex keratitis. Cornea, 2007; 26: 930–934.
4. Ghosh S, Jhanji V, Lamoureux E, Taylor Hr, Vajpayee Rb. Acyclovir therapy in prevention of recurrent herpetic keratitis following penetrating keratoplasty. Am. J. Ophthalmol., 2008; 145: 198–202.
5. Lomholt Ja, Baggesen K, Ehlers N. Recurrence and rejection rates following corneal transplantation for herpes simplex keratitis. Acta Ophthalmol Scand., 1995; 73: 29–32.
6. Boisjoly HM, Tourigny R, Bazin R, et al. Risk factors of corneal graft failure. Ophthalmology, 1993; 100: 1728–1735.
7. Price FW, Whitson WE, Johns S, Gonzales JS. Risk factors for corneal graft failure. J Refract Surg., 1996; 12: 134–143.
8. Yamagami S, Suzuki Y, Tsuru T. Risk factors for graft failure in penetrating keratoplasty. Acta Ophthalmol Scand., 1996; 74: 584–588.
9. Alldredge OC, Krachmer JH. Clinical types of corneal transplant rejection. Their manifestations, frequency, preoperative correlates, and treatment. Arch Ophthalmol., 1981; 99: 599–604.
10. Branco BC, Gaudio PA, Margolis TP. Epidemiology and molecular analysis of herpes simplex keratitis requiring primary penetrating keratoplasty. Br J Ophthalmol., 2004; 88: 1285–1288.
11. Murthy RC, McFarland TJ, Yoken J, et al. Corneal transduction to inhibit angiogenesis and graft failure. Invest Ophthalmol Vis Sci., 2003; 44: 1837–1842.
12. Gebhardt BM, Shi W. Experimental corneal allograft rejection. Immunol Res., 2002; 25: 1–26.
13. Cobo LM, Coster DJ, Rice NS, et al. Prognosis and management of corneal transplantation for herpetic keratitis. Arch Ophthalmol, 1980; 98: 1755–9.
14. Ficker LA, Kirkness CM, Rice NSC, et al. Long term prognosis for corneal grafting in herpes simplex keratitis. Eye, 1988; 2: 400–8.
15. Jones BR, Fison PN, Cobo ML, et al. Efficacy of acycloguanosine (Wellcome 248u) against herpes simplex ulceration. Lancet, 1979; 1: 243–4.