

## WORLD VIEW

## Risk factors for graft infection in India: a case-control study

R B Vajpayee, S K Boral, T Dada, G V S Murthy, R M Pandey, G Satpathy

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W V Good and S RuitSee end of article for  
authors' affiliationsCorrespondence to:  
Rasik B Vajpayee, Dr  
Rajendra Prasad Centre for  
Ophthalmic Sciences, All  
India Institute of Medical  
Sciences, Ansari Nagar,  
New Delhi-110029, India;  
[rasikvajpayee@rediffmail.com](mailto:rasikvajpayee@rediffmail.com)Accepted for publication  
31 October 2001**Aim:** To study the demographic, clinical, and microbiological profile and the risk factors for graft infection following penetrating keratoplasty.**Methods:** 50 eyes of 50 consecutive patients with graft infection after an optical penetrating keratoplasty were included as cases; 50 eyes of 50 patients with no graft infection were included as controls. The main variables evaluated in this study included the clinical and microbiological profile, sociodemographic status, suture related problems, persistent epithelial defects, and ocular surface disorders.**Results:** Cultures were positive in 43 (86%) eyes and *Staphylococcus epidermidis* (67.4%) was the most common organism isolated. Infection could be resolved with treatment in 37 (74%) eyes. In eight (16%) eyes the graft melted and a repeat penetrating keratoplasty had to be performed. Only 6% of the cases could achieve a best corrected visual acuity of 6/18 or better after resolution of the infection. In multivariate logistic regression analysis persistent epithelial defect (OR (95% CI): 3.0 (1.17 to 8.33)), suture related problems (OR (95% CI): 3.6 (1.39 to 9.25)), and ocular surface disorders (OR (95% CI): 2.4 (0.93 to 6.03)) were found to be statistically significant risk factors for graft infection following an optical penetrating keratoplasty.**Conclusions:** *Staphylococcus epidermidis* is the commonest organism responsible for post-keratoplasty microbial keratitis. Persistent epithelial defects, suture related problems, and ocular surface disorders are the major risk factors predisposing to graft infection.

Microbial keratitis following corneal transplant surgery leads to graft failure in the majority of affected patients and is associated with a very poor visual outcome.<sup>1–3</sup> The infection can be acquired at any time following surgery but usually occurs more commonly during the first 6 postoperative months.<sup>4</sup> While early postoperative infection may arise because of recurrence of the host disease, infected donor corneas, or intraoperative contamination late infection is usually caused by pathogens that are acquired from the environment.<sup>5,6</sup>

Several factors that may predispose to graft infection include recurrence of herpes simplex keratitis,<sup>7</sup> suture related problems,<sup>1–6,8–11</sup> persistent epithelial defects,<sup>1,4,6,11</sup> ocular surface disorders including dry eye,<sup>2,6,8,11</sup> graft failure,<sup>2,8</sup> use of soft contact lenses,<sup>1,6,8,12</sup> graft hypoaesthesia,<sup>5,8</sup> and lid abnormalities.<sup>1,6,8</sup>

There is a paucity of studies on graft infection following optical penetrating keratoplasty from the Indian subcontinent.<sup>13</sup> Therefore, in the present work we conducted a case-control study to determine the demographic, clinical, and microbiological risk factors for graft infection in India.

## METHODS

Fifty consecutive eyes of 50 patients who developed microbial keratitis after a successful optical penetrating keratoplasty performed during the study period extending from January 1998 to December 1999 were enrolled as cases in the present study. Fifty patients with a comparable age, who retained a clear graft up to 16 months after the surgery, were included as controls after giving informed and written consent. All subjects enrolled in the study were taken from the cornea service of RP Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi. Paediatric subjects (age  $\leq 15$  years) were excluded from the present study.

All surgical procedures were performed using standard microsurgical technique used for penetrating keratoplasty.

The donor size ranged from 7.5 mm to 9.0 mm in all the subjects enrolled in the study. Sutures of 10-0 monofilament nylon were used in all the cases and the type of suturing technique used was interrupted, continuous, or combined. The donor corneas were decontaminated by sterile saline wash followed by treatment with 5% povidone iodine and 0.3% gentamicin. The donor corneas were stored in McCarey-Kaufman (M-K) medium.

Postoperative medications consisted of 0.1% betamethasone eye drops (initiated at 4 hourly intervals and gradually tapered to twice daily at 6 months), 0.3% ciprofloxacin drops, tear supplements, and antiglaucoma medications where required. The antibiotics drops were stopped between 6–8 weeks after surgery. All patients were switched over to 0.1% fluorometholone 0.1% eye drops twice a day after 6 months and these were continued up to 12 months. Any loose or broken sutures were removed in the postoperative period and exposed suture knots were buried. Tarsorrhaphy was performed for large sized/persistent epithelial defects that did not respond to conservative management (ocular lubricants, patching, and bandage contact lens). All patients were advised to seek treatment immediately if they felt a persistent foreign body sensation, pain, drop in vision, and if a "white spot" was noticed in their eyes.

For the purpose of the study, graft infection was defined as an epithelial defect overlying an area of stromal infiltration and associated anterior chamber reaction on slit lamp biomicroscopic examination. An epithelial defect which did not heal within a week after surgery was categorised as a persistent epithelial defect. Eyes with conjunctival scarring, trichiasis, or dry eye were classified under ocular surface disorders.

The parameters evaluated were age, sex, incidence of graft infection, predisposing risk factors, socioeconomic status (including family literacy score and per capita income score), organisms isolated, success of medical therapy/surgery, and the final visual outcome.

At the initial visit, relevant history, especially with regard to the presence of any predisposing factors such as the presence of ocular surface disorders, trauma, contact lens use, systemic illness and previous history of graft infection, were elicited from the patient. Clinical examination included a record of the pretreatment visual acuity, slit lamp biomicroscopy to determine location/extent of epithelial defect, location and extent of the ulcer/infiltrate, the presence of hypopyon, graft clarity, status of the sutures, presence of any suture abscesses, and digital estimation of intraocular pressure. Ultrasonography of the posterior segment was performed to rule out vitreous exudation suggestive of endophthalmitis.

#### Microbiological investigations and treatment protocol

Corneal scrapings were obtained under 0.5% topical oxybutyprocaine (proparacaine) anaesthesia. Routine Gram staining and potassium hydroxide (KOH) wet mount were done to examine the smears. The specimens obtained were inoculated onto blood agar, chocolate agar plates, and Sabouraud's agar tubes for culture of bacteria and fungi. In vitro disc diffusion tests were performed on culture positive cases to determine the antibiotic sensitivity profile. A positive culture was defined as growth of more than one colony of an organism in the inoculating streak of any culture medium.

Treatment was initiated with a combination of fortified antibiotic drops comprising cephazolin sodium (5%) and tobramycin sulphate (1.3%) at half hourly intervals round the clock in all cases of graft infection. In case of detection of a fungus in a smear or in culture, 5% natamycin eye drops were instilled at 1 hourly intervals in the day and at 2 hourly intervals during the night in addition to the other antibiotic drops. Supportive treatment included cycloplegics, antiglaucoma medications, artificial tears, analgesics, and vitamins in all cases. Treatment was modified according to the culture sensitivity pattern and the clinical response. Repeat cultures were sent where the first culture report was found to be sterile. All patients were admitted and examined twice daily until there were signs of improvement, followed by once in a day until discharge; every 3 days for the first week after discharge, weekly for the first month, monthly till the third month, and then at 6 months. Absence of symptoms, disappearance of circumcorneal congestion, absence of infiltration, and lack of staining with fluorescein were the criteria for the resolution of infection.

#### Socioeconomic profile

Since the research was conducted in a tertiary care centre with patients belonging to both rural and urban background, no single composite scale could be adopted for such a heterogeneous population. A new scale (relative socioeconomic status scale) was developed for the purpose of this study. This was piloted in the hospital attendees and was used in an earlier study.<sup>14</sup> The two items used were (a) per capita income and (b) family literacy score. The monthly family income was divided by the total number of members in the family to give the per capita income. The family literacy score was measured by evaluating the literacy of all members in the household older than 15 years and assigning scores by the pooled judgment of the investigators. The total literacy score was then divided by the total number of family members (aged 15+) who contributed to the total literacy score, which yielded the family literacy score. The family literacy score and the per capita income were combined to give a composite relative socioeconomic score with equal weight given to both. These scores were then divided into two groups based upon whether the value was greater or less than the median value—that is, lower composite socioeconomic status and upper composite socioeconomic status, respectively. Since the comparison of socioeconomic status was within the group itself, it was thought to be more sensitive and discriminatory than using

available scales in the country, which have been specifically designed for use exclusively in urban or in rural areas and are not a composite index. The socioeconomic status scale used here divides all patients (both cases and controls) into two categories based on the median value. Since it only considers experiences of patients recruited for the study, it is much more appropriate than the available scales.

#### Statistical analysis

Data were recorded in a predesigned proforma and managed on an Excel spreadsheet. All entries were checked for any possible error. Risk factor analysis was done using STATA Intercooled Version 6.0 (Stata Corp, Houston, TX, USA). The bivariate relation of various potential risk factors with the graft infection (outcome) was statistically evaluated using the  $\chi^2$  test. Variables showing statistically significant association with graft infection at  $p < 0.20$  were considered as potential risk factors for inclusion in binary multivariate logistic regression analysis. Adjusted odds ratio and 95% CI (confidence interval) were calculated for each of the statistically significant risk factors. In this study a  $p$  value  $< 0.05$  was considered as statistically significant.

#### RESULTS

One hundred subjects participated in this study of which 50 had graft infection (cases) and 50 had no graft infection (controls). The mean age (44.8 (SD 15.2) years) of the subjects with graft infection (cases) was comparable with the subjects with no graft infection—that is, controls (43.6 (17.7) years). There were 36 males and 14 females in the case group while the control group comprised 41 males and nine females. Thirty (60%) patients in the case group and 38 (76%) patients in the control group were from a lower composite socioeconomic status.

The location of the ulcer was central/paracentral in 70% of the cases and in all these cases it was within the palpebral aperture. In 30% of the cases the graft infection was peripheral. All cases had a concomitant epithelial defect with stromal infiltration and an associated anterior chamber reaction. The largest diameter of the epithelial defect varied from 2 mm to 8.5 mm.

The indications for penetrating keratoplasty in the eyes that subsequently developed graft infection were corneal scar (16 eyes), healed viral keratitis (eight eyes), healed bacterial keratitis (nine eyes), failed graft (nine eyes), and bullous keratopathy (eight eyes) (Table 1). Indications for keratoplasty in the control group were corneal scar (12 eyes), healed viral keratitis (eight eyes), healed bacterial keratitis (eight eyes), failed graft (six eyes), and bullous keratopathy (16 eyes). Indications in cases and controls were statistically comparable ( $\chi^2 = 3.89$ ,  $p = 0.42$ ).

Interrupted suturing technique was used in 38 (76%) of the cases compared with 46 (92%) of subjects in the control group ( $\chi^2 = 5.41$ ,  $p = 0.056$ ). Continuous suturing technique was used in nine (18%) patients in the case group and two (4%)

**Table 1** Preoperative diagnosis

Indication for penetrating keratoplasty	Cases	Controls
	n=50 (%)	n=50 (%)
Corneal scar	16 (32)	12 (24)
Healed corneal ulcer	9 (18)	8 (16)
Bullous keratopathy	8 (16)	16 (32)
Healed viral keratitis	8 (16)	8 (16)
Failed graft	9 (18)	6 (12)

Cases and controls were statistically comparable.  $\chi^2$  value 3.80,  $p = 0.42$ .

**Table 2** Microbiological spectrum in culture positive cases

Organisms	No of patients (n=43)
<b>Bacteria</b>	
1 <i>Staphylococcus epidermidis</i>	24
2 <i>Staphylococcus aureus</i>	4
3 <i>Acinetobacter</i> species	3
4 <i>Pseudomonas aeruginosa</i>	2
5 <i>Enterobacter</i> species	1
6 <i>Streptococcus pneumoniae</i>	1
<b>Fungus</b>	
1 <i>Aspergillus fumigatus</i>	2
2 <i>Fusarium solanei</i>	1
<b>Mixed</b>	
1 <i>S epidermidis</i> + <i>Aspergillus</i>	1
2 <i>S epidermidis</i> + <i>Fusarium</i>	1
<b>Polymicrobial</b>	
1 <i>S epidermidis</i> + <i>Ps aeruginosa</i>	1
2 <i>S epidermidis</i> + <i>Acinetobacter</i>	1
3 <i>S epidermidis</i> + <i>Strep pneumoniae</i>	1

patients in the control group ( $\chi^2 = 5.4$ , p value = 0.055). Combined continuous and interrupted suturing technique was used in three (6%) cases and two (4%) controls. Both the groups were essentially similar with respect to the size of the donor cornea used.

Thirty five (70%) eyes developed infection within the first 6 weeks after surgery while 15 (30%) eyes acquired infection 6 weeks after the surgery. In eyes with late onset infection, eight (16%) eyes developed graft infection, 1 year after penetrating keratoplasty. The maximum interval between the penetrating keratoplasty and onset of graft infection was 1 year and 3 months.

### Microbiological profile

On examination of the corneal smears, Gram stain was positive in 15 (30%) cases. Gram positive cocci were isolated in 13 (26%) cases, Gram positive bacilli in one case, and filamentous septate hyphae were detected in one case (also on KOH mount). Cultures were positive in 43 (86%) eyes. The commonest causative organism isolated was *Staphylococcus epidermidis*, seen in 24 (55.8%) eyes, followed by *Staphylococcus aureus* in four eyes, *Acinetobacter* species in three eyes, *Pseudomonas aeruginosa* in two eyes, and *Enterobacter* species and *Streptococcus pneumoniae* in one case each. Fungi were isolated in three cases; *Aspergillus fumigatus* in two cases and *Fusarium solanei* in one case. Polymicrobial infection was present in three cases and mixed infection in two cases (Table 2).

In 21 (42%) of the patients the initial antibiotic therapy, comprising fortified cephazolin and tobramycin, was modified after the antibiotic sensitivity reports. Vancomycin (5%) and ciprofloxacin (0.3%) were the alternative antibiotics used.

### Risk factors for graft infection

Out of the preoperative risk factors, ocular surface disorders (OSD) were present in 30 (60%) eyes in cases and 17 (34%) eyes in the control group ( $\chi^2 = 7.37$ , p value = 0.04). Previous history of graft infection was present in four eyes in the case group and one eye in the control group. Diabetes mellitus (NIDDM) was the only associated systemic disease present in two patients in the case group and one patient in the control group.

Evaluation of the postoperative risk factors revealed that a persistent epithelial defect was present in 37 (74%) eyes in the case group in comparison to 21 (42%) eyes in the control group ( $\chi^2 = 10.50$ , p value=0.001). Suture related problems

**Table 3** Suture status in postoperative period

	Case	Control	p Value
1 Normal	20 (40%)	39 (78%)	NS
2 Loose	25 (50%)	9 (18%)	<0001
3 Broken	3 (6%)	1 (2%)	NS
4 Exposed knot	2 (4%)	1 (2%)	NS

$\chi^2$  test.

(loose/broken suture, exposed knot) were present in the 30 (60%) eyes in the case group in comparison with 11 (22%) eyes in the controls. Loose sutures were significantly higher in the patients with graft infection (50%) compared with the controls (18%) ( $\chi^2 = 11.41$ , p value <0.001) (Table 3). Wound dehiscence was observed in two cases in the case group and none in the control group ( $\chi^2$  value = 2.04, p value=0.47). A bandage contact lens had been used for the treatment of the persistent epithelial defect in two of the cases and none of the controls.

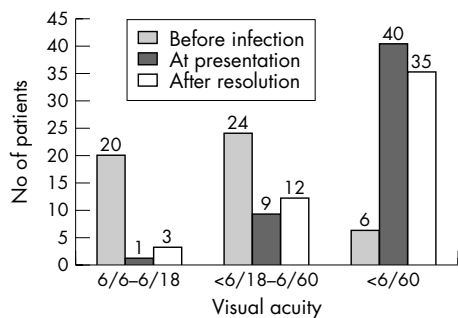
When the variables were simultaneously considered in a multivariate logistic regression analysis, we found that a patient having suture related problem had 3.6-fold odds of developing graft infection compared with a patient who did not have suture related problem (adjusted OR (95%CI): 3.6 (1.39 to 9.25)). Similarly, patients having persistent epithelial defects had threefold odds to develop graft infection compared with a patient who did not have a persistent epithelial defect (adjusted OR (95%CI): 3.0 (1.17 to 8.33)). A patient having an ocular surface disorder had 2.3-fold odds to develop graft infection compared with a patient with no ocular surface disorder (adjusted OR (95%CI): 2.4 (0.93 to 6.03) (Table 4).

### Visual outcome

The overall visual prognosis was very poor in eyes with graft infection, even after an optimal medical/surgical therapy (Fig 1). A best corrected visual acuity better than 6/60 was observed in only 15 patients (30%) after resolution of the infection, while only three patients (6%) achieved a best corrected visual acuity of  $\geq 6/18$  at the final follow up. Although 37 eyes (74%) responded to medical treatment, only 17 eyes (34%) retained clear grafts. Twenty eyes (40%) with central corneal scarring after resolution of the keratitis or graft failure were registered for a repeat penetrating keratoplasty. Eight eyes (16%) with a severe infection and an extensive melting of the corneal graft required a therapeutic penetrating keratoplasty and five eyes (10%) which developed a panophthalmitis had to be eviscerated. Ninety six per cent of the control eyes

**Table 4** Results of bivariate and multivariate logistic regression with graft infection as binary outcome variable

Risk factors	Graft infection		Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)
	Yes (n=50)	No (n=50)		
<b>Ocular surface disorders</b>				
Present	30	17	2.9 (1.29 to 6.57)	2.4 (1.01 to 6.03)
Absent	20	33	1.0	1.0
<b>Suture related problems</b>				
Present	30	14	3.8 (1.66 to 8.91)	3.6 (1.39 to 9.25)
Absent	20	36	1.0	1.0
<b>Persistent epithelial defect</b>				
Present	37	21	4.0 (1.69 to 10)	3.0 (1.17 to 8.33)
Absent	13	29	1.0	1.0
<b>Composite socioeconomic status</b>				
Lower	30	38	2.1 (0.81 to 5.55)	-
Higher	20	12	1.0	-



**Figure 1** Best corrected visual acuity of cases with graft infection: before infection, at presentation, and after resolution.

retained a best corrected visual acuity of 6/60 or better and 56% of these eyes had a visual acuity better than 6/18.

## DISCUSSION

Microbial keratitis is a significant cause of ocular morbidity in cornea transplant patients and constitutes an important cause for graft failure.<sup>1 2 4-6 8 13</sup> It necessitates a prompt diagnosis, thorough microbial investigations, and an intense medical therapy.

In the present study, persistent epithelial defects were present in 74% of the eyes compared to 64% of the cases reported by Tavakkoli and Sugar,<sup>4</sup> 55% of the cases reported by Harris *et al*<sup>5</sup> and 38% reported by Al Hazza and Tabbara.<sup>1</sup> Tavakkoli and Sugar<sup>4</sup> and Harris *et al*<sup>5</sup> found an epithelial defect to be the most common risk factor predisposing to graft infection similar to the results obtained in the present study. The postoperative defect in the epithelial layer may occur because of epithelial cell loss during donor cornea storage, intraoperative trauma, or any kind of minor trauma in the postoperative period, tear film abnormalities, ocular surface disorders, or the effect of topical medications (especially with preservatives).

Suture related problems (loose/broken suture or exposed knots) were significantly higher in cases (60%) compared with controls (38.9%) ( $p < 0.01$ ). The grafts with loose sutures were particularly predisposed to an infection. Although there were more graft infection cases in the eyes with a continuous suturing technique, it was not found to be statistically significant. Continuous sutures may predispose to infection compared with interrupted sutures because, unlike continuous sutures, interrupted sutures can be easily and selectively removed if there is a suture related problem or at the first sign of a suture related infection. Various authors have identified suture related problems as the major risk factor for the development of microbial keratitis.<sup>3 5 8-11</sup> It is therefore essential that any loose or broken sutures should be removed as early as possible. In India this is often a problem owing to the lack of trained ophthalmologists, especially in the rural areas.

A loose or broken suture left unattended may lead to epithelial defect and mucoid deposit around it and becomes a nidus for micro-organisms. Siganos *et al*<sup>9</sup> evaluated presence of micro-organisms in broken or loose sutures and concluded that eroded sutures harbour bacteria and should therefore be removed as early as possible. Leahey *et al*<sup>10</sup> reported that retained sutures following corneal transplant can result in sight threatening infection and recommended that sutures should be considered for removal as soon as the wound is well healed.

Ocular surface disorders were present in 60% of the cases and in 95% of our patients the ulcer was present in the exposed part of the cornea. This may be an indicator for the role of ocular surface disorders in the pathogenesis of graft infection. Bates *et al*<sup>2</sup> found that ocular surface disorders were

a risk factor in 33% of the cases, while Al-Hazza and Tabbara<sup>1</sup> reported the same in 66% of the cases and Tseng and Ling<sup>6</sup> in 29% of the cases in their series on post-keratoplasty graft infection.

In the present study, although graft infection was found to be more common in the patients belonging to a lower composite socioeconomic status (60%) in comparison with those belonging to higher composite socioeconomic status (40%), the role of socioeconomic factors in the pathogenesis of graft infection was not found to be statistically significant. This may be related to the fact that, in a hospital based study on a heterogeneous group, it is often difficult to elicit the exact details required for determining the socioeconomic status. In addition, the difference in socioeconomic status of two groups may not have been apparent owing to the relatively small sample size. In a previous study conducted in India, graft failure was significantly associated with a lower socioeconomic status and related to poor living conditions and inadequate hygiene.<sup>13</sup>

Studies from different parts of the world show a varying microbial pattern with many of the studies showing *Pneumococcus* and *Staphylococcus aureus* as the commonest organisms responsible for graft infection.<sup>1 2 6 11</sup> However, these bacteria were not the major offending pathogens in the present study and *Staphylococcus epidermidis* was the commonest isolate on culture. In India other studies have also shown coagulase negative *Staphylococcus* to be frequently associated with ulcerative keratitis. In a 5 year study conducted at our centre, *Staphylococcus epidermidis* was the most frequently isolated organism (43%).<sup>15</sup>

Visual prognosis remains very poor in eyes with graft infection even after optimal therapy and there is a high rate of graft decompensation.<sup>1-5</sup> In the study conducted by Bates *et al*,<sup>2</sup> only 23% of cases retained a clear graft after infection and 53% of the eyes required a regrant. In another study conducted by Harris *et al*,<sup>5</sup> only 40% of the previously clear grafts retained clarity and 11% eyes lost light perception. In our series only 30% of the patients retained clear grafts with a useful vision (>6/60). Although all eyes received appropriate antibiotic therapy and the keratitis responded to treatment in the majority of the cases, the visual outcome was dismal. This was attributed to the development of a corneal opacity or graft failure after resolution of microbial keratitis. Only 6% of the eyes attained a best corrected visual acuity 6/18 or better at the final follow up examination. Sixteen per cent of the patients had to undergo a therapeutic penetrating keratoplasty owing to extensive graft melting and 10% of the cases had to be eviscerated because of panophthalmitis.

In multivariate logistic regression analysis it was found that persistent epithelial defects, ocular surface disorders, and suture related problems were the significant risk factors predisposing to post-penetrating keratoplasty microbial keratitis in this part of the world. A judicious and prompt management of these problems during the postoperative period may help to prevent this infection. However, once the graft is infected it is difficult to restore useful vision with treatment and most of these cases subsequently require a regrant.

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### Authors' affiliations

**R B Vajpayee, S K Boral, T Dada, G V S Murthy, G Satpathy,**  
Dr Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India  
**R M Pandey,** Department of Biostatistics

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