Risk of Transmission of Infection to Host from Septicaemic Donor Corneas

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ABSTRACT

Purpose: To study the risk of transmission of infection from corneal tissues harvested from donors with septicaemia as cause of death.

Methods: A cross sectional study was carried out on two groups. Group A comprised of corneal tissues harvested from donors with septicaemia as cause of death and Group B was the control group which had corneal tissues harvested from donors who died of causes other than septicemia. Conjunctival swab and aqueous tap as well as blood sample of the deceased donor were inoculated on culture media. In group A , microbiological cultures of corneo scleral tissue was done whereas in group B Corneo sclera rim was cultured post Keratoplasty.MK media was cultured in both groups. Rates and consistency of culture results in innoculum was analyzed using Fisher's exact test.

Results: Thirty four corneas from 17 donors were harvested, with nine donors (18 eyes) in group A and eight donors (16 eyes) in group B. In conjunctival swab microbial growth was obtained in 15 (83.33%) and 5 (31.25%) tissues in group A and B respectively ($p<0.0001$). In Corneo scleral tissue microbial growth was present in 5 (31.25%) tissue of group A. In group B, no microbial growth was seen in Corneo scleral rim. ($p=0.046$). On comparing the blood culture positive and negative donors, the microbial growth in both the groups was statistically significant only for conjunctival swab (90% vs 45%, $p=0.0240$). However the same organism was not grown in blood culture and in conjunctival swab, aqueous tap, Corneo scleral rim or corneal tissue culture.

Conclusion: This study did not show the growth of same organism in blood culture and other ocular tissue cultures. Further study is required to ascertain Septicemia in the donor as a risk factor for corneal transplantation.

INTRODUCTION

Infection is always a serious concern after corneal transplant surgery.[1] All the eye banks rule out contraindications including septicemia before distributing the tissues. Although rare, most infected eyes lose vision or become blind.[2,3] Averting infections associated with transplantation is of paramount concern to all eye banks. One of the preventive strategies includes deferral of donors when septicemia is suspected as there is theoritical risk of transmission of the agent to recipient causing sepsis (bacterial, fungal, and viral agents).

Septicaemia is the tenth leading cause of death among older adults in the United States; its mortality rate has steadily increased over the past decade.[4] According to the International Sepsis Definitions Conference,[5] Septicemia is defined as having the presence of microbes or their toxins in blood and systemic Inflammatory response syndrome (SIRS) which has two or more of the conditions of fever or hypothermia, tachypnea, tachycardia or Leukocytosis - >12,000/micL, Leukopenia - <4000/MicL.

According to a study by Gustave et al,[6] the medical records of 75 potential donors from the North Carolina Eye Bank with signs of possible sepsis were reviewed by an infectious disease consultant and the only sign independently associated with active septicemia was positive blood culture. Sixty-five percent of donors whose charts were reviewed were cleared as appropriate cornea donors. This indicated that only positive blood culture has been seen to predict sepsis and presence of two or more clinical signs of possible sepsis may not be conclusive of septicemia.

According to regulatory requirements tissues collected from the donors diagnosed of septicemia is a contraindication for transplantation (Standards of eye banking in India, NPCB, 2009). Eyebanking world over follows this practice.

It is believed that during the phase of septicemia, organisms may be circulating in the blood vessels in the eye. However, cornea being avascular is expected to remain free of organisms.[7] Keates et al demonstrated positive aqueous cultures in three eyes out of 25 that had positive corneal cultures, suggesting diffuse ocular tissue contamination in septicemia.[8] There was, however, no attempt in the study to demonstrate corneal contamination directly to systemic infection. On the basis of reports that suggested that septicemia can contaminate ocular tissue, most eye banks refuse otherwise good tissue when decedent septicemia is noted.[8,9] This practice has continued for the last three

decades although the evidence to link bacteremia in the deceased to post operative endophthalmitis in the recipient is not clear.[10] In a study by Spelsberg H et al, no organ cultured corneas from septic donor which were transplanted developed endophthalmitis. The rate of immune reactions and graft failure was in the same range when compared to a larger group who received grafts from non-septic donors. They concluded that corneal tissues derived from septic donors was not a contraindication for transplant.[11]

Our study is proposed to determine the risk of transmission of infection from donor tissue harvested from donors with septicemia as cause of death. With this we recognize an opportunity to increase the number of potential donors, without risking the recipients.

MATERIAL AND METHODS

Study Design: A cross sectional study comprising of two groups was conducted at Dr Shroff's Charity Eye Hospital Eyebank, New Delhi, India, over a period of four months. The study was approved by the Institutional Review Board. Approval was taken from Ethics Committee to collect corneo scleral tissues of donors dying of septicemia for study purpose.

Inclusion criteria: The study included two groups. Group A (Septicemic group) comprised of consecutive corneo scleral tissues collected from donors diagnosed of Septicaemia or known focus of infection as cause of death and mentioned by the treating physician on the Death Certificate or Death Summary. Group B was the control group which included consecutive corneo scleral l tissues harvested from donors where cause of death was other than septicemia as mentioned by the treating physician on the Death Certificate or Death Summary.

Exclusion criteria: Donors with HIV, Hepatitis B or Hepatitis C, C-J disease, active viral encephalitis, syphilis, active polio, progressive multifocal leuko-encephalopathy, Rabies, active Reye's syndrome, miliary tuberculosis, cytomegalovirus brain infection, subacute sclerosing panencephalitis (SSPE) were excluded from the study. All non hospital deaths where medical records and history were not available for review were also excluded from the study.

Study procedures: Informed consent from next of kin of the deceased was taken for retrieval of the tissue. Detailed medical history including history of ventilator support was recorded. Pre-death blood counts and blood culture reports, if available, were recorded. Corneo sclera button was retrived using standard aseptic technique. Conjunctival swab, Aquous tap and Blood sample of donor was collected.

Protocol for Conjunctival Swab Collection: Skin of the donor was painted with 5% betadine skin paint. The area around the eye and forehead was cleaned by betadine 10% solution. After waiting for five minutes sterile drape was covered over the eye. Ocular surface was irrigated with 0.5% betadine eye drops and 0.5% moxifloxacin eye drops. Conjunctival swab was collected by inserting the swab into the eye at the lower nasal fornix and passing it along the fornix to its temporal margin after retracting the lower lid. The swab was inserted in the transport medium containing Phosphate buffer saline (PBS) with 20% glycerol.

Protocol for Aqueous Tap Collection: Wire speculum was applied. Conjunctival peritomy (360 degree) with resection to at least 4 mm behind the limbus was done with sterile conjunctival scissors and sterile Lim's forceps. Aqueous sample (0.2ml) was obtained by inserting a 30 G needle in to the anterior chamber through the temporal limbus with the bevel of needle facing up. Before taking the aqueous sample, the surface was disinfected with alcohol swab to rule out surface contamination.

Protocol for Corneoscleral Rim Excision: Tenon's capsule was separated from the sclera adjoining the limbus. An incision was made into the sclera with an 11 number BardParker blade till the uveal tissue was visible. After incision, right and left corneal scissors were used to excise the corneo - scleral button. Care was taken to prevent any vitreous loss and anterior chamber prolapse. The excised button was immediately transferred to the McCarey and Kaufman (M-K) medium.

Blood sample (8-10 ml) was collected from jugular or subclavian vein or directly from the heart of the donor, under aseptic technique. All the samples were labeled and transported to laboratory in ice-box.

Protocol For Inoculation on Culture Medias: Conjunctival swabs and aqueous tap were inoculated on Blood Agar (BA), Chocolate Agar (CA), Thioglycolate Broth (Thio) and Sabouraud's Agar (SDA) within 2 hours of recovery. In group A, microbiological cultures of corneo scleral tissue was done as none of the tissues from septic donors were used for transplantation whereas in group B Corneo scleral rim was cultured after transplant surgery was done. Both were inoculated on Blood Agar (BA), Chocolate Agar (CA), Thioglycolate Broth (Thio) and Sabouraud's Agar (SDA) at 72 hours post recovery before which they were stored at 4 degree celcius in the refrigerator. MK media was cultured in both groups. The inoculation was done under the laminar hood in the laboratory settings.

Standard serological tests and blood cultures were done for each donor.

Statistical analysis: The data was collected in standardized format using Microsoft excel. Statistical analysis was done using SPSS software. Categorical variables were analysed using Fisher's exact test.

Outcome Measurement: The outcome was measured in the form of rate of positive microbial cultures in the two groups and consistency of results was recorded in the form of growth of same organism in Corneo scleral tissue or rim, aqueous, conjunctival swab and blood culture.

RESULTS

Thirty four tissues harvested from seventeen donors were analyzed. Group A consisted of 18 tissues from nine donors. Group B consisted of 16 tissues from eight donors.

Blood culture showed microbial growth in five (29.41%) donors. Blood cultures were positive in three (33%) donors of group A and two (25%) donors in group B (p=0.772, odds ratio = 1.5).

Average duration of hospitalization of group A and group B donors was 9.8 days and 4.5 days respectively. In group A, microbial growth was obtained in 15 (83.33%) conjunctival swabs, that included Staphylococcus sp in four (22.2%), Klebsiella sp in three (16.67%), Candida sp and Enterobacter sp each in two (11.11%), Pseudomonas, Diphtheroids, Penicillium and Citrobacter each in one (5.56%). There was growth of Staphylococcus sp in two (11.11%) and Enterobacter sp in one (5.56%) samples of aqueous tap. C-S button showed growth of Enterobacter sp in two (11.11%), Klebsiella sp, Candida sp and Citrobacter sp each in one (5.56%) sample. In group B, five (31.25%) conjunctival swabs showed microbial growth. There was growth of Staphylococcus and Candida sp each in two (12.5%) and E.Coli in one (6.25%) swabs. No growth was obtained on aqueous tap and C-S rim in group B. The difference between the rates of microbial growth between the two groups was statistically significant for conjunctival swab ($p < 0.0045$; odds ratio= 11.00; 95% CI=2.1570 to 56.0961) and C-S t ($p =0.0465$; odds ratio= 13.4444; 95% CI=0.6807 to 265.5213). The difference between the rate of microbial growths between the two groups in the aqueous tap ($p =0.2299$; odds ratio=7.4516; 95% CI=0.3553 to 156.2874) was statistically not significant (table 1 and 2, figure 1).

In blood culture positivedonors microbial growth was obtained in nine (90%) conjunctival swab and one (10%) Corneo scleral rim . In blood culture negative donors (12), 11 (45.83%) conjunctival swabs and four (16.67%) Corneo scleral rim showed microbial growth. Aqueous tap of three (12.5%) samples of blood culture negative group showed

***Table 1**: Analysis of microbial growth in two groups*

Conj Swab		**P value =** 0.0045 **Odds ratio** =11.00 **95% CI=** 2.1570 to 56.0961	**Aqueous Tap**		**P value** =0.2299 **odds ratio=** 7.4516 **95% CI=** 0.3553 to 156.2874
Group A (n =18)	Group B (n=16)		Group A (n =18)	Group B (n=16)	
15/18 (83.33%)	5/16 (31.25%)		3/18 (16.67%)	0/16 (0%)	

***Table 2**: Analysis of microbial growth in two groups*

Corneo scleral rim or corneo scleral tissue		**P value** =0.0465 **odds ratio**=13.4444 **95% CI=** 0.6807 to 265.5213
Group A (n =18)	Group B (n=16)	
5/18 (27.78%)	0/16 (0%)	

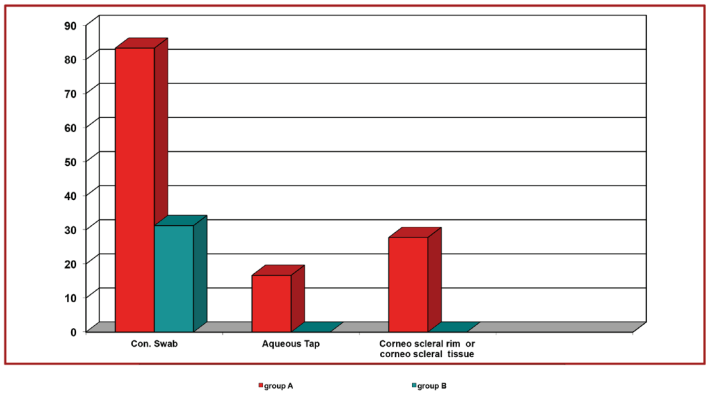

***Figure 1**: Rate of microbial growth in conjunctival swab, aqueous tap and C-S rim in septicaemic and non-septicaemic group*

microbial growth. No aqueous sample in blood culture positive group showed microbial growth. The difference between the rates of microbial growth between blood culture positive and negative samples was statistically significant for conjunctival swab (p =0.0240; odds ratio= 10.6364; 95% CI=1.1592 to 97.5973). The difference between the rate of microbial growths between blood culture positive and negative donors in the aqueous tap, Corneo scleral rim and M-K medium was statistically not significant (table 3 and 4, figure 2).

Overall, conjunctival swab of nine donors (52.94%) showed microbial growth in both eyes. Same organisms were present in swab of five (29.41%) donors while different organisms were present in swabs of four (23.53%) donors (p= 1.000). None of the aqueous samples showed microbial growth in both the eyes. Corneal scleral rim culture of one donor showed microbial growth in both eyes but the organisms present were different in both eyes. None of the corneal tissue collected showed microbial growth of same organism in Corneo scleral rim or tissue and conjunctival swab and aqueous tap or blood culture.

***Table 3**: Analysis of microbial growth in relation to blood culture*

Conj Swab		**p value** =0.0240	**Aqueous Tap**		**p value**=0.54
Blood Culture positive (n=10)	Blood Culture negative (n=24)	**Odds ratio=** 10.6364 **95% CI=** 1.1592 to 97.5973	Blood Culture positive (n=10)	Blood Culture negative (n=24)	**Odds ratio=** 0.29 **95% CI=** 0.0138 to 6.19
9/10 (90%)	11/24 (45.83%)		0/10 (0%)	3/24 (12.5%)	

Table 4 : Microbial growth in relation to blood culture

Corneo scleral rim		**p value** =1.00 **odds ratio**=0.667 **95% CI** = 0.0654 to 6.79
Blood Culture positive (n=10)	Blood Culture negative (n=24)	
1/10 (10%)	4/24 (16.67%)	

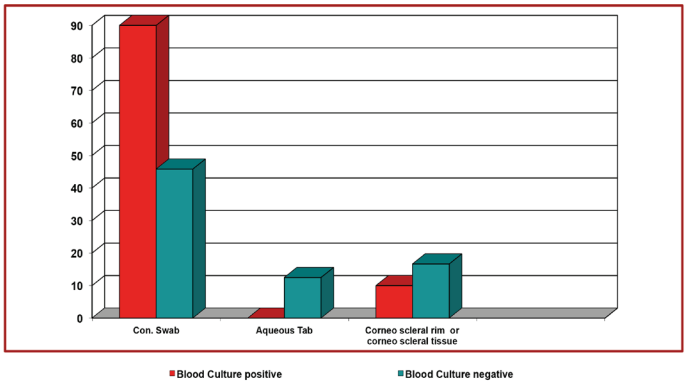

Figure 2 : Rate of microbial growth in conjunctival swabs, aqueous taps, C-S rims and M-K medium in relation to blood culture results

DISCUSSION

Evidence of septicaemia in the deceased is a contraindication to the utilization of corneal tissue. This policy is followed by all eye banks. It has been shown that clinical signs of septicemia may not have a high predictive value of sepsis.[6] Positive blood culture predicts sepsis, but in our study bacterial growth in blood culture was positive in only 34% of septicaemic group. However, 25% of blood cultures taken from non-septicaemic donors also showed microbial growth.

Donor corneoscleral rim cultures are unreliable predictors of endophthalmitis and therefore are not useful in the clinical management of patients having corneal transplants. In a metaanalysis of 17,614 corneal grafts, 2459 (14%) had a positive donor rim culture and only 31 (0.2%) developed endophthalmitis.[16] Ten (100%) of 10 eyes with candidal endophthalmitis had the same species isolated from the donor rim, compared with only 11 (55%) of 20 with bacterial endophthalmitis. The discrepancy between the results of corneoscleral rim cultures and subsequent endophthalmitis renders them invalid as a quality assurance procedure.[17]

Conjunctival swabs and corneoscleral rim cultures in our study showed significantly more growth in the group with septicemia than without septicemia. Only five donors (29.41%) showed microbial growth of same organism in both eyes. However, two (40%) of them belonged to group B. It is interesting to note that in Corneo scleral tissue cultures, only one donor tissue had growth in both eyes. However, the organisms were different in the two eyes. Several studies have cited positive conjunctival cultures in cadavers to range from 12% to 100%.[10,12,13] These results most likely represent surface contamination only and do not appear to be a frequent cause of postoperative endophthalmitis.[14] Robert et al speculated that bacteria found on

the donor tissue arise from peri-mortem bacteraemia, not from underlying infection of the donor. This has no effect on the incidence of endophthalmitis.[15]

In group A with septicemia of our study, 16.67% of aqueous tap cultures were positive and no sample tested positive in the non-septicemic group. Although this finding is significant, all donors with aqueous tap positive samples were blood culture negative. Therefore evidence of increased risk of infection in bacteremia could not be clearly established. Clear evidence is not available in literature whether spread of infection to donor cornea is a result of bacteremia or local invasion of ocular tissues. In a study of aqueous culture in eyes from 50 cadavers, no growth was demonstrated on any sample, although 18 had positive blood cultures and most suffered chronic debilitating illnesses.[10] The present study also showed similar results that aqueous humor is usually sterile in septicemia patients and anterior chamber sterility is not compromised by terminal bacteremia. It is possible that bacterial contamination of donor tissue is a result of suboptimal technique during removal of the tissue from cadaveric donor and not from systemic bacteraemia in the deceased.[18] In our study, none of the tissues collected showed microbial growth of same organism in conjunctival swab, aqueous tap, corneoscleral rim and blood culture. There was no consistency between organism present in blood culture and organism present in conjunctival swab, aqueous tap and Corneo scleral rim or corneao sclera tissue. Our study findings correlate with the results of Spelsberg et al, who also found no correspondence between pathogens isolated from contaminated medium and the pathogens that caused sepsis in the donor.[11] Clark et al have similarly demonstrated that aqueous humor remains sterile in septic donors and contamination of tissue via aqueous or blood circulation is very unlikely.[10]

As 'Hospital Cornea Retrieval Program' (HCRP) is followed by most eyebanks as a major strategy for improving the collection of corneal tissues and Septicemia being one of the major cause notified on death certificate, this study becomes relevant in improving the utilization rate.

There is shortfall in availability of corneal tissue for transplantation in most developing countries. Therefore, it is relevant to increase the collection of corneal tissue by expanding the eligibility criteria for corneal donor tissue.The present study demonstrates a need to improve the criteria to define death due to septicemia and identify high risk cases for transmission of infection among them. The study does not establish a clear link of septicemia as risk factor for transmission of infection to the corneo-scleral tissue. To arrive at a coherent approach a study with larger sample size is recommended.

CONCLUSION

This study shows that the prevalence of positive conjunctival swab and cornea-scleral tissue or rim cultures was significantly greater in donors where cause of death was documented as septicemia. Also the positive conjunctival swab cultures was significantly greater in donors with positive blood cultures. However same organism did not grow in blood culture and ocular tissues. There is a need to reconsider eye donation with septicemia as evidence to transfer infection to donor tissue is not clear. However a larger study is required to come out with clear recommendations for eye banking for increasing utilization of harvested corneal tissue.

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