Original Article

Factors affecting the serological testing of cadaveric donor cornea

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Purpose: The purpose of this study was to evaluate the serological profile of the eye donors and to study the influence of various factors on serological test results. **Methods:** A cross-sectional, observational study was conducted, and data of 509 donors were reviewed from the records of eye bank from December 2012 to June 2017. Various details of donors analyzed included the age, sex of the donor, cause of death, source of tissue, time since blood collection after death, macroscopic appearance of blood sample, and details of discarded tissues. Serological examination of blood was performed for human immunodeficiency virus (HIV), hepatitis B virus, hepatitis C virus (HCV), venereal disease research laboratory (VDRL), and serology reports reactive or nonreactive were analyzed. **Results:** Among the 509 donors, 295 (58%) were male, and 420 (82.50%) belonged to age group ≥60 years. Most donors (354, 69.5%) died due to cardiac arrest. Macroscopically, sera were normal in the majority of 488 (95.9%) cases. Among 509 donors, 475 (93.3%) were nonreactive, 12 (2.4%) donors were found to be reactive to hepatitis B surface antigen (HBsAg), and 1 (0.2%) was reactive to HCV, but no donor serology was reactive to HIV or VDRL. Twenty-one (4.12%) donors' sera were not fit for serological testing. Among all donors, 475 (93.32%) donors were accepted and 34 (6.67%) were rejected or discarded on the basis of serological testing. Cause of death and macroscopic aspect of sera influenced the serological results in a highly significant manner ($P = 0.00$). Acceptance or rejection of the donor was significantly influenced by the serological results of the donor ($P = 0.00$). **Conclusion:** The seroprevalence among eye donor for HBsAg and HCV was 12 (2.4%) and 1 (0.2%), respectively. Factors such as cause of death and macroscopic aspect of sera influence the serological results. Time since blood collection or sampling will not show any impact on viral serological results if postmortem sampling will be done in < 10 hours(h) after death which can improve the safety and utility of the donor cornea.

Key words: Eye bank, hepatitis B virus, serological testing

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Cornea is avascular and immunologically privileged tissue due to which keratoplasty done for various corneal diseases is considered the most successful procedure.[1] Hepatitis B virus (HBV) is known to have been transmitted through corneal tissue, and there is always a risk of transmission of human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infection from the seropositive eye donor though documented evidence of transmission of infection to the recipient does not exist. It is mandatory to perform venereal disease research laboratory (VDRL) for eye donors as it is a surrogate for the sexually transmitted diseases as positive syphilis serology correlates with the risk behavior. If the screening test is positive, a negative confirmatory test must be documented before tissue is released.[2] The cornea has been documented as the vehicle of transmission in 8 cases of rabies, 2 cases of HBV, and one case of Creutzfeldt-Jakob disease.[3] Positive serological results are one of the major reasons for discarding donor corneas.[4] Keeping this in mind in 1990, the Food and Drug Administration provided regulatory regime.[5,6]

In heart-beating donors, sera samples are usually of good quality as emergency virological screening is done before organ harvest.[7] In contrast in cadaveric donors, sera are often of poor quality and frequently yield falsely positive results in serological assays as blood samples are collected at variable times after death.[8] Nonreliability of serological results leads to needless discard of these tissues which eventually enhance the shortage of corneal grafts. In cases of false-negative results, there are chances of transmission of the infection to the recipient or surgeon. Strict quality control can be accomplished with the selection of the donors, careful processing, preservation, and evaluation of parameters, such as donor serology by the eye banks (EB).[9] The aim of this study is to evaluate the serological profile of the eye donor at this only EB of the state and to document the influence of factors such as the timing of blood collection after death and macroscopic aspect of sera on serological test results.

Methods

The research was approved by the institutional research Ethical Committee and was in accordance to the tenets set forth in the Declaration of Helsinki. A cross-sectional, observational,

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descriptive study was conducted in which we retrospectively reviewed data of 509 donors from EB from December 2012 to June 2017.

At this institute, EB received donor tissues from our hospital either through mortuary or intensive care unit under hospital cornea retrieval programme, donor's homes or other hospitals, adjacent areas through voluntary donors, and eye donation centers affiliated to this EB. EB accepted all tissues irrespective of medical or ocular history. In case of medical or serological contraindication for transplantation, relatives were counselled for willingness to donate for research or training purpose, and universal precautions were taken. Enucleation was not performed if denied otherwise the tissue was procured. After gross examination of the donor eye, decisions were made, if grossly the eyes look good with good corneal transparency, formed anterior chamber, few folds the corneoscleral rim excision were performed primarily and if corneas were hazy with collapsed globe, infiltrates, then enucleation was done primarily.

In situ excision was done in an operating theater, a morgue, funeral home, hospital, or donor home. Immediately after enucleation, about 3–4 ml of blood was collected by subclavian or internal jugular vein puncture using a sterile vacutainer for routine serological testing. Serological examination of blood was performed for HIV, HBV, HCV, and VDRL.

Laboratory testing by microbiologist

The cadaveric blood samples were received with storage at 2°C–8°C in microbiology laboratory and were tested and reported within 6–8 h of collection. All the samples were analyzed for any macroscopic abnormalities such as hemolysis/turbidity or cloudiness or lipemic nature. The specimens of all sera were then screened for hepatitis B surface antigen (HBsAg), anti-HCV antibodies, and anti-HIV antibodies using an enhanced chemiluminescence immunoassay (ECI Vitros, Ortho-Clinical Diagnostics, Johnson and Johnson). HIV testing was done as per National AIDS control organization protocol. All the tests were performed, and results were recorded based on the manufacturer's instructions. For detection of antibodies against Treponema pallidum, Rapid plasma reagin testing was done. The samples with macroscopic abnormalities were not accepted by the automated machines and error in the results was displayed. Those samples were considered unfit for serology. Reactive and nonreactive results were given depending on the clumping observed. Information regarding unfit and reactive samples was given to the EB which were discarded and incinerated by EB and personnel involved with enucleation were intimated.

Data analyzed included the age, sex of the donor, cause of death, source of tissue, time since blood collection after death, serology reports reactive or nonreactive, macroscopic appearance of blood sample (hemolyzed/turbid/lipemic), and details of discarded tissues [Tables 1 and 2].

Statistical analysis

Initially, data obtained was entered into an Excel spreadsheet and then transferred to SPSS software (Statistical Package for Social Sciences, version 22, SPSS Inc, Chicago, IL, USA) for analysis. Statistical data were expressed in terms of means ± standard deviations. The descriptive statistics was used to express data in terms of frequency and percentage. Pearson Chi-square test (Fisher exact test) was used to find out the association between categorical variables. $P < 0.05$ was considered statistically significant.

Table 1: Demographics of donors

	n (%)	Total
Age (years)		
≤60	89 (17.5)	509
>60	420 (82.50)	
Sex		
Male	295 (58)	509
Female	214 (42)	
Cause of death		
Cardiac arrest	354 (69.5)	509
Respiratory failure	94 (18.5)	
Malignancies	16 (3.1)	
Roadside accident	14 (2.8)	
Septicemia	31 (6.1)	
Time since sample collection (h)		
≤6	439 (86.2)	509
>6	70 (13.8)	
Serology		
Nonreactive serology	475 (93.32)	509
Reactive serology (HBV + HCV)	13 (2.55)	
Sample unfit for serology	21 (4.1)	
Fate of donor		
Accepted	475 (93.3)	509
Rejected	34 (6.7)	
Macroscopically serology		
Normal	488 (95.9)	509
Hemolyzed	14 (2.8)	
Turbid/lipemic	7 (1.4)	

HBV: Hepatitis B virus, HCV: Hepatitis C virus

Table 2: Depicting the details of discarded tissues

Serology status	*n* (%)
HBsAg reactive	24 (2.4)
HCV reactive	2 (0.2)
Unfit sera for serology	42 (4.2)
Media positive for Gram-negative bacteria after shifting to glycerine	1 (0.09)
Total	69 (6.85)

HCV: Hepatitis C virus, HBsAg: Hepatitis B surface antigen

Results

A total of 509 donors were reviewed from records of this EB from December 2012 to June 2017. Among the 509 donors maximum, 295 (58%) were male. Age group ≥60 years contributed maximally 420 (82.50%) to eye donation. Maximum donors 354 (69.5%) died due to cardiac arrest. Macroscopically, sera were normal in the majority of 488 (95.9%) cases [Table 1].

Details of discarded tissues are depicted in Table 2.

Serological testing results of donors

Among 509 donors, 475 (93.3%) were nonreactive, 12 (2.4%) donors were found to be reactive to HBsAg and 1 (0.2%)

was reactive to HCV but no donor serology was reactive to HIV or VDRL. Twenty-one (4.12%) donors sera were not fit for serological testing. Four hundred and seventy-five (93.32%) donors were accepted, and 34 (6.67%) were rejected or discarded on the basis of serological testing [Table 1].

Cause of death influenced the serological results in a highly significant manner ($P = 0.00$).

Acceptance or rejection of the donor was significantly influenced by the serological results of the donor ($P = 0.00$) [Table 3].

Relationship between macroscopic aspect of sera and results of serological viral testing

The macroscopic aspect of the serum samples was noted in all 509 cases. It was considered normal in 488 (95.9%) cases and was abnormal, i.e., hemolyzed in 14 (2.8%) and turbid or lipemic in 7 (1.4%) donors. Serological results were influenced by macroscopic aspect of sera in a significant manner which were not conclusive ($P = 0.00$). This implicates the relationship between the macroscopic aspect of sera and the utility of the tissue which were actually excluded from utilization [Table 3]. Twenty-one (4.12%) corneal tissues were discarded just on the basis of the macroscopically abnormal sera which were hemolyzed, turbid, or lipemic which were considered unfit. In one case when the tissue was transferred from M. K media to glycerine for long-term preservation, the M. K media as a protocol was sent for Gram's and KOH staining and culture which came out positive for Gram-negative bacteria on gram's staining and that corneal tissue was discarded.

Influence of time of blood sampling on the virological test results

The time of blood collection after death was divided into two groups of ≤6 and >6 h. Time since sample collection showed no significant influence on various serology aspects ($P = 0.13$) [Table 4]. Serology was positive for HBs Ag in 12 (2.4%), and HCV reactivity was seen in 1 (0.2%) cases. The prevalence of positive serological results did not show a significant difference between samples collected ≤6 h and >6 h ($P = 0.76$). Among the 509 donors for whom the time of blood sampling was known, enough sera were available to control equivocal results in 488 (95.87%) cases, but in 21 (4.1%) cases samples were not fit for testing.

Relationship between the macroscopic aspect of serum and the time of blood sampling after death; respective influence on virological results

Time since sample collection showed no significant effect on the reactive serology and on macroscopic status of sera among the donors ($P = 0.76, 0.13$) respectively. Among the donor of reactive serology, no association are computed for the time since sample collection, and macroscopic aspect of the serum as macroscopic aspect of serum was a constant factor which means that all reactive sera were normal in nature amongst serologically reactive donors. Acceptance or rejection of donor tissue was not significantly influenced by time of sample collection ($P = 0.17$) [Table 4].

Discussion

Quality control and serological testing of the corneal tissue is the foremost responsibility of the EB as safety and validity of donor

Table 3: Relationship between serology and various other factors

	Nonreactive, *n* (%)	HBsAg reactive, *n* (%)	HCV reactive, *n* (%)	Unfit for serology, *n* (%)	*P**
Age (years)					
≤60	84 (16.5)	1 (0.19)	0	4 (0.78)	0.81
>60	391 (76.81)	11 (2.16)	1 (0.19)	17 (3.33)	
Sex					
Male	274 (53.83)	8 (1.57)	1 (0.19)	12 (2.35)	0.77
Female	201 (39.48)	4 (0.78)	0	9 (1.76)	
Cause of death					
Cardiac arrest	334 (65.61)	6 (1.17)	0	14 (2.75)	0.00
Respiratory failure	85 (16.69)	6 (1.17)	1 (0.19)	2 (0.39)	
Malignancies	12 (2.35)	0	0	4 (0.78)	
Road side accident	13 (2.55)	0	0	1 (0.19)	
Septicemia	31 (6.09)	0	0	0	
Time since sample collection					
≤6 h	412 (80.94)	11 (2.16)	1 (0.19)	15 (2.94)	0.21
>6 h	63 (12.37)	1 (0.19)	0	6 (1.17)	
Fate of donor					
Accepted donor	475 (93.32)	0	0	0	0.00
Rejected donor	0	12 (2.35)	1 (0.19)	21 (4.12)	
Macroscopic aspect of serum					
Normal	475 (93.32)	12 (2.35)	1 (0.19)	0	0.00
Hemolyzed	0	0	0	14 (2.75)	
Turbid/lipemic	0	0	0	7 (1.37)	

**P* value-calculated by Pearson Chi-square test. HBsAg: Hepatitis B surface antigen, HCV: Hepatitis C virus

Table 4: Influence of time since sample collection on various aspects of donor sera

Time since sample collection	≤6 h, *n* (%)	>6 h, *n* (%)	*P*#
Macroscopic aspect of serum			
Normal	424 (83.30)	64 (12.57)	0.13
Hemolyzed	10 (1.96)	4 (0.78)	
Turbid/lipemic	5 (0.98)	2 (0.39)	
Serology aspects			
Nonreactive	412 (80.94)	63 (12.37)	0.21
HbsAg reactive	11 (2.16)	1 (0.19)	
HCV reactive	1 (0.19)	0	
Unfit for serology	15 (2.94)	6 (1.17)	
Fate of donor			
Accepted	412 (80.94)	27 (5.30)	0.17
Rejected	63 (12.37)	7 (1.37)	

#*P* value-calculated by Pearson Chi-square test. HBsAg: Hepatitis B surface antigen, HCV: Hepatitis C virus

cornea further influence the success of the outcome of keratoplasty. Being the only EB of the state, we hold the responsibility of supplying the serologically safe corneal tissue for corneal transplant. Sometimes, serological testing of donor becomes difficult or impossible due to macroscopic sera alterations. In that situation, the donor cornea cannot be utilized for corneal transplantation and is discarded or incinerated. Keeping that in view this study was planned to evaluate the seroprevalence of the HIV, HBV, HCV, and VDRL among the corneal donors and to evaluate effect of various other factors on serological testing.

A nonreactive serological testing for HIV, HBsAg, VDRL, and HCV is mandatory before tissue transplantation. In this study, 69 (6.85%) of tissues could not be utilized and were incinerated as blood sample showed either reactive serology or insufficient or hemolyzed sera which were quite less as compared to 19% as reported by Jadeja and Bhatt.[10]

In this study, seroprevalence of HBV and HCV viruses in eye donors was 2.4% and 0.2%, respectively and no case showed reactive serology to HIV and VDRL. Mahalakshmi *et al*. in a study done at Shankar Netralaya, Chennai, reported the seroprevalence of HBV and HCV as 3.52% and 1.45%.[11] Bhatt *et al*. reported seroprevalence of HIV, HBV, and HCV viruses in eye donors as 1.31%, 0.49%, and 0.49%, respectively, which is comparable to the present study.[12]

In another study done by EB Association of Australia and New Zealand, the seroprevalence of HBV and HCV was 0.49% and 0.38% which is comparable to the present study.[5] HBV is known to have been transmitted through corneal tissue. HBVc DNA was detected in 6.6% of corneal epithelium and 14.8% of stromal epithelium of seropositive eye donors.[13] There is a significant risk of transmission of HBV to the enucleator and special precautions are required to be taken to handle HBV-infected tissue.

HCV RNA has been detected in 34.5% in the cornea as well as in the tears and aqueous humor of seropositive patients; hence, it is essential to determine the infectious status of the eye donor with the virus. Since Hepatitis C is life-threatening, it is mandatory to screen potential cornea donors for HCV before transplantation.[3]

Challine *et al*. demonstrated that the longer the interval between death and blood sampling, the more likely the serum was abnormal (hemolyzed, icteric, or cloudy). In addition, they also reported for first time the clear and direct relationship between the macroscopic aspect of serum and the results of virological serological testing. Similarly, in the present study, macroscopic aspect also affected the serological result significantly ($P = 0.00$). On the contrary time, since sample collection did not show any significant impact on results of serology and macroscopic aspects of sera or acceptance or rejection of donor tissue which can be due to the fact that maximum time since sample collection in our study was 10 h which was very less as compared to average of 22 h as studied by Challine *et al*.[14]

With the current cornea shortage, it is important to avoid false-positive virological results to ensure that serologically safe corneas are not needlessly discarded. Challine *et al*. suggested that cadaveric donors sampling for serological testing should be done <12 h after death which is consistent with the observation of the present study.[14]

According to the European EB Association, if the postmortem serum is hemolyzed or hemodiluted, then the results of tests done up to 7 days before donation can be used, provided the patient received no transfusions or infusions in the interval.[15] In this situation, ethical question of sampling in view of eye donation arises as patient may not be aware of the forthcoming demise. Edler *et al*. reported postmortem macroscopic changes or hemolysis of blood sample and further changes in serological parameters for HIV, HBV, and HCV up to 48 h which contradicts the results of present study due to the fact of maximum time of sampling was 10 h.[16]

In addition, viral infection between premortem sampling and death cannot be ruled out, and transplant teams would likely feel uncomfortable about using tissues from a donor who was seronegative before death but had a positive or equivocal serological result postmortem.[8] Keeping these things in view quality of postmortem sample and the interval between death and sampling should be taken into account when interpreting apparent seroconversion close to death.

In the available literature, only a few studies are there which highlights the effect of the time of sampling and macroscopic aspect of the blood samples on the serological testing of the cadaveric corneal donors.

Limitations of the study

In this study, the maximum time of sample collection was 10 h after death, but in the available literature, all the studies showed higher time of sample collection due to which results cannot be compared.

Conclusion

The seroprevalence among eye donor for HBsAg and HCV was 12 (2.4%) and 1 (0.2%), respectively. Factors such as cause of death, time of blood sampling after death and macroscopic aspect of sera influence the serological results. The macroscopic aspect of serum collected postmortem influence the serology result significantly and acts as a best predictor of the serological testing in cadaveric cornea donors. Time after blood collection or sampling will not show any

impact on viral serological results if postmortem sampling is done <10 h after death, which can improve the safety and utility of the donor cornea.

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Conflicts of interest

There are no conflicts of interest.

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