Original Article

Comparison of seropositivity of human immunodeficiency virus, hepatitis B virus, hepatitis C virus, and syphilis among Hospital Cornea Retrieval Programme-Donors versus voluntary cornea donors at a large eye bank in Eastern India

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Purpose: To compare the serology profile of donors from Hospital Cornea Retrieval Programme-donors (HCRP-D) and voluntary cornea donors (VC-D) from a large eye bank in Eastern India. **Methods:** This is a retrospective analysis of donor details from January 2011 to December 2016. Donor demographics, cause of death, and serology reports were compiled. Postmortem blood was tested for human immunodeficiency virus 1 and 2 (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and syphilis using government-approved kits as per the National Programme for Control of Blindness Standards of Eye Banking. Donors for whom serology was not possible were excluded. **Results:** A total of 4300 of 4353 donors were included of which 74.3% were hospital donors and 25.7% were voluntary donors. A total of 93 (2.2%) donors with 94 seropositive reports were noted: 79 (84.9%) from HCRP-D and 14 (15.1%) from VC-D which was statistically significantly higher (P = 0.02). Among seropositive reports, HIV, HBV, HCV, and syphilis accounted for 12 (12.8%), 38 (40.4%), 36 (38.3%), and eight (8.5%), respectively. There was no correlation between the cause of death and seropositivity. A statistically significant decreasing trend in seroprevalence among hospital donors was observed over the years (5.3% in 2011 to 1.4% in 2016; P = 0.004). Two (0.47%) of 421 hospital donors with prior negative serology were found to be seropositive. **Conclusion:** Seropositive rates are significantly higher among hospital donors in spite of medical prescreening compared to nonscreened voluntary donors. Serology should be repeated even when prior reports are available.

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Donor serology is one of the most important preoperative investigations for any organ or tissue transplantation. In corneal transplantation, the donor is screened for various blood-borne infections before the tissue is released for surgery. The mandatory serology tests are mentioned in the National Program for Control of Blindness (NPCB) Standards of Eye Banking in India. The following serology tests should be done using enzyme-linked immunosorbent assay (ELISA) or government-approved rapid kits – human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and syphilis. Testing for human T-lymphotropic virus (HTLV I and II) is not mandatory, but tissues from donors with positive reports are not used for surgery.[1] The Eye Bank Association of America (EBAA) tests for HIV 1 and 2, HBV, and HCV for all donors. In addition, tissues from donors who are positive for HTLV I, II, and syphilis are not used for surgery.[2]

In spite of being an avascular tissue, there are various reports of transmission of diseases after cornea transplantation. There are two cases of HBV transmission, eight reports of rabies transmission, three cases of Creutzfeldt–Jakob disease, and a few cases of seroconversion for herpes simplex and cytomegalovirus. However, there are no reports of transmission of HIV, HCV, or syphilis via ocular tissue transplantation so far. There are reports of nine patients who received corneal tissue from HIV-positive donors, but none of them seroconverted.[3] Krajden *et al.* and Tugwell *et al.* both reported cases where HCV-positive multiorgan donors led to the transmission of HCV in other solid organ recipients, but the cornea recipients were unaffected.[4,5] However, Hung Ming Lee *et al.* showed that HCV virus is present in corneas of HCV-seropositive individuals.[6] A few reports of postkeratoplasty tuberculosis infection could be attributed to transmission from donors.[3] Although HTLV I- and II-positive donors are excluded by the NPCB and EBAA, respectively, there are no reports of transmission. Hence, among the four diseases being tested in India, there is documented evidence only for HBV transmission after cornea transplantation from seropositive donors.

Tissue collection through Hospital Cornea Retrieval Programme (HCRP) is growing in India. It accounts for about 25%–30% of cornea donation in the country and up to 70%–75% in some large eye banks.[7] The HCRP has the advantage of

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readily available medical records so that potential donors can be prescreened more accurately. However, all investigations are not always performed in a timely manner in busy hospitals, and admitted patients are more likely to be seropositive than voluntary donors from house calls. In the latter group, serology reports are seldom available, but these are most commonly deaths due to natural causes or chronic diseases.

The aim of this study was to look at the serology profiles of the cornea donors and compare the seroprevalence among hospital donors and voluntary donors at a large eye bank in Eastern India.

Methods

This was a retrospective, observational study done at a large eye bank in Eastern India. Donor details were obtained from the eye bank records; donor demographics, cause of death, and serology reports were compiled. Only donors in whom serology was done were included. The study period was from January 2011 to December 2016. Donors were divided into two groups, HCRP-donors (HCRP-D) and voluntary cornea donors-(VC-D), based on the mode of retrieval.

The HCRP-donors were from three government-run hospitals, one secondary level and two tertiary level. All donors in this group were prescreened using the available medical records and found to be seronegative. Donors without available serology reports had no clinical evidence for these diseases.

Serology was performed with postmortem blood in all cases. Five milliliters of blood was drawn from the jugular or subclavian vein in an aseptic manner. The blood sample was immediately transferred in vacutainer and then sent to the eye bank along with the corneal tissue. All samples were analyzed within 18 h of collection. Serum was separated by centrifuging at 3500 revolutions per minute for 5 min. The following government-approved kits were used for all tests:

1. HIV TRI-DOT (Diagnostic Enterprises, Himachal Pradesh, India) antibody test for gp41 and gp120 of HIV-1 and gp36 of HIV-2
2. HEPACARD (Diagnostic Enterprises, Himachal Pradesh, India) HBsAg detection test
3. HCV TRI-DOT (Diagnostic Enterprises, Himachal Pradesh, India) antibody test for core, NS3, NS4, and NS5 proteins of HCV
4. Rapid Plasma Reagin (RPR) test (Beacon Diagnostics, Maharashtra, India) for antibody against *Treponema pallidum*.

The processing was done as per manufacturer's instructions in an aseptic environment by a trained technician. Tissues from seropositive donors were discarded. The seropositive samples were double checked by ELISA. In case of donors who received transfusion prior to death, hemodilution calculation was performed as per the NPCB eye banking standard guidelines.[1]

Statistical analysis was done using Microsoft Excel (Microsoft Office Professional Plus 2013, Washington, USA). Percentage, mean, and standard deviation were calculated for each group. Fisher's exact test was used to compare between groups.

Results

There were a total of 4353 donors in the 6-year study period, of which 4300 donors with serology reports were included. Serology was not done in 53 donors for reasons such as obvious medical contraindication for transplantation, hemodilution, inadequate blood sample, or coagulated sample. These were excluded from the study and the tissues were not used for surgery. Seven donor samples required hemodilution calculation, of which five were discarded.

There were 2470 (57.4%) male and 1830 (42.6%) female donors. The mean age was 68.9 ± 13.4 years (range: 7.5–103 years), with maximum donors in the 60–80 years' age group (55.8%). There were 3196 (74.3%) HCRP-Ds and 1104 (25.7%) VC-Ds. Prior negative serology reports were available from 421 (13.2%) of the HCRP donors. Table 1 summarizes the demographic breakdown of the donors.

A total of 93 (2.2%) donors with 94 seropositive reports were noted: one HCRP donor was positive for both HIV and HBV. There were 79 (2.47% seroprevalence) seropositive donors among HCRP-Ds compared to 14 (1.27% seroprevalence) among the VC-Ds which was statistically significantly higher (Fisher's exact test, $P = 0.02$). Of the four diseases tested, HIV accounted for 12 (12.8%), HBV for 38 (40.4%), HCV for 36 (38.3%), and syphilis for 8 (8.5%) positive serology reports. Seroprevalence among the study population was found to be 0.28% for HIV, 0.88% for HBV, 0.84% for HCV, and 0.19% for syphilis [Fig. 1].

A total of 421 (13.2%) donors in the HCRP-D group had previous negative serology reports from the hospital medical records. Of them, two (0.47%) were found to be seropositive (1 HBV and 1 HCV).

There was a statistically significant reduction in the annual percentage of seropositive donors from 4.5% in 2011 to 1.4% in 2016 ($P < 0.001$). This is due to both a decrease in the number of seropositive cases and an increase in the total number of donors each year [Table 2].

Seropositivity was found to be relatively higher among donors aged <40 years (4.4%) compared to other age groups (range: 1.9%–2.5%), but it was not statistically significant ($P = 0.08$–0.22) [Table 3]. There was no correlation between the cause of death and the serology status of the

Table 1: Demographic and age distribution of the donors in Hospital Cornea Retrieval Programme and voluntary groups

	Males	Females	Total (%)	Age (mean±SD)	Age group (%)			
					<40	40-60	60-80	>80
HCRP	1831	1365	3196 (74.3)	67.1±13.4	96 (3.0)	701 (21.9)	1788 (55.9)	611 (19.1)
Voluntary	638	466	1104 (25.7)	74.1±13.3	18 (1.6)	88 (8.0)	612 (55.4)	386 (35)
Total (%)	2470 (57.4)	1830 (42.6)	4300	68.9±13.4	114 (2.7)	789 (18.3)	2400 (55.8)	997 (23.2)

SD: Standard deviation, HCRP: Hospital Cornea Retrieval Programme

Table 2: Year-wise breakdown of seropositive reports among Hospital Cornea Retrieval Programme-donors and voluntary donors

Year	Voluntary			HCRP			Total		
	Total donors	Serology positive	Percentage	Total donors	Serology positive	Percentage	Total donors	Serology positive	Percentage
2011	101	1	0.99	452	24	5.31	553	25	4.52
2012	128	3	2.34	487	16	3.29	615	19	3.09
2013	147	1	0.68	552	9	1.63	699	10	1.43
2014	201	2	0.99	551	13	2.36	752	15	1.99
2015	210	3	1.43	584	10	1.71	794	13	1.64
2016	317	4	1.26	570	8	1.40	887	12	1.35
Total	1104	14	1.27	3196	80	2.50	4300	94	2.19

HCRP: Hospital Cornea Retrieval Programme

Table 3: Distribution of seroprevalence among different donor age groups

Age group (years)	HIV	HBV	HCV	Syphilis	Serology positive	Donors	Percentage
<40	4	1	0	0	5	114	4.4
40-60	4	8	4	2	18	789	2.3
60-80	4	20	18	4	46	2400	1.9
>80	0	9	14	2	25	997	2.5

HIV: Human immunodeficiency virus, HBV: Hepatitis B virus, HCV: Hepatitis C virus

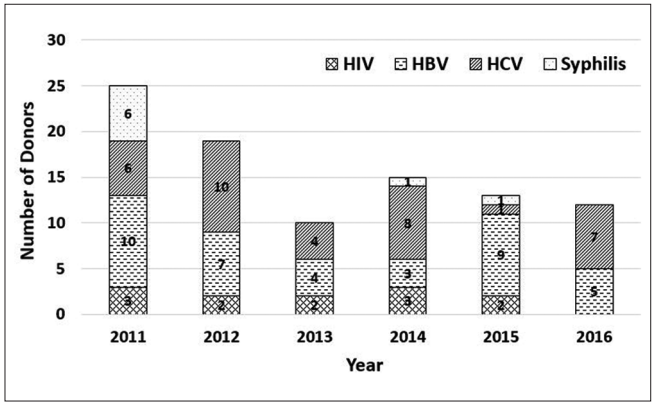

Figure 1: Year-wise distribution of seroprevalence of each disease among cornea donors. (HIV: Human immunodeficiency virus, HBV: Hepatitis B virus, HCV: Hepatitis C virus)

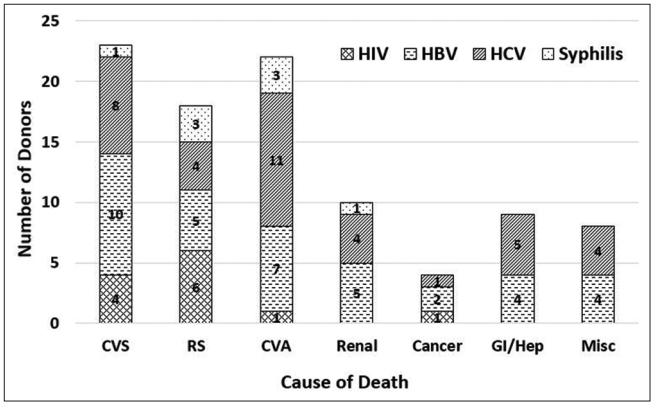

Figure 2: Cause of death of the seropositive donors. (HIV – human immunodeficiency virus; HBV: Hepatitis B virus, HCV: Hepatitis C virus, CVS: Cardiovascular system, RS: Respiratory system, CVA: Cerebrovascular accident, GI/Hep: Gastrointestinal/hepatobiliary, Misc: miscellaneous)

donors. We observed that the nine (9.7%) seropositive donors who died of gastrointestinal or hepatobiliary causes were all positive for either HBV or HCV [Fig. 2]. This was higher than the incidence of death due to similar causes in the total donor population (9.7% vs. 5.7% overall) but it was not statistically significant ($P = 0.11$).

Discussion

Cornea collection, especially through HCRP, has been increasing in India over the last decade.[7,8] There are two previous reports of seroprevalence among cornea donors from India, but both were from voluntary donors only. In this study, we have separately compared the seroprevalence among voluntary and hospital donors. In spite of the available medical records in hospital cases, these are more at risk of being seropositive, and lack of serology being performed routinely in all cases is of further concern.

The National AIDS Control Organization reports adult HIV prevalence to be 0.27% in India, and 0.22% in West Bengal, which is similar to 0.28% observed in this study.[9] The estimated prevalence of HBV carrier state among adults is between 3.0% and 3.7%, and we found a 0.88% positivity for HBsAg.[10,11] The estimated prevalence of HCV in India is 1% and the finding in this study was 0.84%.[11]

Compared to the two previous reports from India, the observed seropositivity in this study is less than that of Mahalakshmi *et al.*'s report (2.2% vs. 5.6%, respectively)[12] and similar to Bhatt's report of 2.3%.[13] In this study, we confirmed the seropositive results with ELISA. Mahalakshmi *et al.* used Western blot for HIV and immunocomb for HBV and HCV confirmation, whereas Bhatt *et al.* used ELISA to confirm the seropositive samples.[12,13] Danneffel and Sugar compared the

Table 4: Comparison of percentage seroprevalence among donors of this study versus other Indian and international eye bank data, blood donor data, and national population seroprevalence

	Current	Mahalakshmi *et al.*[12]	Bhatt *et al.*[13]	EBAA*[2]	Canada[15]	Blood bank[16]	National[9-11]
HIV	0.28	0.62	1.32	0.21	1.12	0.27	0.27
HBV	0.88	3.52	0.49	4.82	2.04	1.38	3-3.7
HCV	0.84	1.45	0.49	2.03	2.08	0.54	1
Syphilis	0.19	NA	NA	0.34	NA	0.32	
Total	2.19	5.59	2.31	7.39	5.23	2.51	

*EBAA: Read as percentage of the total tissues rather than percentage donors; HBV includes HBsAg and HBcAg; NAT done for HIV, HBV, and HCV. EBAA: Eye Bank Association of America, HIV: Human immunodeficiency virus, HBV: Hepatitis B virus, HCV: Hepatitis C virus, HBsAg: Hepatitis B surface antigen, HBcAg: Hepatitis B core antigen, NAT: Nucleic acid test, NA: Not available

Table 5: Relative distribution of donor seropositivity for human immunodeficiency virus, hepatitis B virus, hepatitis C virus, and syphilis in this study versus other Indian and international eye bank data and blood donor data

	Current	Mahalakshmi *et al.*[12]	Bhatt *et al.*[13]	EBAA*[2]	Canada[15]	Blood Bank[16]
HIV (%)	12.8	11.1	57.1	2.8	21.3	10.9
HBV (%)	40.4	63.0	21.4	65.1	39.1	54.9
HCV (%)	38.3	25.9	21.4	27.4	39.6	21.6
Syphilis (%)	8.5	NA	NA	4.6	NA	12.6

*EBAA: Read as percentage of the total tissues rather than percentage donors; HBV includes HBsAg and HBcAg; NAT done for HIV, HBV, and HCV. EBAA: Eye Bank Association of America, HIV: Human immunodeficiency virus, HBV: Hepatitis B virus, HCV: Hepatitis C virus, HBsAg: Hepatitis B surface antigen, HBcAg: Hepatitis B core antigen, NAT: Nucleic acid test, NA: Not available

seroprevalence of HIV among hospital donors and medical examiner cases in the US and did not find any difference between the two groups.[14]

Tables 4 and 5 summarize the comparison of the seropositivity rates from this study with other Indian and international eye bank reports, blood donor, and national population data. It is to be noted that in Western eye banks, serology is determined by antigen-antibody rapid tests, ELISA, and nucleic acid tests.

Chaurasia *et al.* published a report of seroprevalence among blood donors at AIIMS. Total seroprevalence of 2.51% was noted –0.27% HIV, 1.38% HBV, 0.54% HCV, and 0.32% syphilis.[16]

There was an unusual spike of six syphilis cases in 2011. This was probably due to faulty kits, and after the eye bank switched to a different manufacturer, the numbers declined. Interestingly, three of the four women with HIV-positive reports were < 40 years of age, possibly belonging to high-risk groups.

There was a significant reduction in the number of seropositive cases in the HCRP group over the years (5.3% in 2011–1.4% in 2016; $P = 0.004$) whereas the voluntary figure has not changed much (range: 1–4/year). This is probably due to serology being carried out routinely for more patients in the HCRP-designated hospitals.

We also found two seropositive samples among donors with previous negative serology reports. This could be false positive due to postmortem changes in blood as reported by Wilkemeyer *et al.* or it could also be that the initial report was false negative.[17]

Conclusion

Serology tests are a must before tissues are released for surgical use. We found seroprevalence to be significantly higher among HCRP-Ds than VC-Ds. Hence, careful prescreening by the eye donation counselors is also important. And even if prior negative serology reports are available, the tests should be repeated at the eye bank.

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Conflicts of interest

There are no conflicts of interest.

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