Prevalence of Hepatitis B and C Viral Markers in Blood Donors Deferred from Donating Blood on the Basis of a History of Jaundice of Unknown Origin

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Background and aims: The aim of the present study was to know the prevalence of viral hepatitis markers among blood donors deferred on the basis of a history of jaundice of unknown origin and to predict the impact of this deferral on blood safety. **Methods:** Observational study included 200 blood donors deferred on the basis of a history of jaundice of unknown origin and carried out serology and Individual Donation Nucleic Acid Testing (ID-NAT) for hepatitis B and hepatitis C. **Results:** Five (2.5%) out of 200 blood donors deferred on the basis of history of jaundice were reactive on ELISA. Three out of five were reactive for HBsAg; whereas two for anti-HCV antibodies. Out of the 12 ID-NAT initial reactive, 10 samples discriminated on further testing; 4 for HBV, 5 for HCV and 1 was co-infection (HBV + HCV). The odds of being picked up as sero reactive on ELISA was 2.53 (95% CI; 1.04–6.19) and being picked up as ID-NAT yield was 13.08 (95% CI; 5.29–32.37) in donors deferred on a history of jaundice of unknown origin as compared to selected donors without a history of jaundice, with the difference in means achieving statistically significance at $P = 0.03$ and <0.001 respectively. The potential of deferral on a history of jaundice of unknown origin has a capacity to interdict 2–3 HBV and/or HCV reactive blood donors on serology and 5 HBV and/or HCV reactive blood donors on ID-NAT from entering the quarantine blood supply per 100 donors. **Conclusion:** The findings of the present study support that a deferral for a history of jaundice of unknown origin after attaining the age of 12 years in the present scenario of transfusion transmissible infectious disease screening in India. (J CLIN EXP HEPATOL 2019;9:156–161)

Blood Transfusion Services (BTS) have the critical protective dual responsibility of collection of human blood from donors who are apparently at low risk of harbouring infections that have the potential of transmission via allogenic blood transfusion; as well as from those who would be unlikely to put a risk on their own health by donating blood. The suitability of prospective donor is assessed during a medical history recall, and a medical examination in order to select a low risk blood donor, while at the same time ensure that suitable donors are not unnecessarily deferred. Traditionally the historical recall is conducted during medical screening utilizing uniform blood donor questionnaire and consent formats

(UDHQ), and the blood safety is further ensured by the mandatory testing of donated blood that has evolved from serology for indirect markers or direct markers to the novel methods of molecular detection of the blue prints of the organism under consideration.¹ Safe donors who have already donated blood and found to be non reactive at the mandatory testing are further encouraged to donate regularly. Whereas donors found to be at risk for transfusion transmissible infections (TTIs) after a blood donation are encouraged to self-defer from future blood donation. These activities are transformed into action during the time tested approach of information, education and communication (IEC) campaigns undertaken to promote voluntary blood donation or at the time of pre donation counselling.^{2,3} UDHQ are designed to be able to filter donors who could be at higher risk of harbouring infections that could be transmissible by transfusion. Questions have traditionally been included in the questionnaire on basis of precautionary approach and have been added from time to time in order to increase the deferral of high risk blood donors; even though the questions have not been evaluated for their potential benefit to blood and donor safety.² The need to conduct the study was felt when a statistically significant history of jaundice was

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Abbreviations: ELISA: Enzyme Linked Immunosorbent Assay; HBV: Hepatitis B Virus; HCV: Hepatitis C Virus; ID NAT: Individual Donation Nucleic Acid Testing

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noted in blood donors sero-reactive for HBsAg during the post test notification and counselling.³ The aim of the present study was to know the prevalence of viral hepatitis markers among blood donors deferred on the basis of a history of jaundice of unknown origin and to predict the impact of this deferral on blood safety. This study also aims to compare the prevalence of viral hepatitis markers in donors with a history of jaundice of unknown origin with those with no elicitable history of jaundice therefore selected for blood donation.

MATERIAL AND METHODS

This study was conducted at Department of Transfusion Medicine of a tertiary care referral and research institute of northern India, after obtaining the ethical clearance from the institute ethical committee.

Study Design

This was an observational study. The study recruited blood donors deferred on the basis of history jaundice of unknown origin while examining UDHQ, during selection of whole blood donors at blood donation centre, and outdoor voluntary blood donation camps and also included plateletpheresis donors at the apheresis section of the department over a period of one year (April 2015–March 2016).

Study Participant Selection

Voluntary non random (convenience sampling).

Inclusion criteria:

- Age 18–65 years
- Blood donors deferred on history of jaundice, willing to participate in the study after being explained the nature and need of the study, providing written informed consent.

Exclusion criteria:

- Age <18 or, >65 years
- Blood donors deferred on known hepatitis B or hepatitis C infection.

Sample Size Calculation

A sero-reactivity of 10% (± 5) was used for sample size calculations, based on previous study conducted by Zou et al. wherein, four (13.7%) donors were reactive for hepatitis B virus (HBV) and, or hepatitis C virus (HCV) amongst 29 with history of “yellow jaundice, liver disease or hepatitis since age of 11”.¹ The sample size obtained was 139; but the study was conducted on 200 participants.

Study Process Flow

Enzyme Linked Immunosorbant Assay (ELISA, mandatory) and individual donor Nucleic Acid Testing (ID-NAT, additional safety testing) are performed on all blood donations (selected blood donors). For the purpose of the

present study; EDTA and plain evacuated pilot samples were collected from study participants for ELISA and ID-NAT. ID-NAT was performed on the day of collection using Procleix Ultrio Plus Assay (Grifols Diagnostics, USA) and further discriminatory assays were performed for the all initial ID-NAT reactive samples to differentiate between HIV RNA, HBV DNA and HCV RNA on semi automated platform based on transcription mediated amplification. Serum sample was stored for batch testing on I-mark ELISA Reader and Washer (Bio-Rad Laboratories Pvt. Ltd., India) using third generation kits from Transasia Biomedicals Pvt. Ltd., India and SD Bio Standard Diagnostics Pvt. Ltd., India. All tests were performed as per manufacturer's instructions with borderline in house controls placed at two positions in each ELISA run in order to detect inter and intra run variations. The department is enrolled for proficiency testing for infectious disease with the Indian Red Cross Blood Centre (IRCS), Mumbai, the Blood Bank External Quality Assessment Scheme (BEQAS) Jaipur recognized by the National Board for Accreditation of Hospitals and Healthcare providers (NABH), Quality Council of India, and the National Reference Laboratory (NRL) of AIIMS, New Delhi designated by the National AIDS Control Organisation (NACO), India.

RESULTS

Prevalence of Hepatitis Markers in Study Participants

A total 200 blood samples of blood donors deferred on the basis of history of jaundice of unknown origin, were tested for HBV and HCV viral markers by ELISA as well as NAT and the results are detailed in [Table 1](#).

ELISA

Five (2.5%) out of 200 blood donors deferred on the basis of history of jaundice were reactive on ELISA. Three out of five (1.5%) were reactive for HBsAg; whereas two (1%) for anti-HCV antibodies.

ID-NAT

Twelve (6%) of 200 blood donors deferred on the basis of history of jaundice were found to be ID-NAT initial reactive; including all 5 (2.5%) that were reactive on ELISA (concordant reactive). Out of the 12 ID-NAT initial reactive, 10 discriminated on further testing; 4 (2%) for HBV, 5 (2.5%) for HCV and 1 (0.5%) was co-infection (HBV + HCV). Therefore a combined prevalence of 5% (10 out of 200) was obtained on ID-NAT. However 5 were reactive on ELISA, leading to ID-NAT yield of 2.6% (1 in 38 serologically non-reactive blood donors who were deferred from donating blood on the basis of a history of jaundice of unknown origin).

Table 1 Prevalence of Hepatitis Markers in Donors Deferred on the Basis of Jaundice.

Testing platform	Prevalence	P value	OR (95% CI)
ELISA [N = 200]	2.5%	0.03	2.53 (1.04–6.19)
ELISA ^a [N = 60,302]	1.0%		
ID-NAT initial [N = 200]	6.0%	0.82	1.21 (0.51–2.87)
ID-NAT discriminatory [N = 200]	5.0%		
ID-NAT yield [N = 195]	2.6%	<0.001	13.08 (5.29–32.37)
ID NAT yield ^b [N = 60,302]	0.2%		

^aCombined sero prevalence of both HBsAg and Anti HCV antibodies in selected blood donors during the period of the study.

^bID NAT yield in selected blood donors during the period of the study.

Donor Demographic Factors and Viral Markers

There were 185 (92.5%) males and 15 (7.5%) females deferred from donating blood on the basis of history of jaundice including as detailed in Table 2. The prevalence of hepatitis viral markers was not different among the age and gender donors deferred on the basis of history of jaundice of unknown origin. The prevalence of hepatitis viral markers was significantly higher in the uneducated blood donors deferred on the basis of history of jaundice of unknown origin. The prevalence of hepatitis viral markers was not statistically different amongst voluntary or replacement donors deferred on the basis of history of jaundice of unknown origin. The prevalence of hepatitis viral markers was different between first time and repeat blood donors deferred on the basis of history of jaundice of unknown origin. All sero reactive donors had history of jaundice of unknown origin after the age of 12 years.

DISCUSSION

The results of present study demonstrate that blood donors deferred on history of jaundice of unknown origin after the age of 12 years have combined HBV and/or HCV prevalence of 2.5% on serology and 5% on molecular testing. Bajpai et al. report 1.9% prevalence of hepatitis B and C in 207 donors with history of jaundice, 2 (0.96%) for HBsAg and 2 (0.96%) for anti-HCV on serology from New Delhi, India.⁴ Therefore the prevalence of hepatitis markers in both the studies from India are comparable. The population prevalence of HBV is 3.7% and HCV is 1% in India as per the National Centre for Disease Control data.⁵ Whereas Zou et al. have earlier reported 13.7% prevalence for HBV and, or HCV viral marker amongst 29 donors with history of “yellow jaundice, liver disease or hepatitis since age of 11” from American Red Cross, United States of America.¹ However the higher prevalence from USA could be attributed to the difference in demography and ethnicity.

In the present study sero-prevalence of HBV, HCV was 1.5% and 1% respectively; with a combined sero-prevalence

was 2.5%, this reflects that for every 100 blood donors who have a history of jaundice of unknown origin; there could be 2–3 donors who could be reactive on serological testing (ELISA). On the other hand the combined NAT yield for HBV and HCV in our study was 2.6% this reflects that, one in every 37 or 38 serology non-reactive blood donor with history of jaundice of unknown could have HBV DNA or HCV RNA detectable on ID-NAT testing. Only half (50%) of the deferred donors reactive on ID-NAT were reactive on serology, demonstrating the additional layer of safety imparted to blood and blood components with the addition of NAT testing to blood donor infectious disease testing protocol. This is what is termed as NAT yield and the non reactive status of such donors on serology could be attributed to reasons like the donor being in either the first or the second window period (cryptic carriers and occult infections) as understood after the studies on viral replication kinetics and the host response to the infection (antibody production).^{6–9} The odds of being sero reactive was 2.53 (95% CI; 1.04–6.19) in donors deferred on a history of jaundice of unknown origin as compared to selected donors without a history of jaundice, with the difference in means achieving statistical significance at $P = 0.03$. Similarly the odds being picked up as ID-NAT yield in donors deferred on a history of jaundice of unknown origin was 13.08 (95% CI; 5.29–32.37) as compared to selected donors without a history of jaundice, and the difference in means was statistically significant at $P < 0.001$.

The difference in means of the prevalence of markers of hepatitis in blood donors deferred on the basis of history of jaundice of unknown origin amongst age groups, gender, type of donor, previous donation status did not achieve statistical significance. However it was statistically significant when compared across educational status with $P = 0.001$, thought limited by the numbers recruited in the study.

Significantly all blood donors deferred from donating blood on the basis of a history of jaundice of unknown origin after the age of 12 years were found to be reactive on serology and NAT.

Table 2 Prevalence of Hepatitis Markers Stratified on the Basis of Donor Demography.

Age (years)	ELISA	ID-NAT	Disc NAT
18–30 (N = 116)	2 (1.7%)	3 (2.5%)	2 (1.7%)
31–40 (N = 60)	2 (3.3%)	6 (10%)	5 (8.3%)
41–50 (N = 19)	Nil	2 (10.5%)	2 (10.5%)
51–65 (N = 5)	1 (20%)	1 (20%)	1 (20%)
P value	0.06	0.08	0.05
<i>Gender</i>			
Male (N = 185)	3 (1.6%)	10 (5.4%)	8 (4.3%)
Female (N = 15)	2 (13%)	2 (13%)	2 (13%)
P value	0.05	0.22	0.16
<i>Education</i>			
Uneducated (N = 3)	2 (66.7%)	2 (66.7%)	2 (66.7%)
Secondary (N = 22)	Nil	Nil	Nil
Senior Secondary (N = 36)	1 (2.7%)	1 (2.7%)	1 (2.7%)
Graduate (N = 80)	1 (1.2%)	3 (3.7%)	2 (2.5%)
Postgraduate (N = 59)	1 (1.6%)	6 (10.1%)	5 (8.4%)
P value	0.0001	0.0001	0.0001
<i>Donor type</i>			
Voluntary (N = 179)	4 (2.2%)	9 (5%)	7 (3.9%)
Replacement (N = 21)	1 (4.7%)	3 (14.2%)	3 (14.2%)
P value	0.99	0.11	0.07
<i>Donation type</i>			
First time (N = 97)	4 (4.2%)	7 (7.2%)	6 (6.1%)
Repeat (N = 103)	1 (0.9%)	5 (4.8%)	4 (3.9%)
P value	0.20	0.48	0.52
<i>Jaundice (at age)</i>			
<1 (N = 1)	Nil	Nil	Nil
1–12 (N = 14)	Nil	Nil	Nil
>12 (N = 185)	5	12	10
P value	0.81	0.59	0.65

In India a safe blood donor is one who has been selected in strict accordance with the blood donor selection criteria of the Drugs and Cosmetics Act of 1940 and the Rules of 1945 as amended from time to time.¹⁰ As per the act “No person shall donate blood and no blood bank shall draw blood from a person, suffering from Hepatitis infection”. However in practice the blood donors who give a history of jaundice, have difficulties to recall the duration, severity of jaundice and whether the testing to differentiate the infectious cause of hepatitis was done or not; and therefore the deferral criteria to be adopted becomes difficult in practice and could vary as per the discretion of the medical officers in accordance with the SOP (standard operating procedure) of the particular BTS. The SOP of blood donor selection at our department warrants permanent deferral for history of jaundice of unknown origin, this was kept in view of the prevalence of HBV and HCV in our country

and the reasons detailed above regarding the inability of blood donors to recall the type of jaundice at the time of donor selection. The efficiency of transmission of HBV and HCV through transfusion of a reactive unit is about 90 percent. The current risk of transfusion associated hepatitis, today with highly sensitive testing techniques is less but still exists primarily due to the HBV and HCV. The risk of HBV being transmitted by a transfusion today is in the order of 1 per 63,000 units of blood, whereas for HCV it is in order of 1 in 125,000 units of blood based on serology.¹¹ Even slight compromise on the criteria of deferral for history of jaundice of unknown origin may pose serious threat to blood safety as not only known pathogens but emerging pathogens may find way into blood supplies. Knowing the natural history of HBV and HCV which have chronic carrier stage, genetic diversity, mutants and cryptic/occult infection and that during

this the potential of transmission through blood transfusion remains. Therefore need to garner data as evidence to support for permanent deferral for jaundice in the interest of blood safety was realized.

It can be concluded based on the findings obtained in the present study that the potential of deferral on a history of jaundice of unknown origin has a capacity to interdict 2–3 HBV and/or HCV reactive blood donors on serology and 5 HBV and/or HCV reactive blood donors on ID-NAT from entering the quarantine blood supply per 100 donors (2.5% on serology, 5% on ID-NAT initial reactivity and 2.6% ID-NAT yield). This is two and half times higher to the combined sero prevalence of 1% on serology (HBsAg and anti HCV) in accepted blood donors during the period of study and that of 0.93% reported in the earlier publication from our department.^{3,12} Further this is even higher than the observed NAT yield of 0.2% within accepted donors (without elicitable history of jaundice of unknown origin at time of donor selection) during the study period.¹²

Therefore in peripheral blood bank setting and in urgent apheresis scenario; where at present only rapid testing is available in majority of blood banks in our country, there is a possibility that blood donors if not deferred strictly and selected for donation would stand a chance of being non reactive, escape infectious disease screening and thus theoretically have the potential of spreading transfusion transmissible infections in recipients.^{13–15}

Based on the findings obtained in the present study we infer that the permanent deferral of blood donors on the basis of a history of jaundice is an additional step towards pursuit to blood safety, thought more data from different regions will be needed to arrive at a unanimous consensus for the country as a whole. Nevertheless these preliminary results would encourage other researchers to conduct similar studies preferably on a larger sample size and in donors who have a history of jaundice before attaining the age of 12 years.

Finally the study emphasizes the importance of eliciting a history of jaundice while screening the donor prior to blood donation and following the deferral criteria as per the Drugs and Cosmetics Act, Government of India. Any change from the prevalent rules will need to be based on evidence and consensus of opinion.

Limitations

The authors acknowledge that they could not recruit all donors deferred on the basis of a history of jaundice and therefore selection bias may exist. The present study was limited to 200 participants that to representing blood donors from northern India only, therefore the obtained prevalence of HBV, HCV among blood donor deferred on history of jaundice cannot be generalized to the whole of the Indian population. The core antibody for HBV if taken

in account may further increase the prevalence from that observed in the present study.

CONCLUSION

The findings of the present study supports a deferral for a history of jaundice of unknown origin after attaining the age of 12 years in the present scenario of transfusion transmissible infectious disease screening in India, wherein both rapid testing and third generation ELISA are the baseline, with only few blood banks employing chemiluminisence and NAT and the core antibody test for HCV being not mandatory.

CONFLICTS OF INTEREST

The authors have none to declare.

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