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Short Paper

Prevalence of hepatitis B & C infections in prospective blood donors deferred due to history of jaundice

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***Background & objectives*: As per national guidelines, prospective blood donors with a history of jaundice of unknown cause are deferred permanently to prevent the transmission of hepatitis B and C. The validity of this guideline was tested by comparing prevalence rates of hepatitis B and C in prospective blood donors deferred due to a history of jaundice, with that of donors who were found fit.**

***Methods*: Blood samples of 212 consecutive donors (male, n=203) deferred due to a history of jaundice were studied for hepatitis B and C by rapid test kits as well as by chemiluminescence (n=115) or ELISA (n=97). Consecutive healthy donors (n=549; male, n=518) were also studied by ELISA (n=266) or chemiluminescence (n=283).**

***Results*: The cumulative prevalence detected by rapid test kit and ELISA/chemiluminescence tests of hepatitis B (n=10) and C (n=2) among donors deferred due to a history of jaundice (n=212) was 5.7 per cent [95% confidence interval (CI): 2.9, 9.9]. The prevalence of reactive results among healthy donors (n=549) by ELISA/chemiluminescence tests was 3.3 per cent (95% CI: 1.9, 5.2), which included hepatitis B (n=15) and hepatitis C (n=3) cases. Compared to healthy donors, the odds of seropositivity among jaundice-deferred donors was 1.7 (95% CI: 0.8, 3.6), *P*=0.15. For rapid test-negative deferred donors, the odds of seropositivity by ELISA/chemiluminescence declined to 0.4 (0.1, 1.5), *P*=0.19.**

***Interpretation & conclusions*: The prevalence rates of hepatitis B and C in prospective blood donors deferred due to a history of jaundice of unknown aetiology did not differ significantly from that in healthy donors. The current practice of permanently deferring such donors depletes valuable donor pool. A strategy of rejecting only those donors who are found reactive on pre-donation testing by rapid test needs further validation.**

Key words Blood donors - hepatitis B - hepatitis C - jaundice - medical history taking - prevalence

Suitability of a potential blood donor is screened using a questionnaire followed by physical examination. Once the donor is found fit, blood is collected. The current guidelines do not advocate pre-donation testing[1,2]. The unit of blood, thus collected, subsequently undergoes testing in the blood bank for transfusion-transmitted infections (TTIs)[1]. Some blood bags, obtained from 'healthy donors', are routinely

discarded following laboratory testing due to them being reactive for HIV, hepatitis B virus (HBV) and/or hepatitis C virus (HCV)[3]. There is a very low but estimable residual risk of transmission of TTI including hepatitis B and hepatitis C to the patients through the transfusion of the blood products thus screened[4,5]. Increased donor awareness, good quality management protocols and the latest advances in testing for TTI[6] have thus contributed to considerable decline in transfusion mediated transmission of infection.

As per national guidelines, prospective donors with hepatitis B or C infection or jaundice of unknown causes are deferred permanently[1]. Those with jaundice due to confirmed hepatitis A or E infection are deferred temporarily for 12 months. Potential donors with a history of jaundice are accepted if the jaundice is known to have occurred in the neonatal period or is attributed to gallstones, Rh mismatch or infectious mononucleosis.

In Indian setting where most of the potential donors do not possess any laboratory documentation confirming hepatitis or its aetiology, the burden of jaundice of 'unknown' cause tends to be high and a large number of prospective donors thus get permanently deferred during history taking[7,8]. This method of deferral based on history captures not only individuals with possible HBV or HCV infections, but also those with infections due to hepatitis A or E, other viral infections, or medical conditions associated with jaundice that do not cause post-transfusion hepatitis.

Majority of blood donations in India are done on replacement basis[9]. Replacement donors often conceal a history likely to result in their rejection, to facilitate the availability of blood products for their patients[10,11]. On the other hand, general reluctance for voluntary or replacement donation amongst masses in developing countries like India results in low donor pool[12]. Therefore, history alone is unlikely to completely mitigate the risk of TTI and existing guidelines further deplete the donor pool. Therefore, it would be pertinent to check the validity of the current criterion of permanent deferral of donors with a history of jaundice of unknown cause to explore the possibility of reintroduction of such donors in healthy donor pool. To test this, it would be appropriate to demonstrate that the prevalence for HBV and HCV in this group is not significantly different from healthy donors and also that the residual risk of HBV and HCV is similar. While the latter is difficult to demonstrate, in this study, we decided to measure the prevalence of hepatitis B and C among prospective blood donors deferred due to a history of jaundice of unknown cause and compared them with that in donors who were found fit. Additionally we tested donors deferred with a history of jaundice of unknown cause using rapid diagnostic test (RDT) kits for HBV and HCV, to check its performance with respect to ELISA/chemiluminescence results.

Material and Methods

This study was conducted in the Blood Bank of J. N. Medical College Hospital, Aligarh Muslim University, Aligarh, Uttar Pradesh, from February 2015 to July 2017, following approval obtained from the Institutional Ethics Committee and it adhered to the recommended protocols. All prospective donors, after giving informed consent, answered hepatitis risk-directed questions including previous transfusion, hospitalization, surgery and high-risk behaviour (HRB), defined as having multiple sexual partners. Consecutive donors deferred due to a history of jaundice were enrolled and compared with healthy donors who served as controls. Blood samples were collected from all enrolled participants and their serological status for hepatitis B and C were assessed. Screening of TTI shifted from ELISA to chemiluminescence technique after June 2016; accordingly, the study samples were tested by two different techniques corresponding to the respective time periods. The samples of the deferred donors were additionally tested by the Food and Drug Administration (FDA)-approved RDT. For testing hepatitis B surface antigen, SD HBsAg and Alere TrueLine (SD Bioline, S. Korea), Hepaview (Qualpro, India) and HEPA-SCAN (Bhat Biotech, India) rapid test kits were used. For testing hepatitis C antibody, SD Bio (SD Bioline, S. Korea) and Accucare (LabCare, India) rapid test kits were used. ELISA was performed on semi-automated platform (Robonik ELISA Reader and Microplate Washer, India) using third generation ELISA test kits (ErbaLisa, Transasia, India) and QUALISA (Qualpro, India) for HBsAg and SD ELISA (SD Bioline, S. Korea) and Qualisa (Qualpro, India) for anti-HCV. The chemiluminescence testing was done on Vitros ECiQ machine from Ortho Clinical Diagnostics, India.

Sample size: As per the National Centre for Disease Control, the population prevalence of HBV in India is 3.7 per cent and of HCV is 1 per cent[13]. The reported hepatitis B and C prevalence rates amongst donors

deferred due to jaundice in India have varied from 2 to 6 per cent[14,15] a study from the west reported seroprevalence of 13.8 per cent in such donors[16]. The sample size was computed using an online calculator to detect a difference in proportions between the two groups[17]; significance level (alpha) of 0.05 and power of 80 per cent were aimed. Assuming combined seroprevalence of hepatitis B and C among healthy donors as four per cent and among donors deferred due to a history of jaundice as 10 per cent, the study required a sample of 516 healthy donors and 206 donors deferred due to a history of jaundice for enrolment ratio of 2.5, to test the difference between the groups. Accordingly, we enrolled 212 donors with a history of jaundice and 549 healthy donors.

Statistical analysis: The prevalence rates with 95 per cent confidence interval (CI) were calculated for hepatitis B and C among deferred and healthy donors. The prevalence rate difference and odds ratio were computed for hepatitis B and C positivity, among healthy and deferred donors as well as between healthy and deferred donors who tested negative by rapid test kit. The significance was considered at $P<0.05$. All statistical analyses were conducted using IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, NY, USA).

Results & Discussion

Of the 212 deferred donors (male: n=203), blood samples of 115 participants were tested by ELISA and 97 by chemiluminescence. All 212 samples also underwent testing by RDT. Out of the 549 healthy donors (male donors: n=518), blood samples of 266 participants were tested by ELISA and 283 by chemiluminescence technique. The mean±S.D (range) age of the deferred donors was 32.2±8.9 (18-62) yr; and that of healthy donors was 29.3±8.3 (18-58) yr.

Seroprevalence in deferred donors: The cumulative prevalence rate of hepatitis B and hepatitis C in prospective donors deferred due to a history of jaundice was 5.7 per cent (95% CI: 2.9%, 9.9%) using the combined results of rapid and ELISA/chemiluminescence testing, hepatitis B (n=10) and hepatitis C (n=2; Tables I and II). The use of rapid test kits among deferred donors resulted in nine positive cases (HBsAg n=8; anti-HCV n=1). Chemiluminescence testing additionally detected one anti-HCV-reactive and two HBsAg cases that were reported negative by rapid test kits [prevalence of 1.5 per cent (95% CI: 0.3%, 4.3%)] (Table II). Rapid testing detected one case of HBsAg that was not diagnosed by the ELISA. The concordance rate between the two methods of testing for non-reactive samples was 99 per cent (201/203).

Seroprevalence in healthy donors: Among the healthy donors accepted for blood donation, the cumulative prevalence was 3.3 per cent (95% CI: 1.9%, 5.2%), using ELISA/chemiluminescence testing; hepatitis B (n=15) and hepatitis C (n=3).

The odds of hepatitis B or C positivity in donors deferred due to a history of jaundice when compared with healthy donors was 1.7 (95% CI: 0.8, 3.8), $P=0.15$. The odds of a positive result by ELISA/chemiluminescence on the samples that tested negative by rapid test (n=203), when compared with healthy donors, was 0.4 (95% CI: 0.1, 1.5), $P=0.20$ (Table II).

Among the risk factors associated with transfusion-transmitted hepatitis, history of HRB was the most common factor noted in 17.9 per cent (n=38), out of which 7.9 per cent were found reactive for either hepatitis B (n=2) or C (n=1). Four other patients gave a history of risk factors including previous hospitalization (n=2), surgery (n=1) or blood transfusion (n=1); none of them were found seropositive.

High risk donor deferral before donation is one of the blood donor screening strategies to ensure safety of the blood products, but the risk factors vary by country or region[18]. History of jaundice of unknown cause is one of the major reasons for permanent donor deferral in our country and is based on the premise of reducing transfusion-transmitted hepatitis[7,8]. In our study cohort, we observed that the prevalence rates of hepatitis B and C-reactive samples amongst those with a history of jaundice were not statistically significantly different compared to that of healthy donors (5.7 *vs*. 3.3). We noted that prior rapid testing among donors with a history of jaundice to exclude seropositive subjects could further reduce hepatitis B and C positive cases to 1.5 per cent. That the risk of HBV or HCV related TTI would be low from these donors deferred due to jaundice of uncertain cause is reinforced by the fact that majority of them (n=170, 80.2%) had no risk factors associating them with transfusion-transmitted hepatitis.

Technically, nucleic acid amplification technique (NAT) is the best method available to detect TTI

Table I. Test results of deferred donors (n=212) and healthy donors (n=549)*

Donor category	Result	Rapid test	ELISA	Chemi	Total positive cases
Deferred donors		n=212	n=115	n=97	n=212
	HBsAg	8 (3.8)^	5 (4.3)	4 (4.1)	10 (4.7)
	Anti-HCV	1 (0.5)	1 (0.9)	1 (1)	2 (0.9)
	Total	9 (4.2)	6 (5.2)	5 (5.2)	12 (5.7)
Healthy donors			n=266	n=283	n=549
	HBsAg	-	6 (2.3)	9 (3.2)	15 (2.7)
	Anti-HCV	-	0	3 (1.1)	3 (0.5)
	Total	-	6 (5.2)	12 (4.2)	18 (3.3)

*All values represent n (%) of reactive cases; ^1 case was HBsAg positive by rapid test alone. ELISA, enzyme-linked immunosorbent assay; Chemi, chemiluminescence assay; HCV, hepatitis C virus; HBsAg, hepatitis B surface antigen

Table II. Comparison of hepatitis B and C prevalence between healthy and deferred donors

Donor category (n)	Positive result (n)	Prevalence, %(95% CI)	Difference*, %(95% CI)	OR*, %(95% CI)	*P*
Healthy donors (n=549)	18	3.3 (1.9-5.2)	-	-	-
Donors deferred due to history of jaundice (n=212)	12	5.7 (2.9-9.9)	2.4 (−0.6-6.5)	1.7 (0.8-3.6)	0.15
Rapid test-negative deferred donors (n=203)	3	1.5 (0.3-4.3)	1.8 (−1.2-3.9)	0.4 (0.1-1.5)	0.2

*Versus healthy donors. OR, odds ratio; CI, confidence interval

and avoid the risk of transmission, especially in cases of occult infections (high sensitivity) as well as fresh infections (least window period)[19,20]. As per published Indian studies, the difference in prevalence rates by serology versus NAT (NAT yield) among jaundice-deferred donors is around 2.5-4 per cent[14,15]. Assuming the NAT yield in our cohort to be on similar lines, the true prevalence of hepatitis B and C among the deferred donors would be expected to be higher than the prevalence rate of 5.7 per cent observed in our study. Nonetheless, a major proportion of donors deferred due to a previous history of jaundice of uncertain aetiology would still have been inappropriately deferred permanently. It appears from our results that pre-donation rapid testing in jaundice-deferred donors can quickly identify most seropositive subjects who could then be deferred permanently. The remaining seronegative subjects may be assessed further for potential requalification. However, it is pertinent to point out here that the absence of significant difference in seropositivity between healthy and jaundice-deferred donors does not establish their equivalence with respect to true negativity and, by inference, does not equate the residual risk of TTI in these two groups. Interestingly, the USFDA in its guidance for requalification of donors previously deferred for a history of unknown or uncertain viral hepatitis has given non-binding recommendation for accepting such deferred donors after following certain standard operating procedure[21].

Our study had some limitations, the major one being lack of testing of study samples for hepatitis B and C by NAT which in combination with ELISA testing is presently the best strategy to find the true prevalence of hepatitis B and hepatitis C in the donors and maximally reduce the residual risk of transmission of TTI including hepatitis B and hepatitis C[22]. Second, our study did not measure the residual TTI risk. The residual risk of TTI happens either due to assay failures or by donors being in the diagnostic window period[23]. Finally, the technique of TTI testing for hepatitis B or C detection changed over the period of our study. However, a recent study has shown both testing methods to have comparable sensitivity and specificity[24]. Furthermore, the frequency of testing by both assay methods was equally distributed in either group of patients in the present study and hence was unlikely to have affected the final result.

Despite these limitations, it can be inferred from our study that permanently excluding donors with a history of jaundice of unknown cause results in

the loss of a large number of potential donors. This is particularly true in the Indian context given the uncertainties associated with historical screening methods in detecting hepatitis B and C. Nonetheless, the significance of donor screening for jaundice as a first screening step to ensure blood safety cannot be precluded at the moment. Risk–benefit analysis of requalification versus permanent deferral of all such deferred donors needs to be carried out in a larger well-planned investigation with the combined use of ELISA and NAT for an optimum recommendation.

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References

1. NBTC (Internet). *Guidelines for blood donor selection and donor referrals*. Available from: *http://nbtc.naco.gov.in/assets/resources /policy/Letter-reg-%20guidelines-for-blood-donor-selection&referral-2017.pdf*, accessed on February 19, 2019.
2. National AIDS Control Organisation. Ministry of Health & Family Welfare, Government of India. *Standards for blood banks & blood transfusion services*. Available from: *http://www.naco.gov.in/blood-transfusion-services-publications*, accessed on February 19, 2019.
3. World Health Organization. *Blood safety and availability.* Available from: *https://www.who.int/news-room/fact-sheets/detail/blood-safety-and-availability*, accessed on June 16, 2021.
4. Dean CL, Wade J, Roback JD. Transfusion-transmitted infections: An update on product screening, diagnostic techniques, and the path ahead. *J Clin Microbiol* 2018; *56* : e00352-18.
5. Dwyre DM, Fernando LP, Holland PV. Hepatitis B, hepatitis C and HIV transfusion-transmitted infections in the 21st century. *Vox Sang* 2011; *100* : 92-8.
6. Kupek E. Residual risk of hepatitis-B-infected blood donations: Estimation methods and perspectives. *ISRN Infect Dis* 2013; *2013* : 1-15.
7. Sharma T, Singh B, Bhatt GC. Profile of deferral of blood donors in regional blood transfusion center in North India. *Asian J Transfus Sci* 2013; *7* : 163-4.
8. Bahadur S, Jain S, Goel RK, Pahuja S, Jain M. Analysis of blood donor deferral characteristics in Delhi, India. *Southeast Asian J Trop Med Public Health* 2009; *40* : 1087-91.
9. Pahuja S, Sharma M, Baitha B, Jain M. Prevalence and trends of markers of hepatitis C virus, hepatitis B virus and human immunodeficiency virus in Delhi blood donors: A hospital based study. *Jpn J Infect Dis* 2007; *60* : 389-91.
10. Nair SC, Mammen JJ. Repeat voluntary non-remunerated blood donor is the best quality indicator for blood safety. *Indian J Med Res* 2015; *141* : 749-52.
11. Abdel Messih IY, Ismail MA, Saad AA, Azer MR. The degree of safety of family replacement donors versus voluntary non-remunerated donors in an Egyptian population: A comparative study. *Blood Transfus* 2014; *12* : 159-65.
12. Dubey A, Sonker A, Chaurasia R, Chaudhary R. Knowledge, attitude and beliefs of people in North India regarding blood donation. *Blood Transfus* 2014; *12* (Suppl 1) : s21-7.
13. National Centre for Disease Control Newsletter. *Hepatitis in India: Burden, strategies and plans*. Available from *https://ncdc.mohfw.gov.in/ WriteReadData/l892s/NewsLtr0103_20146480274026.pdf*, accessed on February 19, 2019.
14. Prakash C, Sachdev S, Marwaha N, Hans R. 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. *J Clin Exp Hepatol* 2019; *9* : 156-61.
15. Dogra M, Doda V, Kotwal U, Arora S, Bhardwaj S, Aroskar GS. Whole blood donor with a history of jaundice: Reviewing the deferral criteria. *J Appl Hematol* 2016; *7* : 41-2.
16. Zou S, Fujii K, Johnson S, Spencer B, Washington N, Iv EN, *et al*. Prevalence of selected viral infections among blood donors deferred for potential risk to blood safety. *Transfusion* 2006; *46* : 1997-2003.
17. University of California San Fransisco Clinical & Translational Science Institute. *Sample size calculators*. California: UCSF CTSI; 2020. Available from: *https://www.sample-size.net/*, accessed on July 18, 2020.
18. Torre GL, Saulle R. Risk factors from HBV infection among blood donors: A systematic review. *Asian Pac J Trop Biomed* 2016; *6* : 344-9.
19. Datta S, Khillan K, Ranjan V, Wattal C. Nucleic acid amplification test: Bridging the gap in blood safety & re-evaluation of blood screening for cryptic transfusion-transmitted infection among Indian donors. *Indian J Med Res* 2019; *149* : 389-95.
20. Makroo RN, Choudhury N, Jagannathan L, Parihar-Malhotra M, Raina V, Chaudhary RK, *et al*. Multicenter evaluation of individual donor nucleic acid testing (NAT) for simultaneous detection of human immunodeficiency virus -1 & hepatitis B & C viruses in Indian blood donors. *Indian J Med Res* 2008; *127* : 140-7.
21. US Food & Drug Administration. Director, Center for Biologics Evaluation and Research. *Donor suitability related to laboratory testing for viral hepatitis and a history of viral hepatitis: Memorandum to all blood and plasma establishments*. Available from: *https://www.fda.gov/regulatory-information/search-fda-guidance -documents/requalification-donors-previously-deferred-history-viral-hepatitis-after-11th-birthday*, accessed on February 19, 2019.
22. Huang W, Wei W, Shi XT, Jiang T. The analysis of the detection performance of nucleic acid testing and ELISA for HIV, HBV and HCB. *Front Lab Med* 2017; *1* : 200-2.
23. World Health Organization. *WHO technological report series, no. 1004-217, annex 4, guidelines on estimation of residual risk of HIV, HBV, or HCV infections via cellular blood components and plasma*. Available from: *https://*

cdn.who .int/media/docs/default-source/biologicals/blood-products/document -migration/resriskgl_who_trs_1004_web_annex_4.pdf?sfvrsn=55dd09d3_3, accessed on July 20, 2020.

24. Tiwari AK, Upadhyay AP, Arora D, Wadhwa T, Aggarwal G, Pabbi S, *et al*. Head-to-head comparison of Enzyme Linked Immunosorbent Assay (ELISA) and Enhanced Chemiluminescence Immunoassay (ECLIA) for the detection of Transfusion Transmitted Disease (TTD) Markers; HIV, HCV and HBV in blood donors, in India. *J Virol Methods* 2020; *285* : 113962.

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