

The Prevalence of Transfusion-transmitted Infections Among Blood Donors in Hospital Universiti Sains Malaysia, Kelantan

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ABSTRACT

Objectives: Blood bank centres routinely screen for hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) to ensure the safety of blood supply and thus prevent dissemination of these viruses via blood transfusion. The aims of the present study were to evaluate the detection of transfusion-transmitted infection (TTI) markers using standard serological methods and nucleic acid testing (NAT) among blood donors in Hospital Universiti Sains Malaysia. **Methods:** Donated blood units were assessed for the presence or absence of HBV, HCV and HIV using two screening methods; serology and NAT. Reactive blood samples were then subjected to serological confirmatory and NAT discriminatory assays. **Results:** A total of 9,669 donors were recruited from September 2017 to June 2018. Among these 36 donors were reactive either for HBV, HCV or HIV by serological and 8 by NAT

screening. However, only 10 (3 for HBV and 7 for HCV) donors were tested positive using serological confirmatory and 5 (2 for HBV and 3 for HCV) by NAT discriminatory assays. Note that all 5 NAT positive donors detected in the NAT discriminatory assays were confirmed to be serologically reactive. Therefore, the prevalence of HBV, HCV and HIV were found to be 0.03%, 0.07% and 0% (respectively) in our donor pool. **Conclusion:** Overall, the implementation of both serological and NAT screening and confirmatory assays should be used routinely to reduce risk of infection transmission via transfusion of blood and blood components.

Keywords: Transfusion-transmitted infections; Hepatitis B virus; Hepatitis C virus; Human immunodeficiency virus; Serological and nucleic acid assays.

Introduction

Blood transfusion is an important part of patient management in hospitals. However, blood transfusion is not without risks including haemolytic transfusion reaction and transfusion-transmitted infection (TTI) [1-2]. Therefore, all donated blood units are routinely screened for hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) to ensure the safety of the blood supply and prevent dissemination of these viruses via transfusion. Enzyme-linked immunosorbent assay (ELISA) and Line Immuno Assay (LIA) for detections of viral antibodies and antigens are the commonly used serological screening and confirmatory techniques [3]. However, these infectious agents can be difficult to recognise or entirely undetectable serologically; i.e. due to the limited or absence of viral antigens and human antibodies during the early phase (window period) and occult infections [4]. These limitations of phenotyping assays have been recently resolved by testing of viral DNA in donated blood using a molecular technique; i.e. nucleic acid testing (NAT). Several blood donation centres have reported that incidence of transfusion-transmitted HBV, HCV and HIV infection and number of discarded of blood donation units have been reduced by combined serological and NAT screenings [3-5] and the rate has further decreased with the inclusion of serological confirmatory and NAT discriminatory assays [3, 6-9].

From September 2017, The Transfusion Medicine Unit, Hospital Universiti Sains Malaysia (HUSM) has implemented two screening methods for donated blood, serology and NAT. These methods detect the presence or absence of HBV, HCV and HIV. Reactive blood samples are then subjected to serological confirmatory and NAT discriminatory assays. Until present, there are no documented data on the prevalence of transfusion-transmissible HBV,

HCV and HIV among blood donors in Malaysia. Therefore, this study was conducted to provide statistics for these infections among blood donors registered at HUSM from September 2017 to June 2018. This study also aimed to evaluate the relative efficiency of serological and NAT techniques for detecting of TTIs in our donor population.

METHODS

Sample collection

Blood samples were collected from a total of 9,669 voluntary donors registered at Transfusion Medicine Unit, HUSM from September 2017 to June 2018. They were all fulfilled the criteria for blood donation as described by Saleh et al. [5]. A total of 6 milliliters (mL) of peripheral venous blood were collected into three blood collection tubes for laboratory analyses; Tube 1 contains EDTA and was used for ABO and Rh blood re-grouping, Tube 2 contains coagulant separating gel and was used for serological screening and confirmatory testing of HBsAg, anti-HCV and HIV Ag/Ab and Tube 3 contains inert gel and spray-dried K₂EDTA anticoagulant and was used for NAT screening and discriminatory testing. Informed consent was obtained from each participant and this study was reviewed and approved by the Human Research Ethics Committee, Universiti Sains Malaysia (certificate number: USM/JEPEM/18070311).

Serological screening of HBsAg, anti-HCV and combo HIV Ag/Ab

The serological screening of donated blood samples was conducted in Microbiology Laboratory, Transfusion Medicine Unit, HUSM using chemiluminescent microparticle immunoassay technology (Abbott Architect Immunoassay Analyzer, USA). The cut off point for seropositive was set at more than 1 OD.

Serological confirmatory test

The HBsAg, anti-HCV and combo HIV Ag/Ab reactive blood samples were then subjected to the serological confirmatory test. The serological confirmatory test for reactive HBV blood samples was performed in the Microbiology Laboratory, Transfusion Medicine Unit, HUSM using a neutralization test while reactive HCV and HIV blood samples were sent to the

Microbiology Serology Laboratory, HUSM for confirmatory testing using the Line Immuno Assay (Fujirebio, Japan).

NAT screening and discriminatory assays

These NAT tests for HBV, HCV and HIV were performed for us by a referral laboratory (Synapse Sdn. Bhd., Petaling Jaya). This organisation implemented individual donation (ID) NAT screening and discriminatory testing using Procleix[®] Systems (PROCLEIX[®] ULTRIO[®] assay and PROCLEIX[®] HIV-1, HCV, and HBV discriminatory assays, respectively) for target amplification of HBV DNA, HCV RNA and HIV RNA. The NAT discriminatory assay was only used on blood samples found to be reactive by NAT screening. This was done to identify the exact type of viral infection present in that particular blood donor. Results of NAT discriminatory testing were normally obtained within about 2 weeks and results were sent to HUSM staff via email.

Statistical analysis

Prevalence of HBV, HIV and HCV in our donor pool was calculated by direct counting. This was done by dividing number of positive cases by the total number of donated blood samples.

RESULTS

Table 1 shows demographic data for 9,669 blood donors. The majority of our donors were male (56%) and were less than 34 years old. Among the total collection of blood samples, 36 were reactive by serological screening (Table 2). Antibody to hepatitis C virus antibody (anti-HCV) showed the highest number (7) of positives by serological confirmatory screening followed by HBV surface antigen (HBsAg) with 3 positives. No positives were recorded with combo HIV antigen/antibody (HIV Ag/Ab) assays. Thus, out of 36 cases that were identified as reactive by serological screening, only 10 cases were confirmed as positive by serological testing (Table 3). In contrast, initial NAT screening only showed 8 reactive cases and of these 5 were confirmed by NAT discriminatory assays (Tables 2 & 3). All these 5 blood samples were also positive by serological confirmatory testing (Table 3). It is important to note that 5 samples were assigned as indeterminate for HCV by serological confirmatory assays and actual infectious status can only be assured after follow-up testing of their blood samples.

Table 1: Demographic statistics for the 9,669 blood donors participating in this study

Gender	Number and percentage
Male	5,370 (56%)
Female	4,299 (44%)
Age (years)	Number and percentage
17 to 18	1,133 (12%)
19 to 24	3,732 (38%)
25 to 29	1,058 (11%)
30 to 34	977 (10%)
35 to 39	796 (8%)
40 to 44	629 (7%)
45 to 49	529 (5%)
50 to 54	428 (4%)
> 54	387 (4%)

Table 2: The number of reactive blood samples by serological and NAT screening.

Serological screening positive	NAT screening positive
36	8

Table 3: Number of reactive and non-reactive samples detected using serological confirmatory and NAT discriminatory assays.

	Indeterminate NAT-	Serology+ NAT+	Serology+ NAT-	Serology- NAT+	Serology - NAT-
HBV	0	2	1	0	9666
HCV	5	3	4	0	9659
HIV	0	0	0	0	9669

DISCUSSION

The prevalences of confirmed HBV and HCV in our donor pool were 0.03% and 0.07% respectively and none of the donors was found positive for HIV using either serological confirmatory or NAT discriminatory assays. The low prevalence of TTIs in our donor pool could be attributed to effective blood transfusion practices which include screening of donors, an effective deferral system and a policy of unpaid voluntary donation [5]. As recommended by the World Health Organization [10], we have also targeted low risk donor groups, especially university students and government servants. In this context, the frequencies of TTI among blood donors at HUSM reported in this study may not reflect the overall prevalence of HBV, HCV and HIV in the country. Therefore, further studies are urgently needed to get accurate figures for HBV, HCV and HIV occurrence in the general population, such statistics can be used for formulating a better blood recruitment strategy.

In this study, there were three out of the 8 initially NAT positive samples (Table 2) but turned out to be negative by serological confirmatory and NAT discriminatory assays (Table 3). It is claimed that NAT screening is highly sensitive for detection of low viral load during the window period or in occult infections [3,8-9] that may at the time go undetected by the less sensitive serological confirmatory and NAT discriminatory assays. This may sometimes give discrepant results and may mistakenly be taken indicate a false positive in initial NAT screening. Thus, it is recommended that transfusion services should block all NAT-initial reactive blood units from being used regardless of the outcome of serological confirmatory and NAT discriminatory assays [8-9]. In this case, the 3 unconfirmed NAT-initial reactive samples merit further investigation and blood for these donors should be checked carefully when collected in their next visits for repeat blood donation.

Furthermore, we found that NAT assays failed detect 5 cases which were positive by serological screening and confirmatory assays (Table 3). This might be because viral concentration in the infected person was below the detection threshold level at that time [8]. Another possible explanation is the presence of new viral strains formed by point mutation or genetic recombination between different sub-types of viruses. These new strains may not be detectable (even with very slight nucleotide changes) by the oligonucleotide probes used for the NAT assays [11]. In this context, the donors of these samples should be tagged and contacted for another round of blood screening for accurate determination of their TTI status and viral type. Our findings show that serological techniques should continue be used alongside NAT for the detection of TTIs. This is because samples with low viral load but containing adequate levels of antibodies may sometimes not appear reactive by NAT, but can still be detected serologically [8]. Therefore, the implementation of both serological and NAT screening and confirmatory assays should continue be used to improve TTI marker detection as well as to reduce the risk of infection transmission via transfusion of blood and blood components.

CONCLUSION

Blood transfusion is a medical intervention for severe bleeding and anemia. However, this medical procedure requires transfusion of not only compatible blood units, but ones that are also free from bloodborne pathogens. Our results showed a very low prevalence of HBV, HCV and HIV infections among blood donors in HUSM as detected using serological and NAT assays. The present study also indicates that NAT could not replace the well-established serological testing, but both techniques can be used together with NAT further helping to ensure the safety of blood supplies in Malaysia. In summary, findings from the present study provide important information for designing an effective donor recruitment strategy and for achieving zero-risk blood transfusion in HUSM.

Disclosure

The authors have no conflict of interest regarding the publication of this paper.

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