Nucleic Acid Testing in Blood Donors of Northern India: A Single Centre Experience

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ABSTRACT

Introduction: Safety is still the main aim of donor screening programs. Majority of blood banks in India are employing screening by 3rd generation ELISA and only few have ECI testing. Aim of the study was to we report the efficiency of Nucleic Acid Testing (NAT) during the first twelve months of its implementation, to evaluate the risk of transfusion transmitted infections missed by serological screening at Department of Transfusion Medicine, King George's Medical University, and Lucknow

Material and Methods: Blood units were screened by ELISA. A total of 35,722 donations were non reactive by ELISA. These seronegative blood units were tested using the Roche cobas TaqScreen MPX test form from May 2012 till April 2013.

Results: NAT screening detected total 156 NAT yield donations among 35,722 seronegative donations. Among 156 NAT yields cases, 108 (69.2%) reactive for HBV, 46 (29.5%) reactive for HCV and 2(1.28%) reactive for HIV-1.Upon additional testing which employ ECL; 51 of HBV were reactive for HBsAg and twelve were reactive for HCV. (21) HBV and (2) HCV NAT yield samples could not be tested by ECL due to insuffient sample volume. As such, total number of valid NAT yield cases was reduced to ninety three cases with fifty seven (61.3%) HBV NAT yield cases and HCV NAT yield reduced to thirty four cases (36.6%). Therefore, the NAT yield rate in this donor population for HBV was 1:627; HCV was 1:1051 and HIV at 1:17,861

Conclusions: These results reflect high prevalence of HIV, HBV and HCV infections in northern Indian donor population and clearly indicate the benefits of NAT. The use of ECL technology with higher sensitivity performance for serological screening improved the detection of serology yield for both HBsAg and anti-HCV cases, enabling a more accurate understanding of the NAT yield in this donor population.

Keywords: HIV, HCV, HBV, ELISA, NAT (Nucleic Acid Testing), ECI (Electrochemiluminescence immunoassay).

INTRODUCTION

Nucleic Acid Testing (NAT) for blood screening is an essential component in the process of monitoring blood supply safety in addition to serological testing. Safety and adequacy remain the central goal of donor screening programs.[1] Blood donors can be screened for hepatitis B virus (HBV) in blood donors by testing for hepatitis B surface antigen (HBsAg) and also for antibodies against hepatitis B core antigen (anti-HBc). In the present scenario donors who are positive for HBV DNA are not identified during the window period before seroconversion occurs. Additional measures for making blood safer is through use of nucleic acid testing for detection of the human immunodeficiency virus (HIV), hepatitis C virus (HCV) RNA and HBV DNA.[2]

The first country which implemented NAT for HBV along with HCV and HIV-1 also observed a significant amount of decrease in transfusion transmission of this virus.[3] As a screening tool, NAT detects infection before serological tests 10-16 days earlier for HIV-1, 49-65 days for HCV, and 25-36 days for HBV.[4,5] NAT also plays an important role in detecting the incidence of active infection by HIV, HBV and HCV in blood donors. This knowledge is essential as it will determine the policies and guidelines to monitor blood safety.[5]

India is the second most populous nation in the world, with a population of more than 1.2 billion that includes 2.5 million HIV, 43 million Hepatitis B (HBV) and 15 million Hepatitis C (HCV) infected persons. Majority of blood banks in India are employing screening by 3rd generation ELISA and only few have ECI testing.

In this study, we report our experience with NAT during the first twelve months of implementation, to evaluate the risk of transfusion transmitted infections missed by serological screening at Department of Transfusion Medicine, King George's Medical University, and Lucknow. The NAT reactive cases were further screened by ECI technology in order to find out the true NAT yields.

MATERIAL AND METHODS

The work was done in Department of Transfusion Medicine KGMU, Lucknow, U.P this is one of the largest Government sector blood banks in India. The work was approved by the institutional ethical committee. All the blood units were screened by ELISA (hepatitis B surface antigen (HBsAg) by SD HBsAg Kit, hepatitis C virus (HCV) by SD HCV Kit and human immunodeficiency virus (HIV) by SD HIV kit, Bio Standard Diagnostic). A total of 35,722 donations were non reactive by ELISA. These seronegative blood units were tested using the Roche cobas TaqScreen MPX test form from May 2012 till April 2013. This NAT assay performs real-time detection and identification of 5 viruses; HBV, HCV, HIV-1 group M and O and HIV-2. NAT was performed in pools of six and the reactive pools were then resolved to individual donations. Viral target resolution for HIV, HCV and HBV was performed as needed using the respective Cobas MPX Taqscreen assays and discrimination by Cobas Taqman Hepatitis B monitor test, Cobas Taqman Hepatitis C monitor test and Cobas Taqman HIV monitor test. NAT reactive cases were again retested by ECI

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(Cobas e411) using Eclia HBsAg Kit, Eclia HIV kit and Eclia HCV kits in order to find out the true NAT yields.

STATISTICAL ANALYSIS

Microsoft office 2007 was used to make tables. Descriptive statistics were used to interpret results.

RESULTS

NAT screening detected a total of 156 NAT yield donations among 35,722 seronegative donations (non-reactive by serology for HBsAg, anti-HCV and anti-HIV-1 and 2 tests using Alere third generation ELISA Kits). Among these 156 NAT yields cases, 108 (69.2%) were reactive for HBV, 46 (29.5%) were reactive for HCV and 2 (1.28%) were reactive for HIV-1.Upon additional testing using the Roche Elecsys HBsAg II test and Elecsys Anti-HCV assay which employ the electrochemiluminescence technology (ECL); fifty-one of the HBV cases were found to be reactive for HBsAg and twelve cases were reactive for antibodies to HCV. At least twenty one HBV and two HCV NAT yield samples could not be tested by ECL due to insuffient sample volume. As such, total number of valid NAT yield cases was reduced to ninety three cases with fifty seven (61.3%) HBV NAT yield cases and the HCV NAT yield reduced to thirty four cases (36.6%). Therefore, the NAT yield rate in this donor population for HBV was 1:627; HCV was 1:1051 and HIV at 1:17,861 (Table-1).

DISCUSSION

India is a country with a very large population. It has a high prevalence of HIV-1, hepatitis C and B virus which remain undetected in most of the blood donors. NAT has provided a breakthrough and has helped in their detections.

A study by Yang Z et al.[6] showed that a 80 pools of nucleic acid amplification technology (NAT) were identified which were reactive. Amongst them 59 pools (74%) on resolution proved to be reactive. All these samples were reactive for HBV DNA. A quantitative estimation of viral load in each sample was done. The estimated viral loads were in the range from less than 20 to 34,600 IU/mL. 13 of the samples (22%) showed the value of viral loads of more than 20 IU/mL, 27 samples (45.8%) showed viral loads of less than 20 IU/mL, and 19 samples (32.2%) showed undetectable viral loads. Total of 59 NAT-reactive samples obtained, 40 (67.8%) were anti-HBc positive. Fifteen of the these samples did not show a confirmatory test for NAT reactivity either by an alternative NAT test or serology. While in our study we had a total of 156 NAT yield cases in which 108(69.2%) were reactive for HBV and 23 were not tested due to insufficient volume.

A study by Susan L et.al 2011[7] reported 9 donors who showed positivity for HBV DNA (1 in 410,540 donations). These included 6 samples also from blood donors who had received the HBV vaccine or in whom the subclinical infection had already developed but resolved. Of the total HBV DNA–positive donors, probably four of them acquired HBV infection from a sexual partner who was chronically infected. Two of the unvaccinated donors reported clinically significant liver injury. Amongst the 6 vaccinated donors, in 5 of them, a non-A genotype was identified as the dominant strain, while sub genotype A2 (represented in the HBV vaccine) was the dominant strain in unvaccinated donors. Of 75 reactive nucleic acid test results identified in seronegative blood donations, 26 (9 HBV, 15 HCV, and 2 HIV) were confirmed as positive.[2] While our study showed a total of 156 NAT yields cases, 108(69.2%) were reactive for HBV, 46 (29.5%) were reactive for HCV and 2 (1.28%) were reactive for HIV-1.

Blood safety is a challenge in India because of the high prevalence of HIV, HCV, and HBV, the relatively low percentage of voluntary donors[8] and the lack of standardization of screening procedures among the multitude of blood collection centres.[9] Of the 35,722 samples tested from our centre, there were 156 NAT yields donations. Among these 156 NAT yields cases, 108(69.2%) were reactive for HBV, 46(29.5%) were reactive for HCV and 2(1.28%) were reactive for HIV-1. Similar studies in other countries have also demonstrated high yields.[3,11-17]

A study by Rohit Jain et al., showed that enhanced chemiluminescence immunoassay (ECI) was used for detection of HBsAg, anti-HIV, and anti-HCV in donor serum. Combined NAT yield (NAT reactive/seronegative) for HIV, HCV, and HBV was 0.034% (1 in 2972 donations). All the samples tested were positive for HBV DNA, and the HBV viral load was ≥12IU/mL (95% lower limit of detection, 12IU/mL with 5.82 copies per IU conversion factor).[17,18] A study by Xin Zheng et al., showed that a total of 165,371 donor plasma samples from Shenzhen Blood Center were screened as HBsAg negative (HBsAg) with one inter- national and one domestic commercial enzyme immunoassay (EIA) kit. Individual-sample NAT test was performed and thirty three plasma were reported as HBV DNA POSITIVE. Chemiluminescent microplate immunoassay (CMIA) for HBsAg and nested PCR for BCP/PC was done on these 33 samples and amongst them twenty-eight were confirmed as HBsAg and DNA (HBsAg/DNA).[18] In our study out of 156 NAT reactive cases 70 cases were nonreactive by ECI hence were true NAT yields. This comprised of 57(61.3%) HBV, 34(36.6%) HCV and 2(2.15%) HIV. This fact strengthens the support for the use of NAT despite its cost factors. It means preventing the viral spread of these diseases in three times of 70 as 100% component preparation is prevalent in many blood banks.

The potential for NAT yield in India is staggering when compared to other countries that have already implemented the

NAT reactive			ECI not tested		ECI reactive		NAT reactive and ECI non reactive			
	156	%		23		63	133-63=70	70 (NAT reactive and ECI negative) 23 (NAT reactive and ECI not done)		
								70+23=93	93	%
HBV	108	69.2%	HBV	21	HBV	51	70 cases caught to be true NAT yield	HBV	57	61.3%
HCV	46	29.5%	HCV	2	HCV	12		HCV	34	36.6%
HIV	2	1.28%						HIV	2	2.15%

Table-1: Comparison between units tested by NAT (Nucleic Acid Testing) and ECI (Electrochemiluminescence Immunoassay).

technology. Data from studies suggested that the NAT yield for all three viruses in India could be 29 times higher than that observed in Japan, and even higher for HIV-1 alone. Makroo, R.N et al.2008 observed HIV-1 yield was over 515 times that observed in the US and Canada 89 times that observed in Italy, and also observed that HCV yield was 21.5 times that observed in the US and Canada, 26.5 times that of Italy and 125.6 times that of France.[20,9-16] The higher observed yield in India is not surprising given the prevalence of these viruses in the population; 5.7 million[21] with HIV, 12 million with HCV[22], and 40 million with HBV which represents 10 per cent of the world's HBV infected population.[23] India has reported a high percentage of replacement blood donors associated with higher infection rates compared to voluntary blood donors.[24] Many countries, such as Japan and the US, have mostly all voluntary donors[25,9]

CONCLUSIONS

The introduction of NAT enabled detection of a large number of HIV,HBV and HCV cases in these blood donor samples that were undetected by third generation ELISA serological tests. Annually, 50,000 units of blood are collected in KGMU, based on the current NAT yield reported of 1:384, this translates to 130 NAT yields per year. These NAT results reflect the high prevalence of HIV, HBV and HCV infections in this northern Indian donor population and clearly indicate the benefits of NAT by interdiction of a large number of infected transfusion units and the supply of safer blood to patients. Also, the use of ECL technology with higher sensitivity performance for serological screening improved the detection of serology yield for both HBsAg and anti-HCV cases, enabling a more accurate understanding of the NAT yield in this donor population. Although NAT has detected additional serological window period cases, it should be accompanied by properly selected serological assays for maximizing safety in transfusion practices.

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