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PREVALENCE OF HIV, HCV, HBV INFECTION IN BLOOD DONORS DETECTED BY NUCLEIC ACID TESTING: AN INDIAN EXPERIENCE

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ABSTRACT

Nucleic acid testing (NAT) is able to detect viruses during the 'window period' or the time between donor exposure to the virus and the appearance of antibodies. This allows for earlier detection of infection and further decreases the possibility of transmission via transfusion. Out of the 35,722 samples, 700 (1.95%) were reactive by ELISA. Discriminent assays found 40 (0.11%) to be reactive for HIV, 60 (0.16%) to be reactive for HCV and 600 (1.67%) forHBV. Apart from these reactive samples NAT was carried out on the non-reactive samples.Amongst them total of 158 (0.44%) were reactive for NAT. On discriminatory assays 2(0.005%) samples were reactive for HIV-1 and 2 also for HIV -2, 46 (0.12%) samples were reactive for HCV and 108 (0.30%) for HBV.NAT could detect HIV, HBV and HCV cases in blood donor samples that were undetected by serological tests. Third generation ELISA is the mandatory test in India. Our study confirms the utility of NAT and emphasizes the presence of HIV-2 in Indian donors. Study on such a huge population sample further questions the safety of blood supply by regular screening method and stresses on the introduction of NAT as a mandatory test by regulatory authorities.

KEYWORDS: NAT, ELISA, HIV, HCV, HBV.

INTRODUCTION

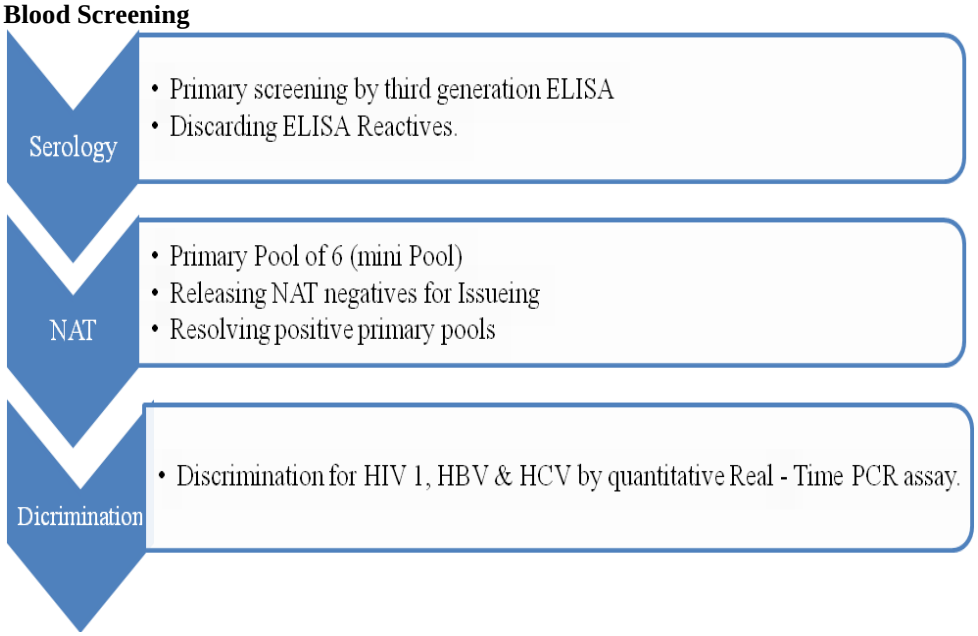
Nucleic acid amplification testing (NAT) and is based upon the technique of direct amplification and detection of viral nucleic acids rather than antibody production by the immune system of the infected person. NAT is thus able to detect viruses during the 'window period' or the time between donor exposure to the virus and the appearance of antibodies. This allows for earlier detection of infection and further decreases the possibility of transmission via transfusion. NAT also detects mutants and occult cases. NAT is being used in developed countries but countries like India have very few centers doing this test. As a consequence not much is known about the true incidence and prevalence of HIV, HCV, and HBV in the general population. The third generation screening assays for HIV, HCV and HBV which are being practiced by most blood banks have significantly reduced the risk of transmission. Nevertheless, there remains a residual risk for transmission of these viruses due to the 'window period' (pre-sero conversion sero negativity). The sero prevalence of anti-HIV-1, HBsAg and anti-HCV among Indian blood donors is 0.5%, 1.4% and 0.4%, respectively.[1] The transfusion of blood containing hepatitis B surface antigen (HBsAg) is associated with post-transfusion infection with hepatitis B virus (HBV). Blood that is free of HBsAg but has high-titer antibodies against hepatitis B core antigen (anti-HBc) in the absence of antibodies against hepatitis B surface antigen (anti-HBs) can also transmit HBV infection.[2, 3] In the present study nucleic acid testing was done for detection ofthe human immunodeficiency virus (HIV) and hepatitis C virus (HCV) and hepatitis B virus (HBV) in blood donors in order to provide additional safety to the recipients.

MATERIAL AND METHODS

A prospective study was carried out on 35,722 blood units collected from voluntary and replacement donors from June 2012 to April 2013. A proper history of the donors was taken regarding their health status to exclude all infectious diseases. Donors with a history of hepatitis or jaundice after 11 years of age were deferred. After a complete physical examination only medically fit donors were allowed to donate blood. Written consent was also taken from them prior to donation. The rate of donor deferral was 5%. Samples were collected in pilot test tubes at the time of bleeding. They were examined in the transfusion transmitted diseases screening laboratory using the third generation enzyme-linked immune adsorbent assay techniques. The blood screening by third generation ELISA was continued (Picture1) as a part of mandatory screening by drug and cosmetic act of India. Blood bags corresponding to positive samples on ELISA were subjected for discarding as per the guidelines. All the available donors corresponding to anti HIV antibody reactive samples were called back and referred to ICTC center. Other donors positive for Hepatitis B & C were

referred to Gastroenterologist for further follow up. HIV positive donors referred to ICTC center were further confirmed by testing guidelines and followed up for sero conversion status. Donors blood units which were ELISA negatives are screened by mini pool of 6 using cobas s201 fully automated blood screening NAT platform, Picture 1. MPX V1.0 intended for screening of HIV I (M & O), HIV II, Hepatitis B & C was used for screening. As our initial algorithm depends on pooling the donor samples positive on a pool were further resolved for identification of positive donor sample. Further to resolution presence of viral target was identified by discrimination test on cobas Taqman 48 Real – Time PCR & CAP-CTM machine based on the availability. All the standard protocols were followed. The infections for which blood was screened by NAT were HIV-1 and -2, HCV, HBV.

Algorithm of Blood Screening



Picture 1: Flow chart showing algorithm of Blood Screening.

RESULTS

Serological testing of Donor samples

A total of 35, 722 samples were tested by J Mitra (New Delhi) ELISA. Out of 35,722 samples, 40(0.11%) to be reactive for HIV, 60 (0.16%) to be reactive for HCV and 600 (1.67%) for HBV. Total Sero positivity for all three markers cumulatively is 1.95% (700/35,722). All sero negative samples were analyzed by mini pool NAT testing. Sero positive donors were further referred to ICTC & Physician follow up. 98% of the donors tested reactive on further testing at ICTC center located at KGMU, Lucknow.

NAT Testing of Donor samples

A total of 158 (0.44%) were reactive for NAT. Sero negative and NAT positive samples were tested on diagnostic quantitative real time PCR assay using cobas Taqman & CAP/CTM instrument. Discrimination with viral load assay resulted in 2 (0.005%) HIV-1, 46 (0.12%) HCV and 108 (0.30%) HBV as shown in (Table1).Details of viral load testing and number of copies presence in the NAT reactives were given in (Table 2).

Table 1: Transfusion Transmitted Infection in Blood Donors by ELISA and NAT

S.No.	Method	HIV-1	HIV-2	HCV	HBV
1	ELISA	40 (0.11%)	Nil	60 (0.16%)	600 (1.67%)
2	NAT	2 (0.005%)	2 (0.005%)	46 (0.12%)	108 (0.30%)
Total number of samples: 35,722.					

Table 2: Viral Load testing result for NAT positive Sample.

Viral Load testing Result for NAT positive samples							
Donar ID	**HBV (IU/ml)**	**HCV (IU/ml)**	**HIV (copies/ml)**	**Donar ID**	**HBV (IU/ml)**	**HCV (IU/ml)**	**HIV (copies/ml)**
14738	>110000000	471711		28367	43.2		
14841	88			28314		1730000IU/ml	
16972	312			28305	24.2		
17023	486			28354		2110000IU/ml	

17062	<6			28333	40.5		
17264	34			28685	62600		
17435	152	Very High +ve		28693	444		
17463	<6	95228		28907	36.2		
17604	7071			29082		22300000	
17661	2116			29118	19.9		
17680	6700			29123	3500		
17746	<6			29131	147		
18030	740	5680916		29844		1100000	
18097	<6	678507		30078	756		
18452	>110000000			30075			
18634	12570			30469	<6		
18701	<6			30471	10600		
18932	>110000000			30493	10100		
19004	1809		3870	30859	88		
19010	7202			31121	73		
19019	125		112000	31073	216		
19094	<6			31072	4480		
19096	>110000000			31174	262		
19337	164			31176	86		
19485	<6			31169	194		
19565		141856		31127	561000		
19569	702			31285		243000	
19692	<6			31919	7680		
20380		1524085		32090	3400		
20580	>110000000			32112	3570		
20818	<6			32178		5650000	
21012	<6			32567	1520		
21013	1442			32617	1960		
21146	10077			32679	673		
21281	<6			**32805**	**5050**		
21282	<6			**33287**	**<6**		
21284	65			**33497**	**65**		
21450	<6			**33510**		<25	
21697		26602		**33518**	10200		
21710	38			**33871**	174		
21870	<6			**34179**		63800	
22045	388			34385		**26100**	
22670	<6.00			34669	184		
22753		5100000		35118	18		
23453	6790			35102	<6.0		
23764	49800			35310	2630		
23904	<6.00			35429	372		
24439		356		35502	17		
24438		13400000		35703		**595000**	
24621		133000		36077	<6.0		
24648	<6.00			36150	573		
24636	<6.00			36546	9340		
24683		895		36583	6310		
25127		134000		36679	18100		
25238	110000000			36714	1700000		

23910	3570			37116		**4090000**	
24901	151			37491		**1740000**	
25457	118			7760		6600000	
25524	126			8723		**727000**	
25743	30000			10931		350000	
25752	47.9			11163		177000	
25753	<6.00			11165		235000	
26696	2020			11176		4660000	
26903	<6.00			11329		**11400**	
27458	<6.00			11369		**2860000**	
27649		981000		11897		**135000**	
27754	2810			10931		350000	
27790		2790000		11163		177000	
28141	562			11165		235000	
28156		1230000		11176		4660000	
28394	7380			11329		**11400**	
28396	18.6			13173	756		
28264	1040			13178		<25	
				13180	<6		
				13185	<6		
				13203		<25	

Our algorithm of NAT testing led us to evaluate the test performance and the reliability of the assay. As shown in the (picture 1). Resolution of initial reactive pool by individual sample testing were helped us to differentiate the false positives and true positives. Even though some of the initial pool positives were turned out negative on resolution the percentage is very less. Further to resolution, discrimination by viral load testing performed outside the blood bank is an additional confirmatory step. Data on false positives by NAT screening is listed in Table 3. Inbuilt authentication present with the algorithm has made a provision for elimination of false positives by repeat testing of the samples. Further to NAT testing available donors were followed up for sero conversion to authenticate the results. Presence of HIV in 2 Donors was further confirmed by rapid testing which can discriminate HIV1 & 2. The results of rapid testing & viral load testing intended for screening HIV 1 (Roche, Basel) confirmed the presence of HIV 1.

DISCUSSION

NAT detects viral ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) by the amplification method. Early in the course of an infection, NAT detects low levels of viral genetic material present in the blood. Nucleic acid testing (NAT) is currently used in conjunction with serological tests in the four continents, North America, Europe, Australia and Asia12. Although NAT screening cannot completely eliminate the risk of transfusion transmitted infection, it has reduced the risk for HIV-1 and HCV where it has been implemented.[4, 5] Japan was the first country to implement routine HBV NAT in addition to HCV and HIV-1 NAT screening and observed a significant reduction in transfusion transmission of this virus as well.[6] South Africa and in a number of European countries also employ the NAT.[7, 8, 9] In addition, NAT is also useful for determining the incidence of active infection by these viruses in blood donor populations. This knowledge is critical to the successful planning of measures to increase blood safety**.**Safe blood implies blood or a blood component transfusion with no transfusion transmitted infection. As TTIs such as HIV-1, HIV-2, HCV and HBV can be easily transmitted through infected blood; considerable effort has been made to reduce their transmission. In the mid-1990s, the risk of transfusion-associated HCV infection was estimated to be more than 1:5000. Until thelate 1990s, blood screening for TTIs depended entirely on serological assays. Except for HBV, where the virus can be detected using HBsAg assays, tests for the detection of other TTIs relied almost exclusively on antibody detection. However, these tests are associated with a relatively long window period because they detect the response of the immune system to an infection.

In the first Indian multicentre ID- NAT study, 12 224 donor blood samples were tested.5 Ofthese, 217 samples (1.78%) were found to have markers of infections, including 8 (0.07%) which had markers of only the viral genome without serological signs of infections. These 8 NAT yield cases consisted of 1 HIV, 1 HIV HCV co-infection and 6 HBV.[10] No reporting of HIV-2 was there as ID-NAT had no provision for the detection of HIV-2 viral infection. In our study, the seroprevalence of HIV, HCV, HBV infection was 0.11%, 0.16%, 1.67% by ELISA but by NAT there was an additional seroprevalence of HIV-1 (0.005%), HIV-2(0.005%), HCV (0.12%), HBV (0.30%) infection among blood

donors. The blood of donors with acute HBV DNA–positive infection during the window period is likely to be highly infectious in transfusion recipients[11, 12] .The significance of infection in vaccinated donor's is less clear. In one study, blood donations that were positive for HBV DNA with detectable levelsof anti-HBs were not infectious in any of 22 recipients, as compared with a rate of infection of27% among 37 recipients of blood that was devoid of anti-HBs.[11] Similarly, the absence of infectivity in the presence of anti- HBs has been observed in other studies.[3, 13, 14, 15, 16] Conversely, blood containing HBV DNA with low-level anti-HBs (<75 IU per liter) may carry arisk of transmission leading to acute hepatitis.[17] These study findings may be relevant todecisions about the need to implement screening for HBV DNA among blood donors.

Minipool NAT showed a high sensitivity and specificity in the samples tested. None of the samples were false positive as confirmed by the viral load on discriminatory assays. NAT also helped us to evaluate our blood donor population. No data was available which emphasized the number of reactive cases which were being missed out in the representative population of state of Uttar Pradesh which has the highest population of state. Our center being the state of art model blood bank received donors from all over the state. Presence of HIV-2 in donors is the first to be detected data in blood donors. ID-NAT has no provision for detection of HIV-2; as a result the data about true incidence of HIV-2 is not available in most of the blood banks.The benefits of NAT are especially important in patients who receive multiple blood transfusions for diseases such as thalassemia and hemophilia. Such patients need regular, repeated and life-long blood transfusions and are at higher risk of being infected with seriousTTIs. In a survey by the National Thalassemia Welfare Society among 551 multiply transfused patients with thalassemia, 33 were HIV-positive, 89 were HCV-positive and 43 were HBV positive.[18]

CONCLUSION

NAT could detect HIV, HBV and HCV cases in blood donor samples that were undetected by serological tests. Third generation ELISA is the mandatory test in India. Our study confirms the utility of NAT and emphasizes the presence of HIV-2 in Indian donors. Study on such a huge population sample further questions the safety of blood supply by regular screening method and stresses on the introduction of NAT as a mandatory test by regulatory authorities.

Competing interests

The authors have declared that no competing interests exist.

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