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Clinical significance of viral markers testing by ELISA and Individual Donation Nucleic Acid Testing (ID-NAT) for blood screening in blood bank: Single center study in Egypt

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Abstract

Prevention of transfusion-transmitted viral infections and insurance of safe blood transfusion are the main goals of all blood banks worldwide. Despite the high sensitivity and specificity of currently used enzyme linked immunosorbent assay (ELISA) for hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) testing, viral transmission could still occur during the window period. Introducing viral individual donation nucleic acid testing (ID-NAT) can greatly decrease such risk providing an additional layer in securing blood transfusion. We aimed to assess the clinical significance of viral markers testing by ELISA and ID-NAT for blood screening in the Blood Bank of Suez Canal University Hospital. We studied all donations (2132) collected during a two-months period. Blood donor samples were screened by ELISA and ID-NAT tests for HBV, HCV, and HIV. Serological testing results for HCV by ELISA revealed 2,122 (99.5 %) negative donations compared to 2,131 (99.95 %) negative donations by ID-NAT testing. Of the positive ELISA samples, only one was NAT positive. For HBV ELISA testing, 2,115 (99.2 %) donations were negative, also by ID-NAT testing 2,115 (99.2 %) donations were HBV DNA negative. Out of the negative ELISA samples, two samples were ID-NAT reactive donors which were missed by serology assay being in the window period. HIV ELISA testing revealed negative 2,130 (99.9 %) donations while ID-NAT testing showed 2,131 (99.95 %) negative donations and one positive donation. In conclusion, this is the first study carried out in the Suez Canal and Sinai region, Egypt to assess the importance of ID-NAT implementation. The introduction of ID-NAT in blood banks is an effective method for increasing safety of the blood transfusion.

Keywords: HIV, HBV, HCV, Nucleic Acid Amplification Test, ELISA.

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Introduction

Prevention of transfusion transmissible infections (TTIs) and blood-borne viruses is the major challenge in blood banks worldwide. It is compulsory to ensure that all units of blood and blood components are secure before transfusion.[1] The transmission of transfusion

transmissible infections is significantly decreased nowadays as the currently available serologic screening methods for detection of viral markers in donated blood are highly sensitive and specific. However, the risk of transmission persists as these methods may miss viral detection during the window period (WP) resulting in viral transmission.[2]

Nucleic acid testing (NAT) is a molecular technique used for viral screening of donated blood reducing the chance of collecting infected blood and improving blood safety.[3]It amplifies specific viral RNA or DNA regions with high sensitivity and specificity allowing viral detection before the other commonly used blood screening methods. It shortens the WP of hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) to 10.34 days, 1.34 days to 2.93 days, respectively.[4]

Occult HBV infection is characterized by very low viral replication, low concentrations of circulating viral DNA and HBsAg. Recipients of contaminated blood or blood components can be infected being a major obstacle in the way of safe blood transfusion.[5,6] The ID-NAT showed a greater HBV yield than mini-pool (MP)-NAT as it reduced HBV WP by 25-36 days compared to MP-NAT that reduced WP by 9-11 days.[7] ID-NAT is more efficient in detection of negative hepatitis B surface antigen (HBsAg), positive HBV DNA samples than MP-NAT, whatever the donor anti-hepatitis B core status.[8] The currently available blood screening serological methods may give false positive results that can be detected by NAT.[9]

In spite of the notion that MP-NAT may be cost-effective compared to ID-NAT, it still has some disadvantages. First, all the donated blood units cannot be used until the NAT results are available. Second, the samples pool is too large which dilutes the viral nucleic acid resulting in decreased sensitivity. Third, the whole pool must be individually retested if it is reactive to detect the reactive blood unit which is a time-consuming process delaying the release of blood units. This can be avoided by ID-NAT, where reactive blood bag is eliminated, and the other bags are released without delay.[10]

In 2019, Hans et al., reported that blood screening by NAT succeeded in detection of 50 donations with positive viral markers every year that were missed by the sole use of serology. They also observed that NAT retesting of ELISA anti-HCV positive NAT negative samples, after 3 months, the samples remained NAT negative indicating its ability to detect false reactive donations by serological methods.[9, 11] Also, an Egyptian study conducted by Ebeid et al., 2019, showed that testing HBV using NAT detected one reactive sample that was false seronegative. Testing for HCV antibodies (HCV Ab) using NAT revealed that all NAT reactive samples were seroreactive by ELISA. Of 1,000 samples tested for HIV by NAT, two samples (0.2 %) were reactive; while ELISA testing revealed only one seropositive sample, i.e., missed the other one which was seronegative. They concluded that ID-NAT added more and more to the blood safety.[12]

In the current work, we aimed to study the importance of ID-NAT implementation in the Blood Bank of Suez Canal University Hospital which serves the Suez Canal and Sinai region, Egypt. We compared ID-NAT to the current sole use of routine antibody/antigen screening of blood donations for the detection of HBV, HCV, and HIV infections. To the best of our knowledge, this is the first carried out study in this region.

Subjects and Methods

Each blood donor filed a questionnaire and then subjected to medical examination prior to blood donation. All blood donations (n=2,132) were collected from volunteer donors at the Blood Bank of Suez Canal University Hospital during a period of two months.

Serology was tested by ELISA and ID-NAT concurrently for each donor as follows:

- A blood sample (5 ml) was collected from each study participant in a plain vacutainer tube. After serum separation, serologic markers of viruses were measured by ELISA. These included HBV antigen (HBsAg), anti-HCV Ab and HIV Ag-Ab by commercial kits (Monolisa HBs Ag ULTRA, catalog no. 72346, Monolisa HCV Ag-Ab Ultra V2, catalog no. 72562, and Genscreen

Ultra HIV Ag-Ab kit, catalog no. 72386, respectively, BioRad Laboratories, France), according to the manufacturer's instructions. The optical density was measured at 450 nm by an automated microplate ELISA reader (Stat Fax2200, Awareness Technology, USA).

- Another venous blood sample (6 ml) was collected from each study participant in K2 EDTA vacationer tube. Collected plasma was tested for simultaneous detection of HBV DNA, HCV RNA, and HIV-1 RNA by ID-NAT using commercial kits (Cobas Taq Screen multiplex (MPX) test, P/N 06998909190, Cobas 6800, 1546, Roche Diagnostics, Mannheim, Germany), according to the manufacturer's instructions. Briefly, proteinase and lysis reagent were used to release viral DNA or RNA that bound to the silica surface of the magnetic glass particles. Unbound particles were removed by repeated washing. The elution buffer was added at elevated temperature, releasing the bound nucleic acid from the magnetic beads. Forward and reverse virus-specific primers were used to amplify the target nucleic acid in the donor sample. This was followed by reverse-transcription and amplification using thermostable DNA polymerase. The cobas® MPX master mix contained specific probes for viral nucleic acid. Each HIV, HCV, HBV, and IC (internal control) probe had its distinct fluorescent dyes, one reporter dye and another quencher dye. Each reporter dye was measured at distinct wavelengths allowing synchronized recognition and differentiation of the amplified viruses and the IC. The fluorescent signal of the unbound reporter dye was concealed by the quencher dye. DNA polymerase cleaved the probes bound to the specific single stranded DNA which separated the reporter from the quencher dye releasing a fluorescent signal. As the amounts of cleaved probes increased with PCR cycles, the reporter dye signal was concurrently increased.

- Serology negative and NAT-positive were defined as NAT yields. NAT negative and serology positive samples were defined as "seroyield".

Statistical Analysis

We analyzed our data using Statistical Package for the Social Sciences (SPSS) software package version 20.0 (Armonk, NY: IBM Corp). Categorical data were represented as numbers and percentages. Two groups comparison was carried out by Chi-square test. Alternatively, we used Fisher Exact test when the cells with expected count less than 5 were more than 20%. The Kappa test was used for agreement between NAT and ELISA. The Kappa was classified as follows: poor agreement if ≤ 0.20; fair agreement from 0.21 to 0.40; moderate agreement from 0.41 to 0.60; good agreement from 0.61 to 0.80; very good agreement > 0.80. The 5 % level was used to judge the results' significance.

Results

A total of 2,132 blood donations were subjected to serologic testing by ELISA for anti-HCV Ab, HBsAg tests and HIV Ag-Ab. ELISA results were compared to ID-NAT HBV, HCV, and HIV-1 nucleic acid results. Regarding HCV, ELISA testing revealed 2,122 (99.5 %) negative donations and 10 (0.5 %) positive donations (seropositve). By ID-NAT testing, 2,122 (99.5 %) donations were negative. Out of the 10 ELISA positive, one (10 %) donation was ID-NAT positive and the remaining 9 (90 %) donations were ID-NAT negative with a total of 2131 (99.9%) donations were ID-NAT negative and one (0.1 %) positive.

For HBV, ELISA testing showed that 2,115 (99.2 %) of the total 2,132 donations were negative and 17 (0.8 %) positive donations. ID-NAT testing revealed that 2,115 (99.2 %) donations were HBV DNA negative and 17 (0.8 %) positive donations which indicated that ID-NAT testing detected two (0.09 %) ELISA false negatives (seronegative) that were reactive by ID-NAT testing (NAT yield). This indicates the ability of ID-NAT to detect samples in the window period. Also, two (11.7 %) donations that were ELISA positive (false positive/seropositive) turned to negative (non-reactive) by ID-NAT testing. In addition, 15 (0.7%) donations were HBV negative by both ELISA and ID-NAT testing.

Finally, HIV ELISA testing revealed 2,130 (99.9%) negative donations and two (0.09 %) positive donations while ID-NAT testing showed 2,131 (99.95 %) negative and one (0.05 %) reactive donation (positive =NAT yield).

Table 1. Relation between diagnosis of hepatitis C virus (HCV), hepatitis B virus (HBV) and human immunodeficiency virus (HIV) by Nucleic acid testing (NAT) and enzyme linked immunosorbent assay (ELISA) testing in 2132 cases.

ELISA	NAT		χ^2	^{FE}p value	Kappa	Percent of agreement
	Negative	Positive				
HCV	(n = 2,131)	(n = 1)				
Negative	2,122	0	212.3	0.005	0.181 (Poor)	99.6
Positive	9	1				
HBV	(n = 2,115)	(n = 17)				
Negative	2,113	2	1656	<0.001	0.881 (Very good)	99.8
Positive	2	15				
HIV	(n = 2,131)	(n = 1)				
Negative	2,129	1	0.001	NS	-0.001 (Poor)	99.9
Positive	2	0				

χ^2: Chi square test FE: Fisher Exact κ: kappa test. *p: p* value for association between different categories
p > 0.05 is not significant (NS).

Discussion

ELISA is commonly used in blood banks to detect HBV, HCV, and HIV infections before blood transfusion to ensure blood safety. The use of ID-NAT will have additional benefits mainly reducing the window period and decreasing the possibility of viral transmission through blood transfusion. This, in turn, will improve health outcomes. We studied 2,132 volunteer donor samples that were subjected to serologic testing by ELISA for anti-HCV Ab, HBsAg tests and HIV Ag-Ab. Their data were compared to ID-NAT results which provide detection of HBV DNA, HCV RNA, and HIV-1 RNA.

Our HBV screening results revealed that two samples were positive by ID –NAT testing although they were ELISA negative. Furthermore, two samples were ID-NAT non-reactive although of being ELISA positive and 15 donations reactive by both NAT and ELISA testing. This showed that NAT helped in detection of ELISA false positive and false negative samples recognizing samples in the window period. In 2019, Ebeid et al., screened 1,000 blood donation samples for HBV. The NAT Procleix Ultrio testing method revealed 6 positive samples (0.6 %); 5 samples (0.5 %) were positive by both NAT and ELISA testing and one sample (0.1 %) was ELISA seronegative and NAT positive with a ratio of 1:1000 HBV NAT yield rate.[12] This agreed with the finding of a study by with O'Flaherty et al., 2018 who used ID-NAT for screening of more than 1·2 million donations for HBV DNA. Of these, 30 samples were HBV positive, two samples NAT HBV DNA positive although of being HBsAg and anti-HBc negative by ELISA. The negative ELISA samples can be explained by being in the window period.[13]

Our results for HCV revealed that one donation was NAT reactive while 10 donations were seropositive by ELISA testing. This indicates the ability of ID-NAT to detect false ELISA positive results. In our study, HIV NAT testing detected one positive donation (NAT yield) compared to two seropositive donations by ELISA testing revealing the advantage of NAT in detecting false ELISA reactive samples.

In a study by Ebeid et al., 2019, NAT HCV testing detected 13 positive samples (1.3 %) that coincidence with ELISA testing for HCVAb. In addition, two samples were NAT HIV positives, and one sample revealed to be HIV NAT yield after being seronegative by ELISA testing.[12]

Our NAT negative samples out of the positive ELISA results were two HBV seroyield samples, 9 HCV seroyeild sample and two HIV seroyield samples. On the other hand, HBV NAT positive results out of seronegative results (ELISA negative) were two with negative yield for HCV and one for HIV. This may indicate that NAT testing could help in reducing the possibility of transfusion transmitted infections. Most of blood screening studies which compare NAT and ELISA results, revealed positive NAT samples that were missed by ELISA increasing the risk of transfusion transmissible infections.[10,12]

In a previous study, ELISA, and NAT results of 6,000 donor samples showed that 60 samples were seroreactive while only 52 samples were NAT positive and ELISA negative with one in 113 NAT yield.[14] In another study, ELISA positive, NAT negative (seroyield) samples were as follows, 9 samples seroyield HBV (0.9 %), 9 samples HCV (0.9 %) and 3 samples seroyield HIV (0.3 %).[12]

The study by Hans et al., 2019, observed a combined NAT yield for all 3 viruses of 0.09 % (1 in 1,031). They reported that using serology alone in blood screening missed nearly 50 donations every year. They also observed that on repeating HCV screening after 3 months by NAT testing on anti-HCV ELISA positive samples, they were still NAT negative.[11] The study by Naizi et al., 2015 showed that NAT yield was one in 2,016 (combined yield, 1 in 2,367 for HBV and 1 in 13,609 for HCV).[15] Similarly, the study by Dong *et al.*, 2014, revealed that NAT yield 1 in 1,056 (HBV)[16] while the study by Zou *et al.*, 2010, documented that NAT yield of 1 in 1,149,000 (HCV) and 1 in 1,467,000 (HIV).[17] The study by El Ekiaby et al*.,* 2009, revealed a combined HBV, HCV and HIV yield of 1:3100.[18]

In our study, HBV NAT yield for two seronegative donor samples could be explained by the higher sensitivity of NAT than the serological ELISA testing for detecting donors in the WP that are missed by serological testing alone. This was also shown by Perazzo et al., 2015, and Hans et al., 2019, who found that HBV NAT yield was high. They reported that ID-NAT was able to identify samples in the WP that could be missed by serological ELISA testing. They also related that to occult HBV infection. This confirmed the value of NAT to pick up seronegative donors.[11,19]

The study by Allain et al., 2005, showed that the discrepant release of complete viral particles and the different structural proteins in the circulation may be responsible for non-concordant HBV ELISA positive samples. They showed that non-capsulated viral DNA is quickly degraded. With the absence of anti-HBs, HBsAg circulates in the blood for a longer time causing HBsAg reactive samples.[20]

In conclusion, our data indicated that ID-NAT could detect transfusion transmitted viruses in screened blood donations that could be missed by immunoassays. It can help avoiding false reactivity results of serological tests. Thus, adding ID-NAT to ELISA in blood banks could provide a big move towards safe blood transfusion.

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Author Contributions

FMA; formulation of the idea, study design, general supervision, data interpretation and editing final approval. MAF; assemble the data, analysis and interpretation of the study data and final editing and revision. SAA; put the study plan, writing the study protocol for ethics committee approval, study population selection, samples analysis, data interpretation, writing, editing and final revision.

Declaration of Conflicting Interests

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Ethical approval

The protocol of the study was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Suez Canal University (Ref. no.5079, dated October 2022).

Informed consent

Each blood donor provided a written informed consent.

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