

ASSESSING NUCLEIC ACID TESTING VERSUS ELIZA FOR BLOOD VIRUSES DETECTION IN SOME BLOOD BANKS

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ABSTRACT

INTRODUCTION: The goal of any transfusion service is to provide adequate and safe blood and blood products that meet the needs of patients. Transfusion-transmissible viral infections, such as hepatitis C virus (HCV), hepatitis B virus (HBV), and human immunodeficiency virus (HIV), remain a major public health problem in developing countries. Nucleic acid testing (NAT) is a molecular technique for screening blood donations to reduce the risk of transfusion transmitted infections (TTIs) in the recipients, thus providing an additional layer of blood safety.

AIM OF THE WORK: To assess the importance of implementing NAT assay to detect donors during window period which are not detected with Enzyme-linked immunoassay (ELISA).

SUBJECTS AND METHODS: This cross sectional study was conducted at the Egyptian Abbassia regional Blood Transfusion center (ARBTC) at Abbassia, Cairo. Blood donation collected from 10000 voluntary donors from January 2016 to June 2016 and tested with both ID NAT and ELISA assays for HBV, HCV and HIV.

RESULTS: NAT testing has the potential to detect viral nucleic acids of HIV 1-2, HBV, and HCV earlier than current screening methods.

CONCLUSION: The implementation of NAT screening for three viruses has improved blood safety and reduced transfusion-transmitted infections.

KEYWORDS: Hepatitis B virus, hepatitis C virus, human immunodeficiency virus, nucleic acid amplification testing, transfusion-transmitted infection, Enzyme-linked immunosorbent assay.

INTRODUCTION

Transfusion-transmissible viral infections, such as hepatitis C virus (HCV), hepatitis B virus (HBV), and human immunodeficiency virus (HIV), remain a major public health problem in developing countries. The prevalence of these viral infections among blood donors may reflect the burden of these diseases among populations (Biadgo *et al.*, 2017). The goal of any transfusion service is to provide adequate and safe blood and blood products to the recipients. With every unit of blood transfusion, there is a 1% chance of transfusion related complications including transfusion-transmitted infections. The process of preventing the TTI presents one of the greatest challenges of transfusion medicine. Blood transfusion departments not only screen TTI, but they also provide information about the prevalence of these infections in populations (Sushma *et al.*, 2016). Availability of safe supplies of blood and blood products cannot be achieved without costs. However, an unsafe or inadequate blood supply is ultimately even more costly, in both human and economic terms (Smit, 2017). The human costs of unsafe blood are incalculable— morbidity and mortality resulting from the transfusion of infected blood have far-reaching Consequences, not only for the recipients themselves, but also their families, their communities and the wider society (Patel *et al.*, 2012). In the last few decades through an awareness of TTIs, a majority of countries have mandated serology based blood screening assays

for HIV, HCV, and HBV. However, despite improved serological assays, the transfusion transmission of HTV, HCV, and HBV continues, primarily due to release of serology negative units that are infectious because of the window period (WP) (Shyatnala, 2014). The WP is that period of time from infection to the time of detection by a given blood screening assay (Weusten *et al.*, 2011). During this period, the risk of infection in donated blood can be missed by the immunoassay testing. These undetected WP infections are responsible for most of the transfusion transmission of these viruses (Chigurupati and Murthy, 2015). Nucleic acid amplification technology (NAT) is highly sensitive and specific for viral nucleic acids. It is based on amplification of targeted regions of viral ribonucleic acid or deoxyribonucleic acid (DNA) (Hans and Marwaha, 2014). It detects them earlier than the other screening methods thus, narrowing the window period of HIV, HBV and HCV infections. NAT also adds the benefit of resolving false reactive donations on serological methods which is very important for donor notification and counseling (Yaseen *et al.*, 2013). The need for NAT depends on the prevalence and incidence rate of infections in blood donor population, available resources and the evidence of benefit added when combined with serology tests. (Hans and Marwaha, 2014).

The aim of this work is to assess the importance of implementing NAT assay to detect donors during window period (the duration between the donor exposures to a virus until antibodies against the virus are produced) which are not detected with ELISA. Also, it detects the impact of NAT on the yield and residual risk of TTIs.

SUBJECTS AND METHODS

This cross sectional study was conducted at the Egyptian Abbassia regional Blood Transfusion center (ARBTC) at Abbasiaa, Cairo. The period of the study was six months from January 2016 to June 2016. Blood was collected from 10000 voluntary donors. They donated blood either in the blood bank or in the camps organized by mobile teams.

Inclusion criteria: the following are some of the criteria universally accepted for donor selection under universal regulation of American Association of blood banks and the instructions on work procedures produced by the general administration of blood banking of the Egyptian Ministry of Health.

- Age between 18-60 years
- Weight of the donor should be at least 50 kilos
- Haemoglobin levels of 12 g/dl for females and 13 g/dl for males.
- Pulse between 50 to 100 / min with no irregularities.
- Blood pressure systolic 90–180 mmHg and diastolic 60–100 mmHg.
- The minimum interval between blood donations varies between 56 days (8 weeks) and 16 weeks and different donation intervals are usually followed for male and female donors
- All donors must pass general and medical examinations prior to donation.

Exclusion criteria include (Donor deferral): Individuals disqualified for donating blood are known as the “deferred” donors. Person is deferred temporarily or permanently will depend on their specific reason for disqualification (e.g. a person may be deferred temporarily because of anemia. A condition that is usually reversible). Criteria for rejecting donors:

- Hypotension, hypertension, anemia and sever chronic disease
- Anyone with positive test for HIV.
- Men and women who have engaged in unprotected sex.
- Anyone who has a past history of hepatitis.

All blood donors, involved with in our research were requested to complete a detailed questionnaire and sign consent before being enrolled in this study.

1-A universally recognized and detailed questionnaire was taken during these donors registration. Information regarding risk factors as past history of surgery, prior hospitalization, history of blood transfusion and donation, occupation, high risk behavior (multi sexual partner), and history of vaccination, or any episode of jaundice was recorded.

2-Sample Collection was carried out at the beginning of phlebotomy by 16-17 gauge needles, 5 ml venous blood was drawn from donors in plain tubes (Becton Dickenson) and used to obtain serum by centrifugation. Blood donor samples were subjected to screening for HIV, hepatitis B virus (HBV) and hepatitis C virus (HCV) by using fully automated enzyme-linked immunosorbent assay (ELISA) processor of FAME, Labtop, India. The tests performed were HBV surface antigen (HBsAg) to detect HBV, anti-HCV to detect HCV, 4th generation ELISA kits to detect p24 antigen and glycoprotein antibodies against HIV and Individual NAT(ID NAT)Using the PROCLEIX® TIGRIS® System, ID-NAT depending on transcription mediated amplification (TMA) Technology. **TMA assay involves three main steps utilizing three proprietary technologies:**

- (a) Target capture based sample preparation,
- (b) Transcription-mediated amplification,
- (c) Hybridization protection assay.

STATISTICAL METHODS

Data were collected, processed and analyzed on PC computer. Statistic package for social science program (SPSS) were used for analysis of this data, numerical data were presented in the form of number and percentage, proper significant test were used for analysis and p-value <0.05 was considered statistically significant (SPSS12,2004).

RESULTS

Table (1): Incidence of blood born viruses in reactive samples by ELISA

	Positive		Negative		χ^2	P
	No.	%	No.	%		
HCV	464	4.64	9536	95.36	0.41	0.521
HBV	156	1.56	9844	98.44		
Total number	620	6.2	9380	93.80		

There was no significant difference in blood born viruses in reactive samples, were $P > 0.05$.

Table (2): Incidence of HCV, HBV and HIV reactive cases according to gender

Gender	Total No of reactive samples		HCV		HBV		HIV		Chi-square test	
	No.	%	No.	%	No.	%	No.	%	χ^2	P-value
Male	542	5.42	404	4.04 %	138	1.38%	0	0%	0.290	0.587
Female	78	0.78	60	0.60%	18	0.18%	0	0%		
Total	620	6.20	464	4.64%	156	1.56 %	0	0 %		

There was no significant difference in viral infections distribution between male and female.

The percentage of reactive male donor were 542 (5.42%) and that of females donors were 78 (0.78%) of total tested samples

Table (3): Incidence of HCV, HBV and HIV reactive cases according to age group

Age (years)	Total No. of reactive samples	HCV		HBV		χ^2	p-value
		No.	%	No.	%		
<20	11	9	0.09%	2	0.02%	63.605	<0.001**
20-<30	323	204	2.04%	119	1.19%		
30-<40	178	169	1.69%	9	0.09%		
40-<50	81	59	0.59%	22	0.22%		
50-<60	27	23	0.23%	4	0.04%		
Total	620	464	4.64%	156	1.56%		

There was highly statistically significant difference in viral infections distribution between age group.

Table (4): NAT discriminatory results

Parameters	No of samples	Percentage% to total tested samples
HBV	3	0.003%
HCV	4	0.004%
Total NAT yield samples	7	0.007%

The fact that single donation is used for generating 3 components, The NAT yield becomes 3 times that is, 21 blood products

Table (5): Incidence of HBV, HCV and HIV false reactive results (reactive ELISA and negative NAT)

Parameters	No of samples	Percentage% to total tested samples	x2	p-value
HBV	29	0.29%	60.37	<0.001**
HCV	76	0.76%		
HIV	18	0.18%		
Total	123	1.23%		

There was significant difference were $p < 0.001$

DISCUSSION

Blood transfusion saves millions of lives worldwide each year. However, transfusion-transmissible infections (TTIs) are a major problem associated with blood transfusion, particularly in developing countries. The magnitude of this problem is directly related to the prevalence of TTIs among blood donors. Each blood transfusion carries a risk of transmitting blood-borne pathogens (Biadgo *et al.*, 2017). The safety of blood and blood components continues to raise debate all over the world. In the past few decades, many measures have been introduced in order to reduce the risk of transmission of blood-borne viruses. Blood safety has been achieved through many factors

including more stringent donor selection criteria, improved sensitivity of the screening tests, and inclusion of NAT testing in the routine screening program (Stanic *et al.*, 2017). This current study shows the value of NAT in blood screening, NAT have the potential to detect viral nucleic acids of HIV 1-2, HBV, and HCV earlier than current screening methods, also to focus on the primary benefit of NAT in reducing residual risk of infectious window period donations, identify NAT yield and save donations from being wasted due to their false reactivity by ELISA testing. The prevalence of transfusion transmitted viruses among the 10000 studied donors were 4.64% for HCV and 1.56% for HBV, that agrees with a pervious study done on voluntary non remunerated donors in National Blood Transfusion Center (NBTC), the prevalence of HCV and HBV were 4.8% and 1.5% respectively (Ashour *et al.*, 2009). These rates can be compared with a study from Pakistan the prevalence of Hepatitis B, Hepatitis C, was found to be 1.84, 1.7, % respectively (Arshad *et al.*, 2016). On the contrary to current study, A study done at Beniseuf University hospital on 30055 blood samples collected from November 2007 to April 2015 reported seroprevalence of anti-HCV and HBsAg of 9.4% and 1.6% respectively (Fouad *et al.*, 2016), another study done at Suez Canal University hospital on 149,381 blood samples collected from January 1996 to December 2011 reported seroprevalence of anti-HCV and HBsAg of 7.2% and 2.3% respectively (Hesham and Atwa, 2013). The prevalence of blood-borne viruses according to gender was male blood donors 86.7% respectively and only 13.24% females were included. The high ratio of male to female blood donors in this study was agreed with studies from other countries for example, in a study from Northern Pakistan done on

56,772 donors, Of these donors, 55,985 (98.6%) were male and 787 (1.4%) were female, with a mean age of 29 years (range, 18-60 years) (Niazi *et al.*, 2015). Also agree with a study done in Suez Canal University Hospital Blood Bank 125, 562 blood donors (84%), were males, compared to 23,819 (16%) females. In Egypt, most women are usually housewives and this may lead them to avoid outdoor activities. Moreover, women have lower hemoglobin levels and a higher number of vasovagal reactions. This may cause the high rate of refusal for women donors (Hesham and Atwa, 2013).

The incidence of HCV, HBV and HIV reactive cases according to age group were the largest age group among donors in our study was between 20 to 30 years 43.2% and the majority of the reactive donors were also from the same age group i.e. 20-30 years. The sero-positive prevalence of HBsAg was 119 donors (1.19%). However, the sero-positive prevalence of HCV is 204 donors (2.04%). There was a significant association among the age of the donor and infection with hepatitis B virus, hepatitis C ($p < 0.001$), this agree with a study in Sudan done on 1204 donations collected in Kosti teaching hospital. Average age of the donors was 28 years (range 18 years to 65 years). The majority of the donors were in their third decade of life. The sero-positive prevalence of HBsAg was 66 donors (5.5%). However, the sero-positive prevalence of HCV is 41 donors (3.4%). All donors with sero-positive HCV were between 20 and 40 years old (3.4%). All donors with sero-positive HBV were between 20 and 40 years old, which is comparable with our study (Bazie *et al.*, 2015). Whereas the study done in Turkey showed decrease in the prevalence of sero-positive donors in the last years (24) and

this due to the high health educations programs (Emekdas *et al.*, 2006). NAT discriminatory results; in our study we identified 7 samples (0.07%) as NAT yield (sero-negative samples and NAT reactive) of total 10000 tested samples. The NAT yield of 7 samples in 10000 samples assumes more significance when one considers the fact that single donation is used for generating 3 components (packed RBCs, plasma, platelet) that can be used by 3 recipients. Hence, in effect the NAT yield becomes 3 times that is, 21 in 10000 donations, Saving 21 recipients from TTIs less statically non-significant but worth to decrease rate of infection.

Our NAT yield rate was 1:2500 for HCV and 1:3333 HBV for which is much higher than NAT yield rate in studies from developed countries that have already implemented NAT technology, in United States, it was seen that over a 10-year period, approximately 66 million donations were screened with NAT and the yield of HCV was 1:270,000 and for HIV 1: 2000000 (Zou *et al.*, 2010). On other hand, in developing countries like South Africa, Thailand, Kuwait, and Malaysia the NAT yield was 1: 52303, 1: 4868, 1: 24275, and 1: 3616 donations, respectively (El Ekiaby *et al.*, 2010).

NAT yield rate = NAT yield: total tested samples (Roth *et al.*, 2012).

The Incidence of HBV, HCV and HIV false reactive results in the current study the total number of false positive tested samples (serology reactive and NAT free samples) were 123 samples (1.23%) of total tested samples which were relatively high and this was agreed with a Malaysian study, 1388 donor samples were tested by serology as well as NAT, authors found 1.37% samples reactive on standard serology methods but non-reactive by NAT,

these samples were confirmed to be “false reactive” on confirmatory serological tests (Yassen *et al.*,2013).

CONCLUSION

Finally, we concluded that with increased prevalence of TTIs and incidence of infection, we need to augment TTIs testing, implementation of more sensitive tests such as NAT testing for HIV, HBV and HCV that detect infection earlier (reduce the window period) will further decrease risks of TTIs and save donations from being wasted due to their false reactivity by ELISA testing.

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تقييم اختبار الحمض النووي مقابل الأليزا للكشف عن فيروسات الدم

في بعض بنوك الدم

[٣]

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المستخلص

يتمثل الهدف من خدمات نقل الدم في توفير الدم ومنتجاته بصورة مناسبة وأمنة تلبي احتياجات المرضى. ولا تزال الإصابات الفيروسية التي تنتقل عن طريق نقل الدم، مثل فيروس التهاب الكبد الوبائي (سي) وفيروس التهاب الكبد الوبائي (ب) وفيروس نقص المناعة البشرية، تمثل إحدى المشكلات الرئيسية للصحة العامة في البلدان النامية. ويُعد اختبار الحمض النووي إحدى التقنيات الجزيئية التي تهدف إلى فحص المتبرعين بالدم للحد من مخاطر انتقال العدوى عن طريق الدم بين المتلقين، وبالتالي توفير معيار آخر للتأكد من سلامة الدم.

الهدف من البحث: تقييم أهمية تطبيق اختبار الحمض النووي للكشف عن المتبرعين خلال فترة النافذة التي لم يتم الكشف عنها بتقنية الممتز المناعي المرتبط بالإنتيم (الإليزا).

الحالات وطرق البحث: أجريت هذه الدراسة المقطعية بمركز نقل الدم بالعباسية، القاهرة. التبرع بالدم الذي تم جمعه من ١٠٠٠٠ من المتبرعين الطوعيين من يناير ٢٠١٦ إلى يونيو ٢٠١٦ واختبارها مع فحوصات الحمض النووي والإليزا لكل من فيروس التهاب الكبد الوبائي (سي) وفيروس التهاب الكبد الوبائي (ب) وفيروس نقص المناعة البشرية.

النتائج: يتميز اختبار الحمض النووي بالقدرة على كشف الأحماض النووية الفيروسيية لفيروس نقص المناعة البشرية، فيروس التهاب الكبد الوبائي (ب) و (سي) في وقت سابق من طرق الفحص الحالية. **الخلاصة:** قد يساهم تطبيق اختبار فحص الحمض النووي لثلاثة فيروسات في تحسين سلامة الدم. **الكلمات الرئيسية:** اختبار الحمض النووي، الإليزا، الفيروسات، بنوك الدم.