

TRANSFUSION-TRANSMITTED HEPATITIS B VIRUS (HBV), HEPATITIS C VIRUS (HCV), HUMAN IMMUNODEFICIENCY VIRUS (HIV) DURING WIDOW PERIOD WITHIN IRAQI BLOOD DONORS

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Abstract:

Volunteers of Blood or who called donors are screened for hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) infection by ELISA in the National center of blood bank in Baghdad. The risk of enzyme immunoassay (EIA) negative samples is not estimated till now in Iraq. The aim of this study is to screen the seronegative plasma of blood donors by a commercially available multiplex NAT test with mini-pooling. One thousands (1000) blood donors plasmas were screened by EIA and then NAT was performed on pools of ten blood plasma. A total of 100 minipools donors plasmas were screened by NAT. Positive HBV NAT minipools of plasma were (3%), 3 HCV (3%), and 3 (3%) HIV minipools by NAT. Accordingly, the use of NAT was shown to detect occult HBV and reduce window period in HCV and HIV in sero-negative plasma from blood donors. Therefore, the implementation of NAT with mini-pooling for routine blood donor screening will provide a safe and cost-effective mean of screening for blood transfusion in Iraq.

Keywords: Blood transfusion, widow period, multiplex PCR Short running title: transfusion- transmissible HBV, HCV and HIV viruses in Iraq

INTRODUCTION

Blood could be became safer than before for transfusion through increasing sensitivity of the assays for screening tests of donated blood, with efficient clinical use of blood. Historically the serological tests had been the foundation of blood screening for transfusion transmitted diseases ⁽¹⁾, but it remains limited during the 1) pre-seroconversion window period (PWP), 2) donors infected with genetic and immunovariant viral strains and 3(cases of immunosilent infections, the tests only provide positive results once the donor's immune system reacts against the respective pathogens ⁽²⁾. over two decades ago, advanced and newer tests like nucleic acid testing (NAT) have helped in shorting the viral “window period” ⁽¹⁾. NAT is a molecular technique that used for viral nucleic acids detection to screen the donated blood with high sensitivity and specificity. It is an amplification technique of targeted regions of viral RNA or DNA and could detect the presence of viruses earlier than the other screening

methods, so it will narrow the window period of HIV, HBV and HCV infection, in addition to the ability for resolving false reactive donations on serological methods ⁽³⁾.

International survey on NAT testing of blood donations implemented from 1999-2009 to screen blood donations for HCV and HIV-1 on more than 300 million blood donors and about 100 million blood donors for HBV, the results revealed there were over 2000 NAT-reactive with serology negative donations that would otherwise have been transfused ⁽⁴⁾ also shown that the infection rates for HCV 1:447 000, 1:111 000 for HIV and for HBV 1:66 000. ⁽⁵⁾ But, NATs are the cost technique with about 5–10-fold greater than that of the most expensive enzyme immunoassay. To overcome the cost problem that related to NATs, there were two strategies have been suggested, the using of pooled plasma samples so that fewer tests are required to screen large numbers of samples with using of multiplex PCR assays that can detect several viruses at the same time in one reaction tube ⁽⁶⁾. In Iraq, recent national epidemiological studies regarding the prevalence of HBV, HCV and HIV were very few, but there were some Iraqi studies in separated areas depended on sero-prevalence of HBV, HCV and HIV; in general, all the studies observed intermediate endemicity of HBV infection which found to be 3% and correlate positively with age and also found that HCV with low endemicity among blood donors 0.5% ^(7, 8, 9). HIV program of WHO in Iraq mentioned that the HIV prevalence is currently less than 0.1% of the population, but HIV infection through Iraqi people is vulnerable and risk factors continue to increase that could obviously related to more active trade relations and increased drug use at the last two decades. Less than 100 people living with HIV were reported in Iraq in December 2014, and noticed that a slight increase occurred in officially reported HIV cases from 1986 to 2014, half of which were nationals and half foreigners, and 57% were infected through blood transfusion and blood products as the reported cases revealed ⁽¹⁰⁾.

serological tests In Iraq till now are the dependable standard methods for screening donated blood; so the question here, are these tests enough to ensure the safety of blood or plasma for transfusion? To answer for this question we designed this study as an attempt to make insight on many important points related to blood transfusion in Iraq.

This study aimed to determine the rate of positive HBV, HCV and HIV in donated blood within a large sample of Iraqi donors, also to elucidate sensitivity and specificity of the currently used serological tests in blood screening in comparison with NAT assay, and the rate of transmission of HBV, HCV, and HIV during the seroconversion window.

MATERIALS AND METHODS

Subjects and Samples collection

Blood volunteers came to National center of blood bank in Baghdad / Bab-ALmuadham during the period from July 2018 to January 2019. Up to 20 ml of blood from apparently healthy Iraqi blood donors, in EDTA tubes, were collected and the plasma was separated by centrifuging at 4000 rpm for 20 min.

Serological screening assay

The serological assays were performed on plasma samples of blood donors in National center of blood bank in Baghdad / Bab-ALmuadham laboratory of virology with using most recent serological kits to detect the HBsAg by ELISA (Lot no.Bs-1904-4, fortress /UK), Advanced diagnostic (lot.no.201704120/USA) advanced kit for detection of antibody to hepatitis C virus by ELISA and detect the presence of HIV-1/2 antibodies and/ or HIV-1 p24

antigen in plasma by EIA through Fourth generation (Lot no. 2017091201, Advanced USA) kit.

One thousands (1000) blood donors who revealed seronegative for HBV, HCV, and HIV were included in this study, most of them were male (98%) and the rest were female (2%), 95% were from Baghdad and, (5%) from other Iraqi provinces (except Kurdistan). The samples were categorized into 500 of plasma samples collected after 6hrs from blood withdrawal and the rest (another 500) were collected after 12hrs from blood withdrawal, then 1ml of plasma was pipetted into 1.5 ml microcentrifuge tube and stored at -80 °c until use.

Pooling of plasma samples

Pooling of plasma from blood donors for nucleic acid extraction and NAT was carried out as shown in table (1); pre-estimated positive samples 10^3 copies/ml for HBV and HCV and 10^4 copies/ml for HIV were used for qualifying the pooling system to obtain the most propitiate numbers and volume of samples that could be used in pooling. The pooling validation was done by mixing positive samples with negative samples, for example: One HIV positive plasma (150 µl) + one HCV positive plasma (150 µl) + one HBV positive plasma (150 µl) + 150 µl from 7 individual negative plasma samples resulting in totally 1500 µl pool for 10 individual samples i.e. mini pool 10 (MP10).

Table 1 pooling system validation used in this study.

Samples volume	No. of pooling	Results for HIV,HCV,HBV
100 µl from each sample	5 samples pooled	Failure (+ - +)
100 µl from each sample	6 samples pooled	Failure (--+)
100 µl from each sample	10 samples pooled	Failure (---)
150 µl from each sample	5 samples pooled	Success (+ + +)
150 µl from each sample	6 samples pooled	Success (+++)
150 µl from each sample	10 samples pooled	Success (+++)

Nucleic acid extraction (RNA and DNA)

QIAamp® MinElute® Virus Spin kit 50 (Lot no.163029117 Qiagen/Germany), for simultaneous purification of viral RNA and DNA from plasma, serum, and cell-free body fluids, was used. It was used for viral nucleic acid extraction from of pooled blood donor's plasma. The extraction of nucleic acid was performed according to the manufacturer's instruction, and then the concentration and the purity of all extracted samples were measured using Quantus Florometer (promega/USA); then, DNA extracts (eluent) were stored at -20 °C.

Detection of HBV, HCV, HIV in plasma of the pooled blood donors using multiplex real-time RT PCR

The **HCV/HBV/HIV Real-TM** (Lot no.10H18H705 sacace /Italy)⁽¹¹⁾ kit was used; it is a qualitative Real-Time RT PCR test for detection of HIV RNA, HCV RNA and HBV DNA in human plasma. The extracted RNA/DNA from plasma is amplified by using RT-amplification then detection of fluorescent reporter dye probes specific for HCV, HBV, HIV and internal control (IC). This kit is intended for use for individual donors or could be used to test pools comprised of equal aliquots. The recommended samples numbers in one pool must be not more than 5-10 (100-200 µl of the plasma for each sample). The extracted RNA/DNA sample (15 µl) were added to tube with prepared Reaction Mix according to manufacturer's instructions and mixed by pipetting avoiding air bubble. The tubes (Samples, positive and negative controls) were transferred to real-time PCR thermalcycler ABI® 7500 (Applied Biosystem /USA). Thermo-cycling profile on Real-time instrument (plate type) was as follows: 1 cycle at 50 C° for 20 min, 1 cycle for 15 min at 95C° , 4 cycles for 20 sec at 95 C°, 40 sec at 46 C° , then 42 cycles for 5 sec at 95 C° , 40 sec at 60C°, 40 sec at 45 C° . on

the Fam (Green) channel HCV cDNA was detected, while the Joe (Yellow)/HEX/TET/Cy3 channel detected the HIV cDNA, on the Rox (Orange)/TexasRed channel the HBV DNA was detected and IC on the Cy5 (Red) channel. Results were accepted if the positive amplification and negative amplification controls along with negative and positive controls of extraction are passed. Sample was considered to be positive for *HCV* or *HIV* or *HBV* if the value of Ct was lower than 33 according to the manufacturer's instructions.

RESULTS

Collected 1000 sero-negative plasma samples from blood donors were pooled in 100 mini-pools (MPs) composed of 10 samples. The pooled samples were screened by NAT tests with commercial multiplex PCR kit (Saccace/Italy) for HBV, HCV and HIV detection. The results of multiplex PCR revealed that about 3/100 (3%) of MP10 seronegative plasmas were HBV, HCV, and HIV positive, as shown in figure 1.

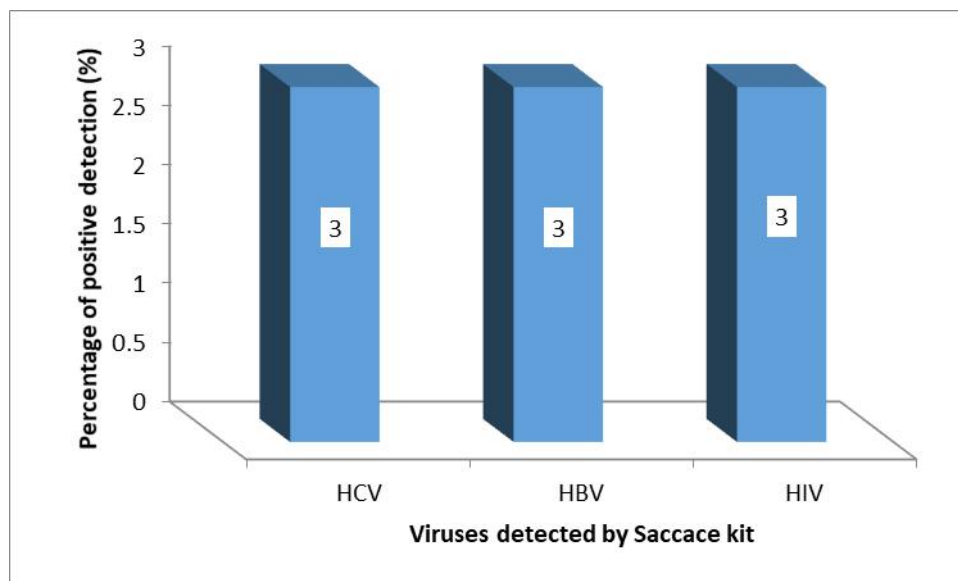


Figure 1: the percentage of positive pooled plasma samples of blood donors that detected by Saccace commercial kit multiplex PCR for HBV, HCV, and HIV

The rate of detection of HBV, HCV, and HIV 6 h versus 12 h after being withdrawn was shown to be borderline different for HIV and HCV. All the three positive minipool, 3/50 (6%) samples for HIV RNA and HCV RNA were found in plasma from the group of 6h blood after withdrawal while zero positive sample was found in 12h group ($P=0.06$), as shown in figure 2. On the other hand, the positive HBV DNA was found in 2/50 (4%) in 12h blood group versus 1/50 (2%) in 6h blood group; however, there was no significant difference in the rate of positive HBV DNA between 6h and 12h blood groups ($P>0.05$), as shown in figure 2.

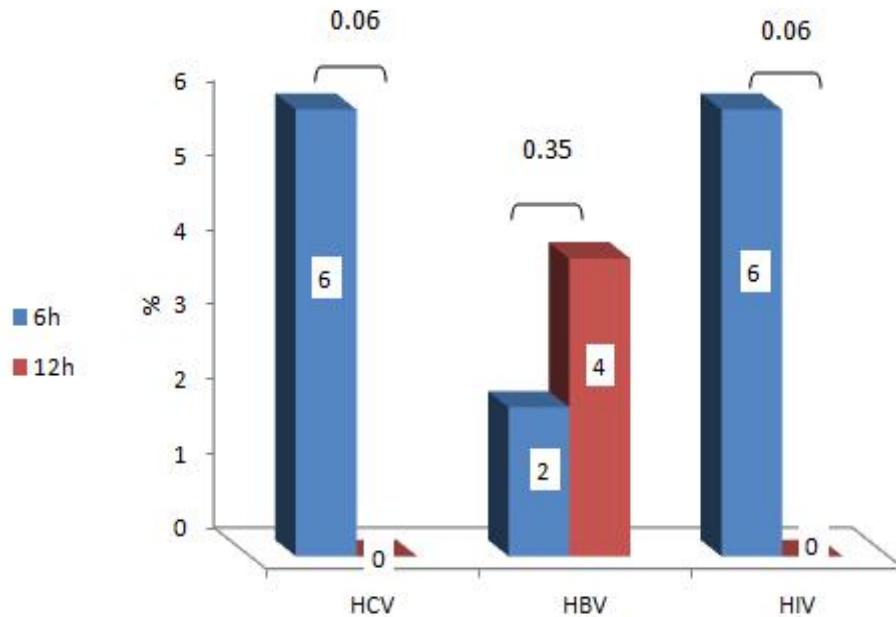


Figure 2: Percentage of positive detection of HCV, HBV, and HIV by Saccace commercial kit within the first 6 versus 12 hours from withdrawing blood. The difference in detection rate of HCV and HIV is borderline significantly better in 6h compared to 12h withdrawal of blood. (P value > 0.05)

DISCUSSION

The mandate for serological screening is important in reducing the risk of transfusion-transmitted infections and improving donation selection. In Iraq, serological screening for blood donors is a standard dependable method for blood screening assay without application of NAT. There is no previous study found to estimate the residual risk of HIV, HCV, and HBV transmission through blood transfusion during viral window periods. The current study was implemented on 1000 seronegative Iraqi blood donors' plasma samples. The rate of positive HBV, HCV, and HIV in these seronegative plasma was estimated using real-time RTPCR. In addition, this study attempted to evaluate the feasibility of pooled samples screened by multiplex PCR. Hence, 100 mini-pools of plasma were screened by multiplex PCR; this study revealed that 3% of MP10 were HBV, HCV, and HIV positive. The current research findings are close to the results of the research done by donor's database of Dubai Blood Donation Centre (DBDC) from 2008-2009; after introducing of multiplex assay in UAE through 2 years, (19%) of blood donors were HBV NAT positive and HBsAg negative. A study applied on 59,283 samples, the potential HBV-positive donors were 187 screened by NAT and serologic assays, 50 HCV-infected donors (12.3%) were reactive for HCV RNA by NAT with negative anti-HCV but only two HIV-infected donors who they were HIV RNA and anti-HIV reactive ⁽¹²⁾. Another study that was conducted in Turkey in 2017, screening of 3000 seronegative donors by NAT was performed on pools of six blood sera, 9 HBV (0.3%) and 1 HCV (0.03%) and 1 HIV (0.03%) were detected and revealed positive results by NAT ⁽¹³⁾. According to WHO guidelines for blood transfusion in 2017, NAT screening method reduces the window period 4-7 days for HIV, 3-5 days for HCV and 17-27 days for HBV while the viral window periods were 14-28 days for HIV, 9-80 days for HCV and 42-55 days for HBV with serological tests ⁽¹⁴⁾.

In western countries the NAT testing became mandatory over years, and began to be performed with commercial CE-marked NAT systems by multiplex PCR, detecting HBV, HCV and HIV genomes on automated machines ⁽¹⁵⁾. The pooling of 6 to 16 specimens that termed minipool nucleic acid testing (MP-NAT) is done in United State, while in some other

countries individual donation testing is performed⁽¹⁶⁾. At the first time, the majority of countries performed the NAT testing in minipools of 96–16 pooled samples; but recently there was a direction towards smaller pools of 6 to individual donations (ID) in order to increase testing sensitivity⁽¹⁵⁾.

The current study also tried to estimate the impact of the duration between the time of blood phlebotomy till the time of screening assays, so we categorized the plasma samples after 6h and 12h from blood withdrawal (according to the dependable system in national Iraqi blood bank), we observed that the detection of HCV and HIV was higher after 6h than 12h without such observation for HBV. *R.W. de Almeida et al* evaluated the HBV DNA in plasma samples stability when it stored at 42 °C for up to 7 days and then at –70C (frozen), their results revealed insignificant decrease in viral load⁽¹⁷⁾. *Gessoni et al.* used samples containing different titers of HCV, HIV-1, and HBV to study the stability of viral genomes. They noticed that HCV and HIV-1 RNA can be stored at 4°C 72h; HBV DNA can be stored until 168 h or about 7 days without reducing in the viral titer⁽¹⁸⁾. The stability of HBV DNA for longer period than HCV and HIV RNA related to the principle that DNA is more stable than RNA and, hence, more resistant to the effects of storage conditions⁽¹⁹⁾.

Finally, in spite of NAT has the ability to detect the TTI viruses during the window periods too earlier than serological screening assay, the blood screening should be done by serological and NAT assay because both NAT and serological assays can complement each other⁽²⁰⁾. This study concluded that there was an estimated risk of HCV, HIV and HBV transmission through blood transfusion in already-tested seronegative donated blood samples. This can originate from the window periods of HBV, HCV, and HIV and it is obviously that NAT screening assay was more sensitive than serological screening assay in the detection of TTI viruses.

Conclusion: Pooling system application was successfully done and gave dependable results and the use of qPCR multiplex PCR along with mini-pooling of samples was cost effective, time-saving and reduced the cross contamination problems.

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