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EVALUATION OF HEPATITIS B AND C VIRUS SEROMARKERS OF DONOR'S BLOOD USING THREE DIFFERENT METHODS

Medical Science

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ABSTRACT

Background: Need for blood transfusion requires no special clarification. But safe transfusion is of utmost importance. Safe blood transfusion program proposed mandatory screening of hepatitis B surface antigens (HBsAg), antibody to hepatitis B core antigen (Anti-HBc) and antibody to hepatitis C virus (Anti- HCV) of donor's blood for transfusion by different laboratory methods.

Aims and objectives: Considering the fact the present study was undertaken to estimate hepatitis B and C virus seromarkers of donor's blood using Chemiluminescence Microparticle Immunoassay (CMIA) and Immunochromatography (ICT) and compared with Enzyme Linked Immunosorbent assay (ELISA) method.

Materials and Methods: In this cross sectional study a total number of 80 blood donors were included in the study following the selection criteria. Sensitivity, specificity, negative predictive value and positive predictive value of the methods were calculated using McNemar Test.

Results: Donors blood was screened for HBsAg, Anti-HBc and Anti-HCV by ELISA, CMIA and ICT methods. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of ELISA, CMIA and ICT methods were evaluated. Of the 80 samples positivity for HBsAg and Anti-HCV by CMIA was found in 1 (1.25%) and 2 (2.5%) samples respectively, whereas all 80 (100%) were negative for both the seromarkers as measured by ELISA as well as ICT. Positivity for Anti-HBc was observed in 6 (7.5%) samples by both the CMIA and ELISA methods. Specificity for CIMA in determination of HBsAg was 98.7% and NPV 100%. Sensitivity and PPV could not be calculated. Determination of anti-HBc by CMIA showed sensitivity and specificity as 100% and NPV and PPV was also 100%. The trend was similar for CMIA when considered anti-HCV. Determination of HBsAg and anti-HCV using ICT showed specificity and NPV of 100% when compared to ELISA. Sensitivity and PPV could not be calculated.

Conclusions: ICT for screening of HBsAg and anti HCV showed closer result with ELISA and CMIA. This small study highlighted the promise of using the relatively less time consuming, less costly and easy to perform ICT method for screening of donor's blood in remote setting.

KEYWORDS:

Chemiluminescence Microparticle Immunoassay (CMIA), Immunochromatography (ICT), Enzyme Linked Immunosorbent assay (ELISA), Hepatitis B virus (HBV), Hepatitis C virus (HCV).

Introduction:

Screening of donor's blood for transfusion transmitted infections is an essential step of laboratory procedures. [1] Transfusion of unscreened blood incurs the risk of transmission of infectious agents like Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Human Immunodeficiency Virus (HIV), Syphilis, Malaria etc to the recipient.[2] HBV and HCV are common blood borne infectious agents causing high morbidity and mortality that constitute the major global health problems. Approximately one fifth of the world populations are being chronically infected with HBV and HCV. Death of 1.5 million people every year attributed to HBV and HCV related chronic liver diseases. [3] Infection with HBV results in a wide spectrum disease from subclinical to fulminant hepatitis leading to death. Since these virus are mostly transmitted by transfusion of contaminated blood and blood product, however, other subjected modes of transmission like intravenous drug abuse, close personal contact, use of shared needle, razor etc cannot be ignored.[2] It commonly causes asymptomatic infection but chronic infection causes scarring of the liver which is generally apparent after many years. Approximately, 75% acutely infected patients develop chronic hepatitis infection that commonly progress to liver cirrhosis and hepatic malignancy.[4]

In 2000, WHO said about 2 billion people worldwide have been infected with the virus and more than 350 million live with chronic lifelong infection.[5] About 3% of the global population is infected by HCV that accounts a total of 170 million of infected persons.[2] These viruses are still causing the most clinically significant transfusion transmissible infections with a per unit risk of 1: 82,000.[6] Looking into the data on the prevalence of the transfusion transmitted infections (TTIs), specifically HBV and HCV, among blood donors permits an assessment of the occurrence of infections in the blood donor population and consequently the safety of the collected donations. Most of the studies conducted in Bangladesh are limited among the professional blood donors, drug addicts, commercial sex workers or hospitalized patients. [7] The rapid spread of HBV and HCV infection and the changed scenario in blood donation practice has inspired us to depict the trends of the prevalence of HBsAg and HCV infection among the healthy blood donors.

The most important laboratory test for the diagnosis of early HBV and HCV infection is the immunoassay for HBsAg and antibodies to HCV.[2] Different analytical methods are being used now a day for the diagnosis of hepatitis namely ICT, ELISA, CMIA and PCR. The ELISA, CMIA and PCR methods are found to be expensive and are used in advanced laboratories and major tertiary care hospitals. ICT, using by the most of the laboratories as a rapid diagnostic analytical method are now considered a good choice because of its low price, less involvement of skilled technician and infrastructure.[8] Since 1990, rapid tests are available for detection of HIV, HBV and HCV infections for screening of blood in many low resource areas to save the resources and overcome lack of funding, equipment and electricity supply. But rapid ICT kits are known to have less sensitivity and specificity than ELISA and CMIA.

In this study, HBsAg measured in the donors samples quantitatively by CMIA (Architect HBsAg QT) and compared with other conventional methods e.g. ELISA & ICT. Architect HBsAg QT was found to be a reliable, reproducible, sensitive and specific assay for HBsAg detection and quantitation. Although confirmation of hepatitis B and C viral infections is based on advanced immunological and molecular techniques.[9] Most of the blood banks use immune-chromatographic test strips to screen hepatitis B and C in blood donors. Their mode of

action is based on common principle of antibody present in the test serum or plasma reacting with the protein coated particle and migrating upward on a membrane chromatographically by capillary action to react with recombinant antigen present on the membrane thereby generating a color line in the test region. The test strips manufactures also claim that these test strips have relatively high sensitivity, specificity and accuracy but controversy often arises regarding these claims.[4] Reports from various studies suggest that this rapid test method not always confirms the accuracy of the test results, increasing the risk of blood borne infection during blood transfusion. Very early stages of infection and patients in the recovering stages usually have low viral titers, reflected by low optical density (Low SOD) in the test results. These low positive cases may not be detected by rapid screening test like ICT; these can be detected by ELISA and CMIA. However it should be kept in mind that method standardization is always crucial before diagnosis of an infection. A major concern in utilizing screening tests is that these tests should have a high performance in detecting infections irrespective of stages of disease. Therefore the intent of this study is to compare rapid test strip screening method with advanced immunological techniques and to recommend for a reliable, cost-effective and less time consuming laboratory method for detection of HBV and HCV seromarkers in donors' blood.

Methods and materials:

This cross-sectional study was conducted by obtaining the blood samples from donors attending at the Square Hospital Ltd, Dhaka. Healthy donors willingly came to donate blood at the blood bank satisfied the qualifying criteria for the donation were included in the study. Both the voluntary blood donors and the replacement blood donors were the study participants. For detection of seromarkers for HBV and HCV by CMIA (the Abbott Architect i2000 Assay), ELISA (Multiskan-Ex, Labsystem, USA; Kit used: Enzo Diagnostics, Inc., USA) and ICT (Excel, China) methods done at the Immunology Laboratory, Bangladesh University of Health Sciences. Results were expressed as the mean ± SD and number (percent) as appropriate. The significance of difference in proportions was tested with use of the $\chi 2$-statistic. Sensitivity, specificity, and positive and negative predictive values of different analytical methods were calculated using McNemer Test. A p-value less than 0.05 considered as statistically significant. The data were managed by using the program statistical package for social science (SPSS).

Results:

Total 80 blood donors were included in this study. Mean (±SD) age was 27.45±7.63 years. Majority (76.3%) of the donors was within the age group of 19-30 years. Among the donors, male donors were predominant (93.8%) than female. Blood groups A, B and O constitutes 36.3%, 23.8% and 33.8% respectively and only 1 (1.3%) was Rh negative who was A for ABO typing.

Table 1: Distribution of seromarkers status by ELISA, CMIA and ICT.

	ELISA		CMIA		ICT	
	Positive	Negative	Positive	Negative	Positive	Negative
HBsAg	0	80	1	79	0	80
Anti HBc	6	74	6	74	-	-
Anti-HCV	0	80	2	78	0	80

Out of 80 samples 6 (7.5%) were detected positive for anti-HBc in both ELISA and CMIA. CMIA detect one positive for HBsAg and two positive for Anti-HCV. Except this all donors are negative for different viral seromarkers. (Table-1)

Comparison of test results evaluated by different methods

ELISA *vs* CIMA *for* HBsAg

All 80 donor samples were negative for HBsAg in ELISA against 1 (1.25%) in CMIA. Specificity of the test was 98.7% and negative predictive value (NPV) calculated 100%. Sensitivity and positive predictive value could not be calculated.

Table 2: Evaluation of CMIA with ELISA for HBsAg

Methods	ELISA		Total
CMIA	*Positive*	*Negative*	
	N (%)	N (%)	N (%)
Positive [N (%)]	0	1 (1.25)	1 (1.25)
Negative [N (%)]	0	79 (98.75)	79 (98.75)
Total N (%)	0	80 (100)	80 (100)

Results were expressed as number and percent.

ELISA vs CIMA for anti-HBc

Of the 80 donor samples 6 (7.5%) positive cases were detected for anti-HBc by both ELISA and CMIA (Table VII). Sensitivity and specificity of CMIA were 100%. Positive and negative predictive value for CMIA methods was also 100%.

Table 3: Evaluation of CMIA with ELISA for Anti Hbc

Methods	ELISA		Total
	Positive	*Negative*	
CMIA	N (%)	N (%)	N (%)
Positive [N (%)]	6 (7.5)	0	6 (7.5)
Negative [N (%)]	0	74 (97.5)	74 (97.5)
Total [N (%)]	6 (7.5)	74 (97.5)	80 (100)

Results were expressed as number and percent.

ELISA vs CIMA for anti-HCV

Of the 80 donor samples none was detected positive for anti-HCV by ELISA. CIMA detected 2 (2.5%) samples positive (Table 4). Specificity CIMA for measurement of anti-HCV was 98.5% and negative predictive vale 100%. Sensitivity and positive predictive value could not be worked out.

Table 4: Evaluation of Anti HCV by CMIA with ELISA

Methods	ELISA		Total
	Positive	*Negative*	
CMIA	N (%)	N (%)	N (%)
Positive [N (%)]	0	2 (2.5)	2 (2.5)
Negative [N (%)]	0	78 (97.5)	78 (97.5)
Total [N (%)]	0	80 (100)	80 (100)

Results were expressed as number and percent.

ICT vs ELISA for anti-HCV

All 80 donor samples were negative for anti-HCV in methods (Table 5). Specificity and negative predictive value for ICT was 100%. Sensitivity and positive predictive value could not be calculated.

Table 5: Evaluation of ICT with ELISA for Anti HCV

Methods	ELISA		Total
	Positive	*Negative*	
ICT	N (%)	N (%)	N (%)
Positive [N (%)]	0	0	0
Negative [N (%)]	0	80 (100)	80 (100)
Total [N (%)]	0	80 (100)	80 (100)

Results were expressed as number and percent.

Discussion:

Blood transfusion is a life saving procedure, however, is not risk free which poses potential danger of disease transmission from infected or carrier donors. Screening for common blood borne infection like HBV and HCV is crucial to ensure the safety of transfusion. The selection of screening tests depends upon a number of factors. Among them, evaluation of test performance, measured by sensitivity and specificity, is the most important factor while kit cost, equipment used, expertise, consumables and disposables are also taken into account during selection of analytical process.[1]

This is for the first time through a structured protocol; ICT and CIMA were compared with ELISA method using donors' blood for HBV and HCV seromarkers (HBsAg, anti-HBc and anti-HCV). Almost all donors were male (93.8%) which is consistent with other reports more specifically by Khan et al where 96.8% of the blood donor population was male as males are the predominant donor both in developed and developing countries. [11, 12] Mean (±SD) age of the donors was 27.45±7.63 and 76.3% (61 out of 80) of them were between the age group 19-30 years. This demonstrated the fact that donors belong to the younger age groups which is consistent to the study done by Sajed and his colleague. In their study 80% donors belonged to the thirties years

of age. [12] Test result for HBsAg was found negative for all 80 samples in both ICT and ELISA method. Only one sample (1.25%) was positive for HBsAg when analyzed by CMIA which highlights the comparable agreement of ICT and CMIA method with ELISA for the determination of HBsAg. Specificity and negative predictive value (NPV) of CMIA in analysis of HBsAg was 98.7% and 100% respectively in this study though the method was equally sensitive as well as specific in analyzing anti-HBc when compared with ELISA. Specificity and NPV of CMIA was found to be 98.5% and 100% respectively when detected anti-HCV. CMIA was done by Abbot Architect that was found to be 98.4% sensitive and 99.1% specific in a syphilis assay reported by Young and his colleagues also correlates our study. [13]

Reference centers or central blood banks found to be widely using most sensitive test methods (ELISA, CMIA and PCR) as quantitative immunoassay globally.[14] Rapid test, ICT is intended for qualitative detection of HBsAg in serum.[15] ELISA, CMIA and other advanced methods are laboratory based, time consuming and require trained laboratory personnel. Chemiluminescence based assays are usually used for screening of blood donors in high volume blood banks owing to automation facilities, higher testing throughput and objective interpretation of results, however, expensive instrumentation is required for them thus limiting their use in resource limited settings.[1]

Rapid test enables early detection at sites where laboratory facilities or trained manpower are not available or there is issue of accessibility. Most rapid tests are based on immunochromatographic principles. [16] The rapid tests reduce the potential loss of follow up of a case when test results are on demand right away. [17, 18]

ICT showed potentially good findings in the present study. No false positive test was observed by this method since it provided negative results of all 80 donors' sample for both HBsAg and anti-HCV that was also revealed to be negative by ELISA. Using ICT for both infections, ICT and CMIA were equally sensitive to ELISA as all the 80 samples showed negative reaction in both ICT and ELISA though 1 (1.25%) sample was positive for HBsAg and 2 (2.5%) for anti-HCV by CMIA. Our results showed comparable performances of the three techniques with almost 99% agreement of results. In evaluating both the seromarkers, specificity and negative predictive value of ICT were 100% that was consistent with a study done in Pakistan where ICT and ELISA were compared for detection of HBsAg in healthy individual from Karachi that showed comparable sensitivity and specificity of ICT kits with ELISA technique. [19] It is again consistent with another study done by Herring et al, 2006 where evaluation of nine rapid syphilis ICT kits reported 93-98% specificity. A meta-analysis reported the sensitivity of different ICT devices ranging from 85-100% and specificity 98-100%. [20] An Indian study reported 100% specificity and 93.4% sensitivity of rapid kits when detecting HBsAg.[21] ICT is suitable for use in remote and developing regions since they are simple to perform, can be transported, stored and performed at room temperature and microscopic and electrical equipment not needed. Moreover, these are cheaper and quicker as compared to other diagnostic procedures. [22]

Findings of the present study are also consistent with study conducted in Iran where 6 rapid strips/devices were compared with gold standard methods. [23] In another study from Seoul for detecting HBsAg, rapid technique showed 97% sensitivity and 100% specificity.[24] In the present study, overall specificity results for both HBsAg and Anti HCV ICT kits were high i.e. 97-100%. These results are different to the study conducted in Lahore, Pakistan by Khan et al (2010) who demonstrated 93% to 100% specificity for HBsAg and Anti HCV by ICT method but the sensitivity was 50% for both HBsAg and Anti HCV. In the present study sensitivity was 100% for HBsAg which is higher than the just mentioned study. The present study was carried out as a pilot basis to compare two methods ICT and CMIA with ELISA for HBV and HCV seromarkers HBsAg, anti-HCV and anti-HBc. Although the number of samples tested was limited yet we could infer that the two methods had performed equally well and in limited resource settings, the ICT could be used as an alternative for HBV and HCV seromarkers screening.

Conclusions:

This study showed ICT and CMIA methods were able to determine HBsAg and Anti HCV negative samples as detected negative by ELISA. CMIA detected positivity, though small number, for HBsAg, anti-HBc and anti-HCV compared to ELISA. It can be recommended that ELISA comparable rapid devices may be allowed to be used for initial screening of hepatitis B and C especially, in remote areas. The present study concludes that demonstrate comparable performances of ICT, CMIA and ELISA for screening of HBV and HCV seromarkers. It may be suggested that ICT method could be used in screening of donors' blood in low resource settings and in situation where time is critically important to save life as supported by the data of the present study. The study recommends an extended study by recruiting large number of samples and to expedite the study multicenter based collection of samples might be an option. All methods (ICT, ELISA and CMIA) should be evaluated against a molecular method e.g., PCR that might be gold standard in future studies.

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