COMPARISON BETWEEN ELISA AND ICT TECHNIQUES FOR THE DETECTION OF ANTI HCV ANTIBODY AMONG BLOOD DONORS

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ABSTRACT

Background and Objective: The most important marker for HCV infection is the detection of anti HCV antibodies. Although HCV detection by immunochromatography (ICT) method is one of the most popular method but enzyme linked immunosorbant assay (ELISA) and nucleic acid testing methods are considered as more reliable. This is a cross sectional study. The aim of this study is to compare the performance of ICT and Elisa techniques for the detection of anti HCV antibody among blood donors in Children hospital Lahore.

Methods: Blood samples from 130 male blood donors were randomly collected according to WHO criteria of donor selection at blood bank of children hospital Lahore. Blood samples were taken from 1st September 2015 to 28 November 2015. All samples were subjected for the detection of HCV by ICT and ELISA.

Results: 100 samples of blood donors showing negative results for HCV were retested with ELISA. Out of these 100 samples only 1 (1%) sample showed positive results for HCV with ELISA. Similarly, 30 blood donor sample showing positive results by ICT technique were also analysed by ELISA and only 1 sample (3.3%) showed negative results with ELISA technique. By using ELISA technique as gold standard for HCV infection our results showed 99% specificity and 96.66% sensitivity of ICT technique.

Conclusion: ICT results for blood donor screening are acceptable just like ELISA due to its comparable sensitivity and specificity with ELISA. It can be used in blood banks with limited facilities because it is rapid and cost effective.

Key words: ELISA, ICT, HCV, blood donors.

INTRODUCTION

Hepatitis C is a major health problem worldwide, especially in developing countries like Pakistan. World health organization reported that approximately 170 million people are infected with HCV worldwide.[1] The prevalence rate of HCV in Asia pacific region is from 4% to 12%.[1] Seroprevalence of HCV are reported from 0.4% to 13.3% in different countries.[2] 6% of total population or more than 10 million people in Pakistan are affected with HCV that leads to high rate of mortality and morbidity.[1]

HCV is mainly affects the liver to cause hepatitis C. It is a single standard RNA virus belongs to family Flaviviridae. The major source of hepatitis C virus (HCV) infection includes the infected blood, its products and the other body fluids. Risk factors like intravenous drug injecting, reuse of syringes, dental procedures, use of infected razor, pricking by sharp objects, infected sexual partner and tattooing also play an important role in occurrence of HCV infection.[3]

Now a day's, different lab techniques are used for screening and diagnostic purpose for HCV including rapid kit, ELISA, chemiluminescence (CLIA) and PCR.[6] Blood donation screening tests have major concern with cost-effectiveness, sensitivity and they should provide the results in short duration of time. We can detect HCV antigen, anti HCV antibody or both by serological assays.[2]

In developing countries many blood banks have limited facilities such as, electric supply, trained manpower and instrumentation. Alternative screening methodology to EIAs and PCR is rapid tests in such situation. These tests are easy to perform without instruments or electric supply and can be read visually within a few minutes. They are based upon any one of the following principles; agglutination, immunofiltration or immuno-chromatography.[5] ELISA tests are expensive as the instruments and chemicals are required to perform the test but it has shorten the window period of HCV.[4] For the detection of two infectious markers, (antigen and antibody) combination EIA of HCV that is also known as 4th generation EIA, is used now a

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days.[2] HCV-RNA can be amplified by using (NAT) nucleic acid amplification technology as well. It shortens the window period to 4 days and it is more sensitive.[2]

Polymerase chain reaction (PCR) can be used for the detection of HCV RNA to detect acute infection with hepatitis C virus (HCV). PCR involves high cost of testing because it requires trained staff and specialized expertise.[6] Many blood banks use rapid screening kits because they are rapid, cost effective and are user friendly, and do not require sophisticated equipment and elaborate training.[7] Antigen that is mostly same as used in 3rd generation ELISA is also used in these tests. The sensitivities of these tests are reported from 98% to 100%. Due to their cost effectiveness and user friendly attribute, rapid kits practically used in all primary and secondary health care facilities in Pakistan.[2]

Early generations of ELISA had a long incubation period that has been reduced in new generations. 3rd generation ELISA have a non-structural antigen (NS5), an additional antigen that showed a reduction in window period to 66 days. Monolisa anti-HCV that is 3rd generation ELISA used in this study that has been reported (100%) sensitive and (98%) specific in different studies.[2,11] Hence rapid test and ELISA are most common and popular methods for the detection of HCV infections are. The major problem that we face is the discordance between results of these two assays that can be solved by using the availability of suitable kits. Therefore, kit evaluation gains importance for determining the diagnostic kits of better performance.[8]

This study was conducted to analyse the effectiveness of these testing kits for screening of blood donors and compare ICT results with ELISA. In present study we used ICT for detection of HCV infection. For the reconfirmation of ICT results we used 3rd generation ELISA as gold standard due to its improvements in performance, in term of "generations" of the assay.

SUBJECTS AND METHODS

This cross sectional study was conducted in Immunology department of Children Hospital Lahore. It was a hospital based study from 1st September 2015 to 8 November 2015e. Sample size of this study was calculated by using the WHO software for sample size determination in health studies (Wanga and Limeshow, 2001). Convenient sampling technique was used. 130 donors were selected. Among the 130, 100 donors were screened as non reactive or healthy while 30 were screened as HCV positive according to ICT results. All blood donors were male. Blood donors were selected on the strict basis of the standard operating procedures described by Blood Transfusion Services, Pakistan. The sample was centrifuged at 2000 rpm for 5 minutes and serum was separated. Donors were screened Hepatitis C using, one step Immunochromatographic device method and Fast step rapid diagnostic test palmed therapeutic Houston USA kit was used. These samples were processed in immunology department by using indirect manual ELISA technique and Monolisa anti HCV PLUS Version 2 kit of BIO RAD Company was used according to manufacturer instructions. On the basis of cut values 0.3321, patient samples were considered as reactive and non reactive for HCV.

Statistical Analysis

Data was entered and analysed by using IBM SPSS statistics 20 (IBM corporation) version. Categorical data was analysed descriptively and frequencies of blood donors were calculated. The Chi-square test was used to find the difference between two variables of study (ICT and ELISA). The P value less than 0.05 were considered as significant.

RESULTS

A total of 130 blood donors were randomly selected in this study from blood bank of The Children Hospital and ICH. All donors were adult males. Among of 130 blood donors samples, 100 were screened as HCV (76.92%) negative and 30 (23.1%) samples screened as HCV positive on ICT. All ICT screened samples were also analysed by indirect ELISA. 100 (76.92) negative screened samples were analysed by ELISA technique, out of them 1 sample (1%) showed positive results as shown in table and fig 1. 30 positive screened samples were reanalysed by ELISA technique, out of them 1 sample (3.33%) showed negative results as shown in table and fig 1.1 A statistically significant association was found between ELISA and ICT results with a p value of 0.02, as shown in table 2. one (n = 1/30) false positive result and one (n = 1/100) false negative result were seen on ICT as compared to ELISA results, as shown in table 2. Considering ELISA as gold standard sensitivity and specificity of ICT (29/30) was 96.66% and specificity (99/100) was 99%, as shown in table 3.

All rapid test (ICT) negative samples are analysed with ELISA technique. Out of 100 ICT negative samples only one sample (1%) was positive for HCV with ELISA technique All ICT positive samples were also analysed by ELISE technique. Results showed that out of 30 ICT positive cases only 1 (3.33%) sample was found to be negative on ELISA for HCV (**Fig. 1).**

Table 1: *Distributions of Results Obtained with ICT and ELISA among Blood Donors.*

HCV Screening	*HCV ELISA*		*Total*	*P value*
	Reactive	*Non-reactive*		
Reactive	29	1	30	*0.02
Non reactive	1	99	100	
Total	30	100	130	

Chi-square test was used and *p value <0.05 is significant.

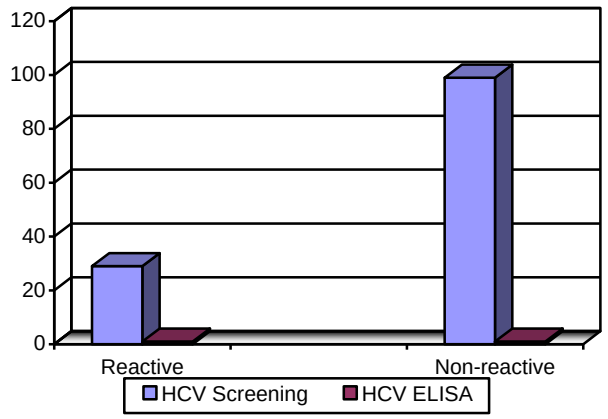

Fig. 1: *Bar Chart Showing Distributions of Results Obtained With ICT and ELISA among Blood Donors.*

Table 2: *False Positive and False Negative Results on ICT in this Study.*

False negative	1/100 (1%)
False positive	1/30(3.33%)

Table 3: *Sensitivity and Specificity of ICT While ELISA Using As Gold Standard.*

Sensitivity	96.7%
Specificity	99%

DISCUSSION

In the present study we use ELISA as gold standard and compared the results of ICT for the screening of HCV infections in blood donors. In this study the ICT screening method showed sensitivity and specificity of 99% and 96.7% respectively for the screening HCV. The screening methods have limitation of false negative and positive results. In this study the rate of false negativity was (1%) and false positivity was only (3.3%) which is very low.

The results of our study are in agreement with the results of Indian study. According to which ICT showed sensitivity and specificity of 100% when results were compared with ELISA.[8] Similar results were observed by a French researcher, who analyzed ICT positive cases again on ELISA to find the sensitivity of ICT. This study results showed 95% sensitivity.[10] Results of both studies are consistent with our results. In another study three rapid strips/devices were compared with gold standard for HCV infection. The final observed sensitivity was 93% and specificity was 98%.[9] In India, all rapid kits were 100%, specific and 87% sensitive for HCV.[13]

A study from Lahore Pakistan reported Sensitivity of ICT, which showed a low detection rate of positive cases in comparison with the ELISA.[6] A study from Lahore reported very low sensitivity as compared to our study (44% to 66%) but specificity was fairly high (93% to 100%).[4] A study from Lahore reported 2.35% false positive cases on ICT as compared to ELISA.[5] Another study from Pakistan showed 0.15% false positive results on ICT in blood donor screening.[14] Both of these study showed low false positive and their results are consistent with our study results.

A study of Hepatitis C in HIV Patients from Cameroon showed 9.1% false positive results in control group and 6.3% in HIV positive patient using ELISA as the gold.[11] The false positive rate is high as compared to our study as previous literature describe the HIV interference decrease the sensitivity of the ICT to 77.5%. This leads to the fact that sensitivity of rapid test for HCV antibodies are decreased due to HIV virus,[11,12,14] other reasons are sample size and manufacturing kit difference.

The published literature show that ELISA is more sensitive but ICT specificity is comparable with ELISA. A similar study in Nigeria with same sample size like our study showed high false negative rate and no false positive results on ICT as compared to ELISA .It is not consistent with our results.

In all the above studies specificity is consistent with our results, but due to sample size difference variation is seen in term of sensitivity which is not the major concern, because in blood bank setup false positive result will be better than a false negative results. Inadequate coating of the antigens on the surface of the immuno-filter or the nature of Antigens used in rapid tests and ELISA can affect the results. Furthermore genetic heterogeneity can affect the serological response.[13] Fall in sensitivity of ICT can also be explained by chance variation, inadequate representation of antigen on rapid device.

Although EIAs shows maximum degree of sensitivity but due to its cost and as it is a time taking procedure it is less preferable. In blood banks where time is the major issue rapid tests is the good substitute of ELISA. Specificity of some rapid kits has increased due to the use of synthetic antigens and due to decrease false negative results therefore the performance of the rapid kits are satisfactory.[8] ICT can be used in blood banks with limited facilities because it is rapid and cheap. It can be used for initial screening only but it could not be the only criteria for diagnosis. Further research with improved sample size and higher techniques are required to found the credibility of such devices for their sensitivity and specificity.

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Author's Contribution

MZ: Did data collection, lab work, manuscript writing. FS: Supervised and did manuscript writing. MS: Did result interpretation, manuscript writing. SA. Statistical analysis. N: Data collection. SH: Review manuscript.

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