2015 Volume I Issue 1 Pages 21-24
Research article

A potential loophole in early diagnosis of the hepatitis B and hepatitis C

Shakeel Waqqar[1*], Farheen Ansari[1], Muhammad Akram Muneer[1], Sharoon Hanook[2], Hajra Azhar[2], Shaihid Hussain Sheikh[3], Maira Gul[3]

[1]Center of Research in Molecular Medicine, University of Lahore, Lahore 54000, Pakistan
[2] Department of Statistics, Government College University, Lahore 54000, Pakistan
[3]NIDS Treatment and Research cEnter, 126 Habitat Flats, Jail Road, Lahore 54000, Pakistan

Abstract
Pakistan is among the countries with high prevalence rates of Hepatitis B and C virus infections. Proper diagnosis of the disease is very crucial in combating with the high prevalence rates in a country. In Pakistan, numbers of diagnostic tests like Immuno-chromatography technique (ICT), enzyme linked immuno-sorbent assay (ELISA) and polymerase chain reaction (PCR) are in use to demonstrate the presence of hepatitis in the subjects. In the present study, we used ICT, ELISA and PCR techniques and compared the sensitivity and specificity of these techniques in order to evaluate best suited method for local situations. Sensitivity analysis of these tests has shown that ICT has a low detection rate of positive cases in comparison with the ELISA and PCR based nucleic acid detection techniques. We suggest that ELISA and PCR should be used instead of ICT for screening purposes in hospitals, blood banks and diagnostic laboratories.
Key Words: Sensitivity and specificity, ICT, ELISA, PCR, hepatitis.

Received January 06, 2015; **Revised** February 02, 2015; **Accepted** March 07, 2015
[*]**Correspondence**: Shakeel Waqqar; **Email**: shakeelwaqqar@gmail.com; **Phone**: 00923454772276

***To cite this manuscript*:** Waqqar S, Ansari F, Muneer MA, Hanook S, Azhar H, Sheikh SH, Gul M. A potential loophole in early diagnosis of the hepatitis B and hepatitis C. Biomed Lett 2015; 1(1):21-24.

Introduction

Prevalence of Hepatitis C in Pakistani population varies in selective target groups, including health professionals, drug abusers, and garbage scavengers. Studies show Hepatitis prevalence of up to 40% in some groups [1, 2]. About 1 million deaths are reported worldwide every year due to Hepatitis B & C [3]. Chronic liver disease like Hepatitis B & C are a greater challenge. Together Hepatitis B and Hepatitis C have been implicated as the leading cause of liver cancer (Hepatocellular Carcinoma) occurring in 78% of the infected cases around the world. Transmission of Hepatitis B is associated with body fluids, semen, and needle stick injury and from mother to infant during early childhood [1, 4]. Hepatitis C is considered as a blood born pathogen and transmitted through blood transfusion, needle stick injury, open skin contact with blood and sexual contact. Treatment of the Hepatitis B is believed to reduce risk of liver cancer, but only 20 to 30% of the patients are believed to get real benefits of the current available treatment. The efficiency further reduces to 10% with co-infection of human immunodeficiency virus (HIV) and HCV. HCV is considered as the treatable condition, but for most it comes at a very high cost and majority of the people cannot afford it in developing countries [3, 5].

Rapid Immuno-chromatography tests are the best suitable test format to be used for screening for blood donors in resource-limited settings. Rapid detection of the hepatitis C infection is serologically based on detection of the IgG. Various techniques are in use for rapid detection of the infection, using immunoassays, immuno-blot assays and more recently rapid devices for detection of hepatitis C are based on Immuno-chromatography technique [6, 10]. For detection of acute infection with hepatitis C virus (HCV) requires detection of HCV RNA by polymerase chain reaction which requires specialized facilities and trained manpower, ultimately resulting in higher cost of testing. The diagnosis of acute HCV infection without the demonstration sero-conversion remains elusive [5, 6]. To detect viremia, antigen based rapid tests are being introduced and evaluated for HCV [6], they are reported as reliable but currently such test are not in common use of diagnostic laboratories [5, 10].

The hepatitis B surface antigen (HBsAg) and hepatitis B e antigen based kits are commonly being used for rapid detection of hepatitis B infection [7]. These kits found their place at blood banks and epidemiological

studies [8]. Detection rates of these kits are reasonably good, but the problem arises when they miss some positive cases. For evaluation of hepatitis B viremia PCR testing is considered as most accurate and gold standard [7, 8] but these are expensive techniques and the majority of the population in developing countries cannot afford such high cost testing.

In Pakistan, health care facilities are often substandard and implementations of the health laws are greatly neglected [2]. There are no set guidelines for use of test techniques which shall be practiced as standard. Most of the laboratories, clinics and hospitals adopt their own choice of test technique. This choice is greatly influenced by low purchase prices and high profitability. In run to increase the profit and lower the cost quality testing is compromised [1, 2]. In Pakistan rapid test devices are preferred by most of the blood banks and hospitals as standard screening tool [2, 13]. We conducted this study to evaluate the efficiency and drawbacks of Immuno-chromatography kits for hepatitis B and hepatitis C as a standard screening method, compared with enzyme linked immuno-sorbent assays and polymerase chain reaction techniques.

Materials and Methods

In this study, 426 samples were used for evaluation of commercially available kits of hepatitis B, 366 samples were tested negative and 66 were tested positive for hepatitis B. 426 samples were tested for hepatitis C, of which 353 were tested negative and 73 were tested positive. All Samples were collected from Neuro immune dysfunction syndrome treatment and research center (NIDS) laboratory. 5 ml of blood was collected in a clot activator gel tube. Samples were centrifuged at 3000 RPM for 5 minutes to obtain clear serum. Serum was separated in a 2 ml serum cup and kept at -20 C^0 before analysis. We used Accue-Check Immuno-chromatography kit by Roche Diagnostics, GmbH D-68305 Mannheim for detection of antibodies against hepatitis C. Antigen detecting kit Acon by ACON Laboratories, Inc. (ACON, San Diego, CA) was used to detect surface antigen of hepatitis B (HBsAg). In this study, PCR technique was used as a reference technique. For detection of HCV RNA, artus® HCV RG RT-PCR was used, manufactured by QIAGEN Hilden, Kit was used on Rotor-Gene Q instrument, manufactured by QIAGEN. For DNA detection of the HBV artus® HBV RG PCR Kit from the QIAGEN, Hilden was used and Rotor-Gene Q instrument from QIAGEN was used for reaction cycles. ELISA tests for HBsAg and Anti HCV were performed using Abbott Murex 4th Generation ELISA kits by Abbott Laboratories on Architect ci8200 instrument from Abbott Diagnostics, USA. For all the testing techniques instructions from manufacturer were followed. Statistical analysis was performed for sensitivity and specificity of diagnostic tests using MEDCAL software.

Results

In this present study, we analyzed 426 samples for hepatitis B, Using polymerase chain reaction and found that 366 (85.91%) samples were tested negative and 66 (15.49%) were tested positive for DNA detection of HBV. Same samples were analyzed by Immuno-chromatography, which showed 384 (90.14%) tested negative and 42 (9.8%) tested positive that is 24 (5.69%) cases were detected false negative. Statistical analysis shows that sensitivity of the HBsAg Immuno-chromatography is 76.92% with 95% CI: 66% to 85.71%. Specificity of HBsAg Immuno-chromatography was 100.00% with 95% CI: 98.99% to 100%. The negative likelihood ratio was 0.23 with 95% CI: 0.15 to 0.35. Disease prevalence was 17.57 with 95% CI: 14.14% to 21.43%. Positive predictive value for HBsAg by Immuno-chromatography was 100.00% with 95% CI: 93.98% to 100%. Negative Predictive Value of the HBsAg kit was 95.31% with 95% CI: 92.69% to 97.20% as shown in Table 1. Sensitivity of the ELISA test for HBsAg was 100% with 95% CI: 93.98% to 100.00%. Specificity of the ELISA for HBsAg was 100% with 95% CI: 98.99% to 100% as shown in Table 2.

Polymerase chain reaction for the detection of HCV RNA showed that 353 (82.86 %) samples were negative and 73 (17.1%) were positive. Detection of antibodies by ELISA showed 100% sensitivity and specificity (at 95% CI: 95.02 % to 100.00%; 98.95% to 100.00%). Immuno-chromatography kit for anti HCV detected 55 (12.9%) positive cases only that is 18 (4.2%) positive cases were given false positive.

Accue-Check Immuno-chromatography kit for the detection of Anti HCV showed sensitivity of 80.22% with 95% CI: 70.55% to 87.83% and specificity of 100.00% with 95% CI: 98.95% to 100.00%. Negative Likelihood Ratio was 0.20 with 95% CI: 0.13 to 0.30. Disease prevalence was at 20.50% (16.84% to 24.55%). Positive Predictive Value of the test was 100% with 95% CI: 95.02% to 100.00% whereas Negative Predictive Value stands at 95.15% with 95% CI: 92.44% to 97.10% as shown in Table 3.

Table 1: Sensitivity and specificity of HBsAG ICT

Sensitivity	76.92%	95% CI: 66.00 % to 85.7%
Specificity	100%	95% CI: 98.99 % to 100 %
Negative Likelihood Ratio	0.23	95% CI: 0.15 to 0.35
Disease prevalence	17.57%	95% CI: 14.14 % to 21.4%
Positive Predictive Value	100%	95% CI: 93.98 % to 100 %
Negative Predictive Value	95.31%	95% CI: 92.69 % to 97.2%

Table 2: Sensitivity and specificity of HBsAg ELISA

Sensitivity	100%	95% CI: 93.98 % to 100%
Specificity	100%	95% CI: 98.99 % to 100%
Negative Likelihood Ratio	0	-
Disease prevalence	14.08%	95% CI: 10.92 % to 17.7%
Positive Predictive Value	100%	95% CI: 93.98 % to 100%
Negative Predictive Value	100%	95% CI: 98.99 % to 100%

Table 3: Sensitivity and specificity of HCV Immuno-chromatography

Sensitivity	80.2%	95%CI: 70.55% to 87.8%
Specificity	100%	95% CI: 98.95% to 100%
Negative Likelihood Ratio	0.20	95% CI: 0.13 to 0.30
Disease prevalence	20.5%	95%CI: 16.84% to 24.5%
Positive Predictive Value	100%	95% CI: 95.02% to 100%
Negative Predictive Value	95.1%	95%CI: 92.44% to 97.1%

Table 4: Sensitivity and specificity of Anti HCV ELISA

Sensitivity	100%	95%CI: 95.02% to 100%
Specificity	100%	95%CI: 98.95% to 100%
Negative Likelihood Ratio	0	-
Disease prevalence	17.14%	95%CI: 13.68% to 21%
Positive Predictive Value	100%	95%CI: 95.02% to 100%
Negative Predictive Value	100%	95%CI: 98.95% to 100%

Sensitivity of the ELISA for Anti HCV was 100.00 % with 95% CI: 95.02 % to 100 % and specificity was 100% with 95% CI: 98.95 % to 100 % as shown in Table 4. Our results showed that Immuno-chromatography test for antigen detection of HBV and anti HCV is not a good choice for blood banks, clinics and hospital based diagnostic protocols.

Discussion

Rapid testing of hepatitis B and hepatitis C by Immuno-chromatography technique is frequently in use by hospitals, blood banks and diagnostic laboratories. Most of the health care facilities are adapting their own protocols as per their convenience and cost efficiency. Diagnosis of HBV infection is usually through serological and virological markers. The hepatitis B surface antigen (HBsAg) is the hallmark of HBV infection and is the first serological marker to appear in acute hepatitis B, and persistence of HBsAg for more than 6 months suggests chronic HBV infection [8].

In this study, we tested the sensitivity and specificity of Immuno-chromatography kits for HBsAg and anti HCV. We found, sensitivity of Immuno-chromatography for HBsAg was 66.00 % to 85.71 %and specificity was 98.99 % to 100.00 %. A study from Madagascar by Randrianirina in 2008 reported that the sensitivity of HBsAg was 97.8% and specificity was 100%. In the present study, we had Positive predictive value of HBsAg at 93.98 % to 100.00 % and negative predictive value of 92.69 % to 97.20 % which is complimentary to the study from Madagascar [9]. A study by Lau *et al* in 2003 reported that rapid Immuno-chromatography technique was 95% to 100% sensitive and specific for fresh and frozen human blood samples, compared with EIA technique [7]. According to Leu *et al* Immuno-chromatography technique for detection of HBsAg is best suited for large scale epidemiological studies because of its rapid results and low cost compared with EIA [7]. An Indian study by Raj *et al* in 2001 showed that HBsAg Immuno-chromatography has sensitivity of 79% (CU: 57.3-92%) and specificity of 98.9% (CI: 97.9-99.4%) compared with the ELISA technique [10].

A review study by Colins *et al* in 2001 stated that third generation immunoblot assays are 65% to 89% sensitive in hemodialysed patients while ELISA was 94% to 100% sensitive [11]. According to a report by the CDC in 2001, the specificity of third generation ELISA was $\geq$ 99 %, but even with this value it fails to provide a desired predictive value for positive cases [12]. A study from Pakistan by Batool *et al* in 2009 showed that immuno-chromatography test for hepatitis C antibody detection gives overall 2.35% false positive results [13] while we found no false positive results although we found 4.2% false negative results by ICT compared with ELISA technique. Among immuno-competent population reliability of ICT is 95%; while in immuno-compromised, it drops to 77.5 % [14, 16] Performance characteristics of pre-market rapid anti-HCV varied in their sensitivity is (78.9-99.3%) and specificity (80-100%) [14-16].

Conclusion

In this study, we found that ICT has a low detection rate of positive cases in comparison with the ELISA and PCR based nucleic acid detection techniques.

This makes ICT an inferior choice as a diagnostic tool of hepatitis, especially in Pakistan where disease burden of hepatitis is very high. We suggest that ELISA should be used instead of ICT for screening purposes in hospitals, blood banks and diagnostic laboratories.

References

[1] Abbas Z, Jeswani NL, Kakepoto GN, Islam M, Mehdi K, Jafri W. Prevalence and mode of spread of Hepatitis B and C in rural Sindh, Pakistan. Trop Gastroenterol. 2008; 29: 210-216.

[2] Rauf MU, Saleem MD, Anwar MO, Ahmad G, Aziz S, Memon MA, HIV, Hepatitis B and Hepatitis C in garbage scavengers of Karachi. J Pak Med Assoc 2013; 63 (6): 798-802.

[3] World Health Organization. Introduction of Hepatitis B vaccine into childhood immunization services. Geneva, WHO, 2001.

[4] Rachiotis G, Papagiannis D, Markas D, Thanasias E, Dounias G, Hadjichristodoulou C. Hepatitis B virus infection and waste collection: prevalence, risk factors, and infection pathway. Am J Ind Med 2012; 55: 650-655.

[5] Faridulla, Salih M, Malik IA, Hussain I. Increasing prevalence of chronic Hepatitis and associated risk factors. Pak J Med Res 2002; 41: 46-50.

[6] Valcavi P1, Medici MC, Casula F, Arcangeletti MC, De Conto F, Pinardi F, Calderaro A, Chezzi C, Dettori G. Evaluation of a total hepatitis C virus (HCV) core antigen assay for the detection of antigen aemia in anti-HCV positive individuals. J Med Virol 2004; 73(3):397-403.

[7] Lau DT1, Ma H, Lemon SM, Doo E, Ghany MG, Miskovsky E, Woods GL, Park Y, Hoofnagle JH. A rapid immuno-chromatographic assay for hepatitis B virus screening. J Viral Hepat 2003; 10(4):331-334.

[8] Kao JH. Diagnosis of hepatitis B virus infection through serological and virological markers. Expert Rev Gastroenterol Hepatol 2008; 2(4):553-562.

[9] Randrianirina F1, Carod JF, Ratsima E, Chrétien JB, Richard V, Talarmin A.Evaluation of the performance of four rapid tests for detection of hepatitis B surface antigen in Antananarivo, Madagascar. J Virol Methods 2008; 151(2):294-297.

[10] Raj AA, Subramaniam T, Raghuraman S, Abraham P. Evaluation of an indigenously manufactured rapid immunochro-matographic test for detection of HBsAg. Ind J Pathol Microbiol 2001; 44(4):413-414.

[11] Colin C1, Lanoir D, Touzet S, Meyaud-Kraemer L, Bailly F, Trepo C; HEPATITIS Group. Sensitivity and specificity of third-generation hepatitis C virus antibody detection assays: an analysis of the literature. J Viral Hepat 2001;8(2):87-95

[12] CDC, MMWR, Recommendations and Reports, 2001;1-43

[13] Batool A, Khan MI, Bano KA. Efficacy of immunoassay chromatography test for hepatitis-C antibodies detection. J Ayub Med Coll Abbottabad 2009; 21(3):38-39.

[14] Desbois D, Vaghefi P, Savary J, Dussaix E, Roque-Afonso AM. Sensitivity of a rapid immuno-chromatographic test for hepatitis C antibodies detection. J Clin Virol 2008; 41(2):129-33.

[15] Smith BD1, Jewett A, Drobeniuc J, Kamili S. Rapid diagnostic HCV antibody assays. Antivir Ther 2012; 1409-1413.

[16] Kamili S1, Drobeniuc J, Araujo AC, Hayden TM. Laboratory diagnostics for hepatitis C virus infection Clin Infect Dis 2012; 43-8.