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ORIGINAL ARTICLE

Comparative Analysis of Electrochemiluminescence Assay and Chemiluminescent Microparticle Immunoassay for the Screening of Hepatitis C

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Abstract The primary objective of the study was to identify a highly sensitive and specific screening technique for the detection of Hepatitis C infection in healthy blood donors in a low prevalence area for HCV. In this study, two of the most commonly used methods for Anti-HCV screening, i.e., Electrochemiluminescence Immunoassay (ECLIA) and Chemiluminescent Microparticle Immunoassay (CMIA) were performed among 517 selected healthy blood donors. The clinical performance of ECLIA and CMIA was compared on the basis of their operational variables, i.e., Sensitivity, Specificity, Accuracy, Youden's J index, Positive and Negative predictive values and False discovery, False positive and False negative rate, etc., Both ECLIA and CMIA are highly sensitive (100%) and specific (98%) in terms of anti HCV detection among the blood donors. According to the clinical performance of ECLIA and CMIA, both are efficient in detecting anti-HCV antibodies among the asymptomatic population of healthy blood donors. But as both of them are associated with false positive results, it is recommended to have Polymerase chain reaction on the reactive samples to detect the HCV RNA.

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Keywords Anti- HCV antibody screening · Electrochemiluminescence immunoassay · Chemiluminescence immunoassay · Healthy blood donor

Introduction

Blood transfusion is considered to be among the major treatment modalities for various life-threatening conditions [1]. The international governing body of transfusion medicine, i.e., AABB (American Association of Blood Banks), FDA (Food and Drug Administration) and WHO (World Health Organization) have emphasized in the selection of safe and compatible blood components for the recipient [2]. The initial selection criterion is aimed to choose a healthy blood donor on the basis of history questionnaire and relevant clinical examination to prevent any adverse effect [3]. Among the adverse effects of blood transfusion, Transfusion-Transmitted Infections (TTIs) are reported as a likely complication. TTIs can be caused by Bacteria, Viruses, Parasites and Prions [4]. Therefore, to minimize their risk, a systematic blood transfusion service should be ensured that manages the timely supply of safe blood. World Health Organization (WHO) has recommended blood screening for HIV, hepatitis B, hepatitis C, syphilis and malaria in the Subcontinent region [5].

Hepatitis C Virus (HCV) is one of the major causes of post-transfusion hepatitis leading to morbidity and mortality [6]. It is a viral infection characterized by the inflammation of liver parenchyma [7]. Hepatitis C Virus is a single-stranded enveloped RNA virus belongs to the genus Hepacivirus of Flaviviruses [8]. Seven different genotypes (1–7) have been isolated which are identifiable on the basis of their nucleotide sequence [9]. HCV is composed of a Ribonucleic acid (RNA) core encapsulated

by an icosahedral protein shell and bordered with a lipid envelope [10]. The incubation period of virus ranges from 2 to 12 weeks [11]. The disease is staged in two phases: i.e., acute phase and chronic phase [12].

Globally, more than 185 million people are affected with HCV with a frequency of 2.8%. South East Asia has a moderate prevalence rate from 1.5 to 3.5% [13]. In Pakistan, more than 10 million people are suffering from it that comprises 6% of the Pakistani population [14]. Due to an increasing trend of this viral illness in the region, it is essential to identify the infected individual. Various different methods are used to diagnose Hepatitis C [15, 16]. Some are based on serological testing for Anti HCV antibodies and others detect HCV RNA. Anti HCV antibodies among blood donors are commonly screened by Immunochromatographic Technique (ICT), semiautomatic Enzyme Linked Immunosorbant Assay (ELISA) [15–17], automated Electro Chemiluminescence Immunoassay (ECLIA) or Chemiluminescence Microparticle Immunoassay (CMIA) [18–21]. However, HCV-RNA is commonly detected by Nucleic acid Amplification Testing (NAT) [22] or Reverse Trancriptase Polymerase Chain Reaction (RT PCR) [15]. A number of studies report automated Chemiluminescense Immunoassays like CMIA and ECLIA to be highly sensitive (100%) and specific (98–99%) [18] when compared to ELISA with sensitivity of 78.9% and specificity of 100% [23].

With the scarcity of resources in Pakistan, it is a great challenge for the blood banks to properly screen blood. Therefore, this research was designed to find out the most sensitive and specific commercially available anti HCV screening techniques between the two (CMIA versus ECLIA) which may assist in prompt diagnosis of HCV and thereby decreasing the financial burden on health care centers for unnecessary molecular analysis.

Material and Methods

This multi-center study was conducted in various blood bank organizations of Karachi, Pakistan from January 2015 to January 2016. The study was approved by the Ethics Committee of Baqai Medical University. Five hundred and seventeen healthy individuals were selected as per the recommended criteria of AABB by random sampling.

The following parameters were strictly evaluated for selection, i.e. An age group of 18–50 years with no past history of any major illness, Weight $>$ 50 kg, hemoglobin level $>$ 12.5 g/dL, systolic blood pressure ranges from 100 to 160 mm Hg, diastolic blood pressure of 60–90 mm Hg, pulse between 61 and 99 beats/min and temperature ranges between 95 and 99 °F. Rejection criteria include the following reasons for donor deferral i.e., History of blood donation in the past 12 weeks or known Hepatitis B, hepatitis C or HIV infection, malaria in the past 1 year, any known major illness or individual belongs to the high-risk group, i.e., Intravenous drug abusers, commercial sex workers, homosexuals or history of multiple sex partners.

Study design: Random sampling was carried out to have an actual representation of the test population

Step I: Both voluntary, non-remunerated and replacement blood donors were included in the research as per the defined selection and rejection criteria. Informed written consent was taken from all donors. A unique identification number was assigned to each subject.

Step II: All the blood samples were taken as a part of the normal protocol for screening TTI's. 5 ml venous blood was collected in a plain tube from each donor under all aseptic measures. Serum was then separated by centrifuging the gel tube at 2000 RPM for 10 min. Serum sample was stored in 3 aliquots at $-$ 20 °C for subsequent screening.

Step III: ECLIA is an advanced sandwich immunoassay. It is used for detecting anti HCV antibodies by measuring the emitted light as a result of an electrochemical reaction to the cutoff signal. Electrochemiluminescence Immunoassay (ECLIA) was performed by Cobas e411 (Roche Diagnostics, Germany) as per the recommended protocol of the manufacturer. The results were calculated as COI (Cut off index). Serum with COI $\geq$ 0.9 $<$ 1.0 were considered borderline high and were repeated. Test samples with COI $\geq$ 1.0 were interpreted as reactive with Anti HCV antibodies.

Step IV: CMIA is a two stepped immunoassay using chemiluminescent microparticles. Anti HCV antibodies are determined by comparing the chemiluminescent signal in the reaction to the cutoff signal. Serial anti-HCV Chemiluminescent microparticle assay (CMIA) was carried out on Architect i 2000 SR system (Abbott Laboratories, Abbott Park, Illinois U.S.A). Reactive and nonreactive results were interpreted on the basis of the observed signal to cutoff ratio (S/Co ratio) as defined by the manufacturer. All samples with the S/Co ratio of $\geq$ 1.0 were declared as Anti HCV reactive.

Step V: Following the recommendations by the Centers for Disease Control and Prevention (CDC), all the samples were confirmed for HCV RNA on Real-time Polymerase chain reaction (PCR) by Automated Roche Cobas TaqMan system (Roche Diagnostics, Germany). The calibration and controls of the test were performed as per the manufacturer's recommendations.

Table 1 summarized the characteristic features of Electrochemiluminescence immunoassay by Cobas e 411

Table 1 Comparison of the characteristic features of ECLIA and CMIA

Characteristics	ECLIA	CMIA
Analyzer	Cobas e 411	Architect i 2000 SR
Manufacturer	Roche diagnostics	Abbott diagnostic division
Assay Kit	100 tests	100 tests
Test speed	88 tests/h	200 tests/h
Intra-assay time	18 min	40 min
Principle	Electrochemiluminescence immunoassay	Chemiluminescent microparticle immunoassay
HCV antigens used	Core, NS3 and NS4	Core, NS3 and NS4
Radiolabeled constituent	Ruthenium complex	Anti-human Acridinium
Reaction	Electrochemiluminescence signal/count	Relative light units
Sensitivity*	100%	99.1%
Specificity*	99.8%	99.2%
Results measured as	Cut off index (COI)	Signal to cut off (S/Co) ratio
Interpretation	Nonreactive COI < 0.90	Nonreactive S/Co ratio < 1.0
	Borderline COI $\geq 0.90 < 1.0$	Reactive S/Co ratio ≥ 1.0
	Reactive COI > 1.0	

* As indicated by the manufacturer

and Chemiluminescence microparticle immunoassay by Architect i 2000 SR.

Statistical Analysis

The data were assembled categorically on Microsoft Excel on the basis of the two screening techniques. Comparative analysis between the two screening methods was done in an attempt to determine the most reliable technique for anti/HCV screening. Statistical software MedCalc version 17.9.7 was used to calculate sensitivity, specificity, accuracy, Youden's J index, positive and negative predictive values, false discovery rate and false positive & false negative rate of both immunoassays. The two screening techniques were compared by Chisquare on Statistical package for social sciences (SPSS).

Results

Among the 517 healthy blood donors, 15 were reactive by Architect CMIA. Out of the same 15 reactive samples, only 13 were found to be reactive for Anti-HCV by ECLIA Cobas e411 Remaining of the samples were nonreactive on both the techniques as shown in Table 2.

It was observed that among all the samples which are reactive for Anti HCV, only 8 individuals have detectable HCV RNA by PCR on Cobas TaqMan. The rest of the samples had no detectable HCV RNA. On the basis of PCR, the results were interpreted as true positive, true negative, false positive and false negative that are compared in Table 2 and Figs. 1 and 2. None of the sample was nonreactive for Anti HCV with the two immunoassays and positive at PCR so no false negative case. It is also noticed that higher signal to cut off (S/Co) ratio in CMIA and coefficient index (COI) in ECLIA were highly suggestive of hepatitis C infection as shown in Table 3. The sensitivity of both ECLIA and CMIA was 100%. However, the specificity of ECLIA was 99.02% and CMIA was 98.62%. In terms of detecting the is observed that higher signal to cut off (S/Co) ratio in CMIA and coefficient index (COI) in ECLIA were highly suggestive of hepatitis C infection as shown in Table 3.

Comparison of ECLIA and CMIA is shown in Table 4. The sensitivity of both ECLIA and CMIA was 100%. However, the specificity of ECLIA was 99.02% and CMIA was 98.62%. In terms of detecting the actually infected blood donors (true positive), ECLIA was more efficient than CMIA, as it had a precision of 61.5%. False positive rates were slightly higher in CMIA. Both negative predictive value and false negative rate were highly significant in both the techniques. The false discovery rate was a common issue with CMIA in contrast to ECLIA. ECLIA had a higher diagnostic efficacy with an accuracy of 99.03% than CMIA 98.65%.

F1 score, Matthews correlation coefficient, Youden's J index, degree of freedom and significance of the two methods were tabulated in Table 5. With respect to these variables, ECLIA surpassed CMIA for an effective anti HCV screening.

The study concludes that both ECLIA and CMIA are very competent for anti-HCV detection as per the recommended criteria of FDA for the Subcontinent region.

Table 2 Anti HCV antibody screening by ECLIA and CMIA among healthy blood donors

Anti HCV antibodies	ECLIA	CMIA
Reactive	13 (2.5%)	15 (2.9%)
Nonreactive	504 (97.5%)	502 (97.1%)
PCR based results		
True positive (TP)	8 (1.55%)	8 (1.55%)
False positive (FP)	5 (0.97%)	7 (1.35%)
True negative (TN)	504 (97.48%)	502 (97.1%)
False Negative (FN)	0 (0%)	0 (0%)
Difference	− 1.35%	− 0.97%
95% Confidence interval	− 2.35% to − 0.36%	− 1.81% to − 0.12%
Exact probability	0.0156	0.0625

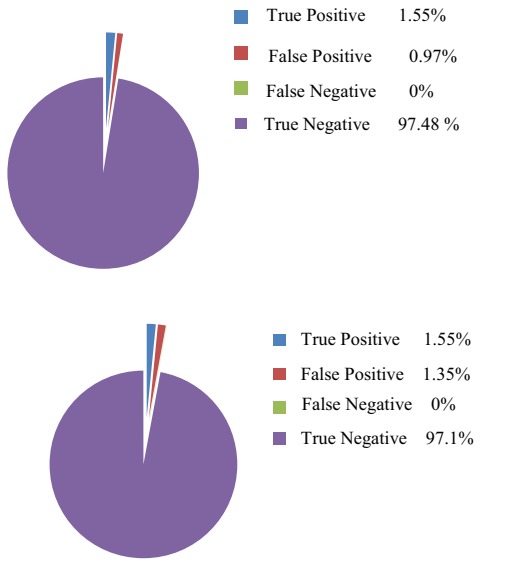

Fig. 1 Screening results of CMIA and ECLIA

However, CMIA shows slightly lower diagnostic efficiency due to increase number of false positive results.

Discussion

Hepatitis C is a serious health issue worldwide [24]. The recently published global epidemiology has narrated an infected population of 170 million people with chronic infection rate of 3% [25]. The prevalence rate of chronic Hepatitis C in the Asia Pacific region is as low as 4% in the less prevalent areas to as high as 12% in regions with high incidence [17]. As the window period for HCV virus is long, several attempts have been made for an earlier detection of Anti HCV antibodies in the affected

Table 3 Correlation of HCV infection status with S/Co ratio of Anti HCV by CMIA (Architect I 2000 SR) and ECLIA (Cobas e 411)

Electrochemiluniescence immunoassay				
Result	COI	Cases	HCV RNA (+)	HCV RNA (−)
Non-reactive	< 1.0	504	0 (0%)	504 (100%)
	1.0–200	3	1 (33.3%)	2 (66.6%)
Reactive	200–400	7	4 (57.1%)	3 (42.8%)
	> 400	3	3 (100%)	0 (0%)
Chemiluminescent Microparticle Immunoassay				
Result	S/Co ratio	Cases	HCV RNA (+)	HCV RNA (−)
Non-reactive	< 1.0	502	0 (0%)	502 (100%)
	1.0–3.0	4	1 (25%)	3 (75%)
Reactive	3.0–10.0	8	4 (50%)	4 (50%)
	> 10.0	3	3 (100%)	0 (0%)

individual. Initially in 1990, HCV recombinant antigen derived 1st generation Enzyme Immunoassay was introduced to detect the antibodies directed against non-structural HCV protein NS4 and recombinant c100-3 [26]. With new advances, the 2nd generation Anti-HCV ELISA was launched with additional NS5 and c 22-3. This was approved in 1992 by Food and Drug Administration (FDA) for screening HCV infection [27]. Later, advanced Chemiluminescence Assays were launched that had captured the market very rapidly because of their higher sensitivity and specificity rate [19].

This study was undertaken with the objective of determining a screening technique that is able to detect the positive results efficiently in order to have no false negative results. Simultaneously the chances of a false positive result should also be minimized in the technique so that the infected individual can be isolated from the normal individuals. In addition to this, a cost effective Anti-HCV technique can be determined that can decrease the load of

Table 4 Evaluation of the diagnostic efficacy of ECLIA and CMIA, the two automated chemiluminescence immunoassays for the detection of Anti HCV antibodies among blood donors

Statistic	ECLIA		CMIA	
	Value	95% CI	Value	95% CI
Sensitivity	100.00%	63.06–100.00%	100.00%	63.06–100.00%
Specificity	99.02%	97.72–99.68%	98.62%	97.19–99.45%
Accuracy	99.03%	97.76–99.69%	98.65%	97.23–99.45%
Positive likelihood ratio	101.8	42.55–243.53	72.71	34.84–151.75
Negative likelihood ratio	0.00	–	0.00	–
Hepatitis C prevalence	1.55%	0.67–3.03%	1.55%	0.67–3.03%
Positive Predictive Value	61.54%	40.08–79.29%	53.33%	35.38–70.46%
Negative Predictive Value	100%	–	100%	–
False positive rate	0.98%	0.13–1.83%	1.38%	0.37–2.39%
False negative rate	0%	–	0%	–
False discovery rate	38.46%	34.27–42.65%	46.67%	42.37–50.97%

Table 5 Comparative analysis of the clinical performance of CMIA and ECLIA for the screening of Anti HCV antibodies among healthy blood donors

Clinical performance of ECLIA and CMIA		
Clinical performance	CMIA	ECLIA
Youden's J statistics	0.98	0.99
F1 Score	0.695	0.761
Matthew's correlation coefficient	0.725	0.781
Chi square	458.741[a]	466.308[a]
Degree of freedom ( )	1	1
Asymp significance	0.000	0.000

molecular laboratories for unnecessary PCR or NAT testing. So a comparative analysis was conducted between ECLIA (Cobas) and CMIA (Architect) among the low prevalent population for HCV i.e., healthy blood donors.

The ECLIA preference among another competitor is well documented in various studies [18–21, 28]. Yoo et al. [28] has strongly recommended the use of ECLIA because of its high sensitivity, i.e., 100% and specificity 99.6%. A similar study was conducted by Kim et al. [18] that again concluded very high sensitivity of ECLIA and CMIA i.e., 100%, but variation was observed between the clinical specificity of ECLIA i.e., 98.2% and Architect 98.8%. Another multicenter study was published by Schmidt et al. regarding the comparison of ECLIA and other immunoassays. The study emphasizes on the use of ECLIA for determining the infectious status of blood donors [20]. Sharma et al. [21] also emphasized on the usage of automated immunoassays for anti HCV antibodies.

In our study, it was observed that both of the immunoassays were effective in detecting Anti-HCV with 100% sensitivity and > 98% specificity. However, ECLIA technique was slightly superior due to less false positive results. The false positive results in immunoassays may be justified by the following mechanisms. Firstly, as naturally occurring structural determinants has not been isolated from HCV and recombinant antigens are being used for testing hence this increases the false seropositivity [19]. Secondly, ECLIA characteristically detect Anti HCV antibodies earlier, sometimes within the window period when the individual is asymptomatic [28]. Thirdly, in Occult HCV infection (OCI), the immune system becomes activated to generate anti-HCV, but because of the very low concentration of HCV RNA in the serum, it remains undetectable by PCR [29]. Fourthly, persistent Anti-HCV antibodies in the serum despite of undetectable HCV RNA can be a result of the self-resolution of the viral illness in a few individuals or it may be due to the presence of anti-HCV in previously treated hepatitis C patients [30].

Although, CDC guidelines have recommended the use of a Recombinant Immunoblot Assay (RIBA) as a supplementary technique for positive results, the presence of HCV RNA is confirmed by either Nucleic acid Amplification Testing (NAT) or Polymerase Chain Reaction (PCR) [31]. This is also relevant to detect the false positive results which are again a big issue with various commercially available immunoassays. This overburdens the health care centers for managing a noninfectious individual.

Conclusion

The study concludes that both ECLIA by Cobas e411 and CMIA by Architect are equally sensitive, i.e., 100% in detecting the infectious status in an asymptomatic individual. Despite, 100% sensitivity and equally high specificity (ECLIA is slightly better as compared to CMIA),

NAT/PCR studies have to be done on all positive results due to high false positivity rate of both the methods.

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Compliance with Ethical Standards

Conflict of interest All the Authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

Ethical approval All procedures performed in the study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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