Original Article

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Evaluation of Performance Characteristics of Enzyme Chemiluminescence Immunoassay (ECLIA) and Rapid Diagnostic Test (RDT) for HBV, HIV and HCV Infections

Microbiology Section

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ABSTRACT

Introduction: The incidence of Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) in India has increased over the past two decades. These infections cause significant mortality and morbidity. This increasing trend is alarming and is emerging as a global health problem. India has one of the largest reservoirs of HIV, HBV and HCV and many of its cases remain undetected. Due to the large sero-prevalence in India, serological tests for HBsAg, anti-HCV and anti-HIV are done for diagnosing the respective diseases, and screening of antenatal, preoperative cases and of blood donors.

Aim: To find out the efficacy of Rapid Diagnostic Test (RDT) and Enzyme Chemiluminescence Immunoassay (ECLIA) in comparison to Enzyme Linked Immunosorbent Assay (ELISA) for diagnosis of HBV, HCV and HIV infections in patients.

Materials and Methods: This cross-sectional study presented a comparative analysis of test results of HBsAg, anti-HCV and anti-HIV antibodies by different methods namely RDT and ECLIA taking ELISA as reference standard. A total of 198 serum samples were taken from patients and tests were done for HBsAg, anti-HCV and anti-HIV by three different methods i.e. ECLIA, RDT and ELISA and sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and accuracy were calculated. The agreement between the results was computed using Kappa coefficient.

Results: The findings demonstrated that for HBsAg, the results of RDT and ECLIA were same. For anti-HIV 1 and 2, sensitivity of RDT and ECLIA was similar. For anti-HCV, ECLIA showed better sensitivity than RDT and RDT showed better specificity than ECLIA. Strength of agreement was almost perfect for HBsAg and anti-HIV, where as for anti-HCV it was substantial.

Conclusion: There were some variations in certain results by different methods. It was observed that RDT and ECLIA are good screening tests for HIV and HBV infections. On the other hand, we inferred that for HCV, ECLIA is a better screening test than RDT.

Keywords: Antenatal, Enzyme linked immunosorbent assay, Hepatitis, Negative predictive value, Positive predictive value, Serological tests, Sensitivity, Specificity

INTRODUCTION

The incidence of HIV, HBV and HCV in India has increased over the past two decades and is emerging as a global health problem. These infections cause significant mortality and morbidity [1]. These infections can also be acquired through blood transfusion. Data in India suggests that, about 0.36% of the population is HIV infected, 2.4% (extending to 15.9% in certain population groups) is HBV infected and 1.2% is HCV infected [2]. In the present scenario there are about 21.17 lakhs HIV cases, 50 million HBV cases and 12-18 million HCV cases within the country [3-5]. Hence, India, being a population of 1.2 billion has one of the largest reservoirs of HIV, HBV and HCV and many of its cases remain undetected [2,6]. Due to the large sero-prevalence in India, serological tests for HBsAg, anti-HCV and anti-HIV are done for diagnosing the respective diseases, antenatal and preoperative screening. When the prevalence of these blood borne infections is 1 in 1000 (0.1%) or more in the general population, screening tests are advisable [7].

Owing to resource constraints, in a country like India, rapid card tests based on immunochromatography methods only are used to screen these viral infections in most of the laboratories, both in private and public sector. It has been found that rapid immuno-chromatographic kits for HBsAg and HCV have only limited efficacy and should be backed by superior methods like Enzyme Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR) [8]. For HIV infection the performance of Rapid Diagnostic Tests (RDT) in comparison to ELISA is suboptimal and RDT based serial testing algorithm cannot parallel the testing accuracy of an ELISA based approach [9]. Hence, it is required to formulate an integrated strategy for India.

Another method is emerging globally for screening of these viral infections, namely Enzyme Chemiluminescence Immunoassay (ECLIA) which is a recent FDA approved method for detecting HBsAg, anti-HCV and anti-HIV antibodies for its higher sensitivity and specificity [8,10].

There are very few studies in India evaluating the role of ECLIA to screen HBV, HIV and HCV infections in patients and blood donors [11,12]. Due to better testing output and objective interpretation of results, most of the laboratories in developed countries have adopted this method. But due to expensive instrumentation, use of this method in India is limited.

The study aimed to assess the performance characteristics of a fully automated rapid immunodiagnostic chemiluminescence system Vitros®ECi (Ortho diagnostics Ltd.,) and RDT for screening of HBs Ag, HCV antibodies and HIV antibodies in terms of sensitivity, specificity and accuracy compared to ELISA. RDT uses the immunochromatographic method to detect HBsAg, anti-HCV and anti-HIV antibodies. ELISA uses combination of the specificity of antibodies or antigens with the sensitivity of enzyme assays to detect HBsAg, antibodies to HCV and HIV 1 and 2 in human serum. These antigen antibody reactions occur in a coated microwell with a positive and negative control being run with each batch of tests following the principle of 'Sandwich ELISA'. ELISA was validated

by the acceptance criteria laid down by the manufacturers. ECLIA uses sophisticated hardware and software and enhanced chemiluminescence detection technology to process samples.

MATERIALS AND METHODS

This cross-sectional pilot study was done at Department of Microbiology of a tertiary care medical college in West Bardhhaman district of West Bengal, India for a period of four months from November 2016 till February 2017. One hundred and ninety-eight blood samples during aforementioned period were collected from inpatient and outpatient in plain vacutainers and were received at microbiology laboratory and information documented. Serum was immediately separated from blood and subjected to respective tests and results were recorded. Purposes of doing the tests were also recorded as diagnosing diseases, antenatal and preoperative screening. HIV pre-test consent was taken according to NACO guidelines [13]. Demographic details of all the patients were noted. Study was initiated after permission from the Institutional Ethics Committee.

Inclusion Criteria: All those with unknown serological tests giving consent were included in the study.

Exclusion Criteria: All those who declined their consent and those who were already infected were excluded from the study.

All samples were tested by three different methods i.e., ECLIA by Vitros®ECiQ/ECi Immunodiagnostic Systems (Ortho Clinical Diagnostics Ltd), ELISA (manufactured by J.Mitra Diagnostics fourth Generation Microlisa-HIVAg & Ab kit for HIV, J.Mitra Diagnostics Hepalisa kit for HBsAg ELISA, J.Mitra Diagnostics 3rd generation HCV Microlisa kit for HCV) and RDT (J mitra & Co. Ltd.,) for HBsAg, anti-HCV and HIV antibodies. The validity of the ELISA tests was assessed by means of acceptance criteria which were laid down by the manufacturer for the absorbance of the reagent blank as well as for the mean absorbance of the positive and negative controls which were supplied with the test kits. The cut off value for reporting the positive results was calculated as per the manufacturer's directions. Both known positive and negative controls were used as the external controls. Sensitivity, specificity, PPV, NPV and diagnostic accuracy were calculated based on the results of the tests taking ELISA as gold standard [14-17]. In Vitros®ECiQ/ECi Immunodiagnostic Systems (Ortho Clinical Diagnostics Ltd.,) for HBsAg, anti-HCV and anti-HIV antibodies, sample with signal cut off ratio less than 0.80 was considered non-reactive. Sample with signal cutoff ratio more than 1 was considered reactive and sample with signal cut-off ratio from 0.80 to 1 was considered indeterminate [11]. Indeterminate samples were tested in duplicate.

STATISTICAL ANALYSIS

Collected data were compiled on Microsoft excel worksheets (Microsoft Office Excel 2007, Redwoods, WA, USA). Validity of the tests like RDT and ECLIA was expressed by sensitivity and specificity by taking ELISA as gold standard. Kappa co-efficient was also computed to see the extent of agreement between the values of two different methods beyond which we would expect by chance alone. Cohen's kappa coefficient (k) was calculated using a free tool available from Graph Pad software website [18]. A p-value <0.001 was considered as statistically significant.

RESULTS

Among 198 patients 114(58%) were male and 84(42%) were female. The population comprising patients aged between 10 to 85 years is shown in [Table/Fig-1]. The purpose of doing the tests was documented and they are shown in [Table/Fig-2]. In most of the cases (53.5%), reasons for doing the tests were not mentioned in the requisition forms.

RDT, ECLIA and ELISA were done for all 198 samples for HBsAg, HIV 1and 2 antibodies and HCV antibodies taking ELISA as gold standard. Sensitivity, specificity, PPV, NPV, accuracy and kappa coefficient were calculated and are tabulated in [Table/Fig-3].

Age Groups	Number of cases	Percentage
≤20 yrs	14	7.1
21-30 yrs	42	21.2
31-40 yrs	24	12.1
41-50 yrs	50	25.3
51-60 yrs	34	17.2
61-70 yrs	24	12.1
>70 yrs	10	5.1
Total	198	100%
Mean±SD	43.62±17.50	

[Table/Fig-1]: Age distribution of patients.

Purpose of testing	Number	Percentage
Antenatal screening	12	6.1
Symptomatic	44	22.2
Preoperative screening	36	18.2
Purpose of screening not known	106	53.5

[Table/Fig-2]: Purpose of doing HBsAg, anti-HIV and anti-HCV tests.

Our findings demonstrated that for HBsAg sensitivity, specificity and accuracy for RDT and ECLIA were same. For anti-HIV 1 and 2, RDT showed 100% accuracy and ECLIA showed 99% accuracy. For anti-HCV, ECLIA by ECiVitros showed better sensitivity than corresponding RDT (Sensitivity100% versus Sensitivity 66.7% respectively). On the contrary, RDT showed better specificity than ECLIA by ECiVitros (Specificity 98.9% versus Specificity 94.6% respectively).

Kappa co-efficient was also computed to observe to what extent the reading of two different methods (RDT and ECLIA) for HBsAg, anti-HIV1 and 2 and anti-HCV agreed beyond which we would expect by chance alone. Strength of agreement for HBsAg by RDT and ECLIA was almost perfect (0.88). Again, strength of agreement for RDT and ECLIA for anti-HIV 1 and 2 and anti-HCV were also substantial [19]. All results were documented according to the kit manufacturers' directions.

DISCUSSION

In developing countries use of RDT as the first line screening assay for HBV, HCV and HIV is very much in use. The present study attempted to compare the test results of RDT and a newer technique (ECLIA) as screening tests for these infections. In the study 114(58%) patients were male and 84(42%) patients were female. The maximum number of patients belonged to 41-50 years age group [Table/Fig-1]. Most of the studies with a similar scope has emphasised only on evaluating different techniques like RDT, ECLIA and ELISA [12,16].

Currently, the information of use of chemiluminescence (ECLIA) technology for the detection of HBsAg, anti-HIV 1&2 and anti-HCV in patients is limited [11]. For screening of HBsAg, HIV and HCV antibodies, RDT are routinely used in developing country like India. In the study, we attempted to evaluate for the first time, the role of ECLIA along with RDT in screening of these infections in patients of this region with ELISA as gold standard. On the other hand, few similar studies have been done for screening of these infections in the blood donor group [16,20].

In the study, report of test results for HIV was done as per NACO guidelines [13]. We observed sensitivity of 80% and specificity of 100% by ECLIA and RDT respectively for HBsAg.

Sensitivity of 100% and specificity of 94.6% by ECLIA vs. sensitivity 66.7% and specificity 98.9% by RDT was observed for anti-HCV. Sensitivity of 100%, specificity of 99% by ECLIA and sensitivity and

Tests	True Positive	True Negative	False Positive	False Negative	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %	Cohens kappa coefficient	p-value
RDT (HBs Ag)	16	178	0	4	80	100	100	97.8	98	0.878	<0.001
ECLIA (HBsAg)	16	178	0	4	80	100	100	97.8	98	0.878	<0.001
RDT (anti-HIV 1&2)	6	192	0	0	100	100	100	100	100	1	<0.001
ECLIA (anti-HIV 1&2)	6	190	2	0	100	99	75	100	99	0.852	<0.001
RDT (anti-HCV)	8	186	2	4	66.7	98.9	80	97.9	97	0.711	<0.001
ECLIA (anti-HCV)	12	186	10	0	100	94.6	54.5	100	94.7	0.681	<0.001

[Table/Fig-3]: Sensitivity, Specificity, Accuracy and Kappa coefficient of different tests.
PPV: Positive Predictive Value; NPV: Negative Predictive Value; RDT: Rapid Diagnostic Test; ECLIA: Enzyme Chemiluminiscence Assay; HBsAg: Hepatitis B surface antigen; HIV: Human Immunodeficiency Virus; HCV: Hepatitis C Virus

specificity both 100% by RDT was recorded in case of anti-HIV. These results are also documented in [Table/Fig-3]. In another study Ismail N et al., observed sensitivity 97.4% and specificity 100% for HBsAg done by ECLIA. Results of the current study showed a little lower sensitivity but same specificity for HBsAg. Ismail N et al., also mentioned sensitivity of 100% and specificity of 98.9% for anti-HCV by ECLIA performed by ECiVitros (Ortho diagnostics). This result for anti-HCV also showed similar sensitivity and specificity by ECLIA [21].

There is no false negative result for anti-HCV by ECLIA but four false negative tests were found by RDT. Due to the findings it can be concluded that RDT has poor specificity as compared to ECLIA for screening of HCV infections. Hence, RDT may miss some HCV infection. As a result use of only RDT for screening of blood donors and patients for transfusion purposes and for antenatal cases, may result in alarming consequences. It is also concluded that RDT may not be a suitable screening test for anti-HCV antibodies.

Regarding anti-HIV, our study revealed 100% sensitivity and 100% specificity by RDT which was comparable with ECLIA which showed 100% sensitivity and 99% specificity. Khan JK et al., mentioned in their study that there was around 50% less sensitivity with rapid kits as compared to ELISA for anti-HIV and HBsAg [8]. However, we observed that both ECLIA and RDT gave good sensitivity and specificity for HBsAg and anti-HIV.

It was also noticed that if cut off/OD was high for the tests by ECLIA and ELISA then the results were reactive by RDT also. But, when OD level was only marginally high in ELISA, it was observed that both RDT and ECLIA showed negative or nonreactive result. This type of finding for ECLIA is unlikely as ECLIA is considered a more sensitive test than ELISA [22,23]. Ideal screening tests should show high degree of PPV and low degree of false negative results. Taking ELISA as gold standard diagnostic test may be the cause of such finding. In such cases it is advisable to confirm the discrepant results by other specific tests.

The essence of this study was to enlighten the poor resource countries that rapid test kits as sole diagnostic test for detection of HBsAg and HCV should be interpreted with caution both in patients and in donors and role of ELISA as gold standard should be re-evaluated [23].

It is suggested that hospitals and blood banks which can provide for ECLIA should prefer it over other test procedures particularly for anti-HCV because it is rapid, highly sensitive, user friendly and quality assured. The inbuilt monitoring system in ECLIA by Vitros ECi assures that proper calibration and running of control a 'must do' before running the tests. This ensures maximum reliability and minimum subjective errors.

LIMITATION

One of the limitations of the study was that confirmatory tests like RIBA and qualitative nucleic acid testing for HCV, Western blot for HIV, anti-HBc antibody and HBV DNA for HBV infection could not be done owing to resource constraint [9,24-27]. Clinical follow up of reactive cases and staging of the disease was not done.

CONCLUSION

In the study, it was concluded that both RDT and ECLIA are good screening tests for HIV infection. For HCV infection, ECLIA is a better screening tool than RDT. Regarding HBsAg, RDT should be used in resource limited areas, but in resourceful areas ECLIA should be adopted as the preferred method of screening test as it is fully automated and requires minimum technical expertise. The role of ELISA as reference standard may also be re-evaluated with Nucleic Acid Amplification Technique (NAT). It is also stressed that ECLIA is more rapid and user friendly than ELISA.

In case of discrepant test results performed by different methods, each laboratory should develop a protocol to interpret and confirm the results in consultation with clinicians so as to serve the patient with the most accurate and standardised reports.

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