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REPORTING OF LOW REACTIVE ANTI-HEPATITIS C ANTIBODY SAMPLES BY CHEMILUMINESCENCE IMMUNOASSAY: A DIAGNOSTIC DILEMMA

KEY WORDS: Anti-HCV antibody, Chemiluminescence immunoassay (CLIA), Diagnostic dilemma, False positive, Hepatitis-C

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ABSTRACT

Background: Viral hepatitis due to hepatitis C virus (HCV) is a leading communicable disease exhibiting an increasing trend of morbidity and mortality. Chemiluminescence immunoassays (CLIA) aid in early detection of anti-HCV antibodies as it covers more antigenic spectrum. However, this has increased the rate of false positivity requiring further confirmation with more specific tests. Resource poor settings without confirmatory tests often face a diagnostic dilemma lacking an objective conclusion. **Aim:** To analyse the occurrence and reporting of low reactive anti-HCV samples by CLIA. **Methods:** A laboratory-based study was conducted from January-August 2024 analysing anti-HCV antibody samples by CLIA. Low reactive samples (signal for test sample/cutoff value <10) were subjected to repeat CLIA from same sample, repeat CLIA from fresh sample (if available), testing by rapid immunochromatographic tests (ICT), and liver function tests (LFTs). Prior history of exposure was also taken. Collected data was entered in the MS Excel and analysed. **Results:** During the study period, a total of 5512 samples were received out of which 101 (1.8%) samples came low reactive. Of these, 40.6% had previous history of reactivity, repeat CLIA on the same sample was reactive in 46.5%, fresh sample was available in 7.9%, LFTs were deranged in 26.7%, ICT were reactive in 21.8%, and 57.4% patients were on dialysis. Final reporting was done using taking into consideration various factors of patient profile. **Conclusion:** Reporting of low reactive samples remains a challenge especially in resource poor settings where molecular facilities are scarcely available. Presently, we recommend reporting after subjecting the sample to at least two different diagnostic assays to increase the chances predicting Hepatitis-C infection.

INTRODUCTION

Hepatitis C virus (HCV) is a single stranded, positive-sense, enveloped, RNA virus, and belongs to Flaviviridae family. It can cause progressive liver disease such as chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. According to the Global Hepatitis Report of 2024 by World Health Organization (WHO), about 1.3 million people died of viral hepatitis of which 17% were caused by HCV alone. It is astonishing to know that between 2015-2022, only 36% of the people living with HCV have been diagnosed and 20% have received curative treatment. India is among the top 10 focus countries and accounts for 2/3rd of global HCV burden.[1]

HCV Diagnostic Modalities

HCV infection is mainly detected through anti-HCV antibodies in serum samples. This can be achieved through various screening methods such as rapid immunochromato graphic tests (ICT), chemiluminescence immunoassays (CLIA), and enzyme linked immunosorbent assays (ELISA). Confirmation of positive result is done through more specific assays such as RNA polymerase chain reaction (PCR), recombinant immunoblotting assay (RIBA), and real time PCR for viral load estimation.[2] CLIA being a third-generation test has an advantage of early detection of anti-HCV antibodies (7-8 weeks after infection) as it covers a greater antigenic spectrum (configured core, NS3, and NS5 antigens). Additionally, it is more precise, reliable, fully automated, less prone to contamination, easy to operate, has short turnaround time, and a greater positive predictive value than conventional enzyme immunoassays.[3] However, it has its own shortcomings such as increased rate of false positivity requiring further confirmation with more specific tests.[4,5]

Problem At Hand

Current recommendations suggest confirmatory testing of all positive HCV antibody samples.[4] However, resource poor settings cannot afford molecular confirmation of all such samples increasing the diagnostic as well as clinical dilemma. Additionally, it puts financial as well as psychological burden on the patient for periodic laboratory testing. In view of the above, this present study was conducted to analyse the occurrence of low reactive anti-HCV samples by CLIA and suggest a possible reporting strategy.

MATERIALS AND METHODS

Study Design

A retrospective laboratory-based study was conducted in the department of microbiology in a tertiary care hospital from January to August 2024 with the objective of analysing anti-HCV antibody samples by CLIA methodology in VITROS® ECiQ Immunodiagnostic assay system (Ortho Clinical Diagnostics, Felindre Meadows, Pencoed, Bridgend, UK).

Study Population

Samples from all inpatient wards and outpatient clinics were analysed. All HCV reactive samples with CLIA value < 10 (signal for test sample/cutoff value) were included and samples with values >10 were excluded from the study.

Study Procedure

Serum samples were received from all outpatient and inpatient department settings for routine viral marker testing. The manufacturer's instructions were followed and protocol described was used for loading of reagent pack, signal reagent, calibration programming, programming of controls, programming of samples, and reviewing of results. The results were calculated automatically by the instrument.

Low reactive anti-HCV antibody samples (<10) were subjected to repeat CLIA from same sample, repeat CLIA from fresh sample (if available), and rapid ICT. Results for liver function tests (LFTs) were recorded. Prior history of reactivity and other exposures (dialysis, blood transfusion, intravenous drug abuse, high risk behaviour, invasive procedures) were also taken.

Quality Control

Internal positive and negative controls were run daily before commencing processing of clinical samples in the instrument.

Data Analysis

Collected data was entered in the MS Excel and analysed. Data was presented in the form of tables.

RESULTS

From January-August 2024, 5512 samples were received out of which 101 (1.8%) came low reactive. Table 1 shows that 40.6% had previous history of reactivity, repeat CLIA was reactive in 46.5%, fresh sample was available in 7.9%, LFTs were deranged in 26.7%, rapid ICT was reactive in 21.8%, and 57.4% patients were on dialysis (both in house and outside our facility). Table 2 shows the demographic characteristics

of low reactive HCV samples. Most of the patients were from outpatient departments (75.2%) predominantly nephrology (65.3%).

Table 1: Showing The Percentage Of Reactive Results In Various Strategies Adopted For Reporting Low Reactive HCV Samples (N=101)

S. No	Parameter	Positive finding N (%)
1	Repeat CLIA same sample reactive	47 (46.5%)
2	Rapid ICT reactive	22 (21.8%)
3	Repeat CLIA from fresh sample reactive	8 (7.9%)
4	Previous history of reactivity	41 (40.6%)
5	Deranged LFTs	27 (26.7%)
6	Patient on Dialysis and other exposures	58 (57.4%)

Table2: Showing Demographic Characteristics Of All Low Reactive HCV Samples (N=101)

S. No	Demographic characteristics	Positive finding N(%)
OPD/IPD		
1	Outpatient department	76 (75.2%)
2	Inpatient department	25 (24.7%)
DEPARTMENT		
3	Nephrology	66 (65.3%)
4	Cardiology	14 (13.8%)
5	Gastroenterology	13 (12.8%)
6	Neurology	8 (7.9%)
CO-INFECTION		
7	Coinfection with Hepatitis-B	2 (1.98%)

DISCUSSION

During the natural course of HCV infection, a wide spectrum of serological antibody changes are observed. Higher antibody levels are observed with greater viral stimulation.[6] As per European Union Standards, anti-HCV assays should have 100% sensitivity and 99.5% specificity for market approval.[7] We have reported 1.8% low reactive anti-HCV samples in our study. False positivity from CLIA as high as 6.25% has been reported in literature.[2] A study proposed that antibody threshold set at a signal to cut off (S/CO) ratio of 4.5 differentiates the samples that do not require additional confirmatory tests.[4] In our study, 23.7% showed CLIA values between 7-9, 21.7% were between 4-6, 50.4% were between 1-3, and 3.9% were <1. Various studies have demonstrated that false positivity is higher in low prevalence settings where confirmatory tests are often required. [8, 9] However, testing of reactive samples by two different assays might replace the need to undergo confirmatory testing by expensive molecular tests.[10] Co-relation of clinical findings and laboratory results has been attempted in literature for discordant HCV samples with repeat CLIA advised after 2-4 weeks.

False positivity can be due to cross reactive circulating antigens and antibodies with certain conditions such as nephrotic syndrome, pregnancy, hepatitis-B virus infection, herpes simplex virus infection, human immunodeficiency virus (HIV), autoimmune diseases, allergies, immunoglobulin administration, and influenza vaccination. [11-13] We reported 2 (1.98%) cases of Hepatitis-B co-infection in our study. Additionally, CLIA might be subjected to interferences from high background, short light emitting process, and matrix effects (high levels of proteins or lipids in sample) which increase the chances of false positivity. Lot-to-lot variability in calibrations and controls due to reagent change, high instrument failure rate, limited shelf life of chemiluminescent substrates, prevalence of analyte, technical incompetence, and improper cleaning of instrument may also contribute to increased false positivity.[14]

Only 58 (57.4 %) patients were on dialysis in our study with most having a history of dialysis outside our facility. Prevalence of HCV infection among haemodialysis patients has been widely analysed with seroconversion rate ranging from 1-53% depending on country-to-country data.[15] Low reactive samples in our set up are reported based on various strategies along with interpretative comments such as confirmation by molecular tests.

India has recently launched National Action Plan in 2018 with the aim of eliminating HCV infection by the year 2030. Under its umbrella, HCV screening, diagnosis, and treatment would be made available at all government healthcare facilities free of cost. Three Indian states including Delhi have initiated Hepatitis C Elimination Through Access to Diagnostics (HEAD- Start) project with the dual objective of filling the diagnostics gap and generating ample evidence for healthcare policy decisions both at state and the national level.[16] There is a dire need for scaling up of one-stop shop models where all the diagnostic facilities including screening and confirmation are made available to the patient at a single healthcare facility.

All the patients could not be followed up to predict the course of natural infection or decay in antibody levels which could be a possible limitation of the study.

CONCLUSION

Although CLIA has numerous advantages as a diagnostic assay but its pitfalls of false reactivity especially in viral marker reporting poses a diagnostic dilemma and is a hindrance to arrive at an objective conclusion. Reporting of low reactive samples remains a challenge especially in resource poor settings where molecular facilities are scarcely available. Access to high quality HCV testing diagnostics with simplified algorithms is the need of the hour. At present, we recommend reporting by at least two different diagnostic assays to increase the chances predicting Hepatitis-C infection. Knowledge of patient history can also aid in arriving at a definitive conclusion.

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None

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