ORIGINAL ARTICLE
COMPARISON OF HEPATITIS C ANTIBODY ASSAYS AND EVALUATION OF AGREEMENT BETWEEN HEPATITIS C ANTIGEN AND PCR RESULTS

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Background: Hepatitis C is associated with a wide range of health repercussions. Pakistan is one of the highly prevalent countries of Hepatitis C Virus (HCV) infection. The availability of cost-effective, robust, and reliable screening and diagnostic tests for hepatitis C is important to address the disease burden. Standardization of screening and diagnostic assays in clinical laboratories is crucial for achieving big goals. Objectives of this study are to correlate the results of two different HCV antibody (HCV Ab) assays and to examine the correlation of HCV core antigen (HCV c Ag) results with HCV PCR for HCV infection diagnosis. **Methods:** This descriptive cross-sectional study was carried out from November to December 2020 at Dow University of Health Sciences. Total number of 40 HCV Ab samples were analysed by both chemiluminescence (CMIA) and electrochemiluminescence (ECLIA) immunoassays. Tests for HCV RNA PCR and HCV c Ag were performed on all samples. Results of screening and diagnostic assays were correlated and agreements were examined. Statistical analysis for agreement was carried out by using R software version 3.6.3 through AC1 Gwetz Statistic. The study was approved by the institutional ethical review committee. **Results:** An agreement of 0.73 and 0.95 was found between two different HCV Ab immunoassays and HCV c Ag and HCV PCR, respectively. **Conclusion:** We found a good correlation between CMIA and ECLIA for HCV Ab. An excellent correlation was found between HCV c Ag and HCV PCR. Based on our study findings, HCV c Ag is a candidate test for the diagnosis of active HCV infection.
Keywords: HCV antibodies; Laboratory diagnosis; HCV Core Antigen; HCV PCR

INTRODUCTION

Hepatitis C is associated with a wide range of health repercussions. The worldwide prevalence of hepatitis C is around 2.5%.[1] An increased prevalence is observed in developing countries.[2] In Pakistan hepatitis C, prevalence is observed as 5–8 %.[3]

World health organization (WHO) has set the target of 2030 for the global eradication of hepatitis C.[4] To meet the timeline, the burden of the disease is imperative to know, especially in high prevalent countries like Pakistan. The availability of cost effective and reliable screening and diagnostic tests for hepatitis C is important to proceed for the disease treatment.[5] Centre of disease control (CDC) recommends HCV antibody (HCV Ab) as a first line screening blood test for HCV infection. Only FDA approved assays are recommended by CDC to use for the screening of HCV Ab with the maximum (>95%) positive and negative percent agreements.[6] No further testing is required if HCV Ab is found negative. However, false negative HCV Ab results may be found in immune-compromised patients or in very early infection when antibodies may not be detectable. The period during which antibodies are not detectable instead of infection is called window

period. To overcome the false negative results during window period, advanced immunoassays have been developed to detect the antibodies at a very early stage. Currently, with the use of 3rd and 4th generation HCV Ab assays, the window period has been reduced to approximate 8–3 weeks respectively.[7] On the other hand, the presence of HCV Ab is indicative of infection (active or chronic) or past resolved hepatitis C infection and required to be confirmed. A recheck HCV Ab test with any other methodology is recommended to endorse the seropositive result before any diagnostic testing. Many HCV Ab tests are available based on different methodologies by manufacturing companies. The sensitivity and specificity of screening tests are very important to pick the true cases for further workup.

HCV RNA is considered as a gold standard and reliable confirmatory test for HCV infection.[8,9] In studies, HCV core antigen (HCV c Ag) is found promising for the diagnosis of active viral infection and may be used as a reflex test to confirm acute infection in seropositive patients.[10,11]

The purpose of this study is to compare the HCV Ab results performed by two different methodologies, CMIA and ECLIA, and to determine the

correlation of HCV c Ag and HCV PCR. Agreement between the antibody's assays may provide an opportunity to use them alternative to each other in the clinical laboratory. Similarly, a substantial agreement of HCV c Ag with HCV RNA may provide a cost-effective, robust and reliable assay to replace the PCR. The rationale of this study is to highlight a cost effective and efficient approach to diagnose Hepatitis C. Establishing single methodology set ups in the small-scale laboratories located in far flung areas may be helpful for prompt screening and diagnosis of Hepatitis C among Pakistani population.

MATERIAL AND METHODS

This was a descriptive cross-sectional study, carried out between November to December 2020, at the sections of Chemical Pathology and Molecular Pathology, Dow international Medical College of Dow University of Health Sciences, Karachi. Through nonprobability purposive technique, 40 HCV Ab positive results were selected from the laboratory information system. Sample size of 40 was adapted from approved Clinical and Laboratory Standards Institute guidelines for method comparison.[12] These results were generated from Abbott Architect i2000 based on chemiluminescence immunoassay. The respective samples were separated and sera were stored at - 40˚ C for further analyses. All seropositive results were rechecked for HCV Ab with electrochemiluminescence immunoassay on Roche e411. Chemiluminescence and electrochemiluminescence were considered as primary and secondary HCV Ab immunoassays, respectively.

All samples were analysed for HCV PCR and HCV c Ag tests. Abbott Architect i2000 and Roche Cobas 6800 were used for HCV c Ag and PCR testing, respectively. Characteristics of all assays and result interpretations are described in Table 1. Data was compiled and analysed on SPSS version 20. Gender and age of the subjects were recorded. Frequencies, percentages and median with inter quartile range (IQR) were calculated for gender and age, respectively. HCV Ab results of two different assays were compared and absolute number and frequencies were calculated. Agreement between qualitative results of two different HCV Ab assays and HCV c Ag and HCV PCR was examined by AC1 Gwetz Statistic, by using R software version 3.6.3. Correlation was determined as poor, fair, good and excellent if values were found as <0.4, 0.4-0.6, >0.6-8.0 and >8.0-1.0, respectively.[13] Diagnostic specificity, sensitivity, positive predictive value, negative predictive value and accuracy of HCV c Ag were checked against HCV PCR test by 2x2 table. This study was approved by institutional ethical review board.

RESULTS

The median age of study subjects was found 45 (IQR: 23-67) years. Among all subjects, 24 (60%) and 16 (40%) were males and females, respectively. Correlation between two HCV Ab assays was examined and agreement was calculated as shown in Table 2. Agreement was also calculated for HCV c Ag and HCV PCR results (Table-2).

HCV c Ag assay characteristics were calculated by comparing the results with HCV PCR as shown in Table-3.

Table-1: Characteristics of Assays

Assays	Principle	Sample volume (ul)	Reaction time	Interpretation
Architect system (anti-HCV qualitative)	CMIA	70	29 minutes	S/CO values: <1.00 = non-Reactive ≥ 1.00 = Reactive
Cobas system (anti-HCV qualitative)	ECLIA	40	18 minutes	S/CO values: <0.9 =non-Reactive ≥ 0.9 - <1.0 =Borderline ≥1.0= Reactive
Architect system (HCV c Ag qualitative)	CMIA	160	29 minutes	Concentration values: < 3.00 fmol/L= Nonreactive ≥ 3.00 fmol/L =Reactive
COBAS® AmpliPrep / TaqMan® Qualitative	Real-time PCR	650	05 hours	Lower detection limit: 15 IU/ml <15 =Non detected ≥ 15 =Detected

Anti- HCV: HCV Antibody, HCV c Ag: HCV core antigen, CMIA: Chemiluminescence immunoassay, ECLIA: Electrochemiluminescence immunoassay, PCR: Polymerase chain reaction

Table-2: Correlations and agreements between results of two HCV Ab assays and HCV c Ag & HCV PCR assays

Correlation of HCV Ab results (n=40)			
Assay	Positive (n)	Negative (n)	Agreement
CMIA	40	0	
ECLIA	38	2	0.73
Correlation of HCV c Ag and HCV PCR results (n=40)			
Test	Positive (n)	Negative (n)	Agreement
HCV c Ag	23	17	
HCV PCR	22	18	0.95

Table 3: Compared characteristics of HCV c Ag assay against HCV PCR (n=40)

Test Assay (HCV c Ag) Outcome	Number of Observations	HCV c Ag Assay Characteristics	Characteristic's result
True positive	21	Specificity	88.8%
False negative	1	Sensitivity	95.4%
False positive	2	Positive predictive value	91.3%
True negative	16	Negative predictive value	94.1%
		Accuracy	92.5%

DISCUSSION

In Pakistan, HCV is one of the infections that contributed a major burden of liver diseases in the country.[3,14] To eradicate HCV disease, it is important to develop and implement a protocol for the screening and diagnosis of HCV. Clinical laboratories in Pakistan must have facilities to offer cost effective and reliable screening and diagnostic tests for HCV infection.

From 40 seropositive results (analysed by primary assay), 38 were also found seropositive by secondary assay. Hence, we found a good correlation (0.73) between two different HCV antibodies assays in this study. A similar finding is observed in other studies.[15–17]

A positive HCV Ab result may be seen in patients with current or resolved HCV infection. However, false HCV Ab positive results may be found if the reliability of assay is compromised.[6] Conversely, a positive result of HCV PCR is mostly present in patients with current infection only. A seropositive result with negative PCR is either interpreted as resolved HCV infection or a falsely positive result from HCV Ab assay. The latter interpretation can be confirmed by testing with another antibody assay.[5]

The two discrepant results of HCV Ab were found negative for both HCV PCR and HCV c antigen. This study was not designed to calculate the specificity and sensitivity of HCV Ab assay, however; the true negative results were detected by ECLIA in this study. All positive PCR (22) results were found screened positive with both HCV Ab assays. Hence, we found 100% sensitivity of both primary and secondary assays in this study. Similar findings are also observed in other studies.[18,19]

We also compared the results of HCV c Ag with HCV PCR, and found an excellent correlation (0.95). Considering the accuracy (92.5%) of HCV c Ag, this assay may be used as an alternative to HCV PCR for the diagnosis of HCV infection. Other studies also show a considerable agreement between HCV PCR and HCV c Ag assays.[20–22]

WHO is committed to eradicating the HCV infection by the year 2030.[23] However in developing countries with higher HCV prevalence, like Pakistan, cost is the major problem to screen, diagnose and treat the diseases. The cost of screening and diagnosis is found considerably more than treatment.[24] So far, no cost-effective testing protocol is established in Pakistan for HCV eradication, but some are proposed. The clinical laboratories in Pakistan are required to be equipped with HCV screening and diagnostic facilities. In this study, we found an excellent correlation between HCV c Ag assay and HCV PCR. Accuracy, cost effectiveness and rapid turnaround time are the major favourable factors for HCV c Ag assay to replace HCV PCR to detect active viremia in Pakistan.[25,26] Providing a single platform for HCV screening and diagnostic testing by same methodology useful to increase clinical laboratory facility centres and decrease turnaround time. The limitation of this study is a small sample size. Studies at a larger scale are required to recommend the HCV c Ag as an alternative approach for the detection of active HCV infection.

CONCLUSION

We found a good correlation between CMIA and ECLIA for HCV Ab. An excellent correlation was found between HCV c Ag and HCV PCR. Based on our study findings, HCV c Ag is a candidate test for the diagnosis of active HCV infection.

AUTHORS' CONTRIBUTION

SI: Concept, design and write-up. SB: Statistical analysis. SN: Literature search. TN: Data organization AF: Statistical analysis. SK: Critical review.

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