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Antiviral Therapeutic Monitoring: Role of Hepatitis C Virus Core Antigen versus Hepatitis C Virus RNA by Polymerase Chain Reaction

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ABSTRACT

Objective: To study the association between hepatitis C virus viral load by real-time PCR and core antigen value of HCV and define an antiviral treatment monitoring cut-off value for HCV core antigen.
Study Design: Cross-sectional validation study.
***Place and Duration of Study*:** Department of Infectious Diseases, Chughtai Lab Lahore Pakistan, from Jun 2017 to Mar 2018.
Methodology: To establish the association between these two parameters, we took a hundred positive plasma samples for HCV RNA and subjected them to an HCV Core antigen test. Furthermore, the samples were divided into three categories based on their viral load; <2000 IU/ml, 2000-10,000 IU/ml and >10000 IU/ml.
Results: Our results showed that the hepatitis C virus core antigen was concordant with hepatitis C virus RNA by PCR when the viral load was above 2000 IU/ml. Below the HCV RNA load of 2000 IU/ml, the HCV core antigen had a sensitivity of 94.95% and specificity of 88.89%.
Conclusion: In patients having a viral load above 2,000 IU/ml, the hepatitis C virus core antigen value can be used as a marker for diagnosis and monitoring antiviral therapy. After the antiviral treatment, HCV RNA real-time PCR should be performed to validate viral clearance.

Keywords: Hepatitis C virus, Hepatitis C virus core antigen, Polymerase chain reaction.

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INTRODUCTION

One of the leading causes of chronic liver disease is hepatitis C virus (HCV), nearly affecting 130-150 million people worldwide.[1] Belonging to the Flaviviridae family, HCV is a positive-stranded RNA virus. So far, there is no effective vaccine against HCV and once infected with this virus, the person often develops persistent infection leading to chronic hepatitis, hepatocellular carcinoma and cirrhosis.[2] In Pakistan, the prevalence of hepatitis C ranges between 5-7%.[3] HCV is typically diagnosed by specific anti-HCV antibody testing. However, the window period between HCV infection and seroconversion is highly variable, and people who have cleared previous HCV infection remain Anti-HCV positive indefinitely. Therefore, measuring HCV RNA by PCR becomes essential to diagnose and monitor ongoing active infection.[4]

In contrast to Anti-HCV testing, nucleic acid-based tests (NAT) can detect an active hepatitis C infection.[5] Recently, as an alternative to HCV RNA, HCV core antigen has also shown high sensitivity and specificity in diagnosing active HCV infection.[6] During the infection, HCV produces an antigen called p22, commonly known as core antigen, in the plasma which can be detected in the pre-seroconversion period thereby reducing the window period of about 70 days in the case of anti-HCV antibodies.[7] This detection method is based on the principle of chemiluminescence immunoassays.[8] Furthermore, as stated in EASL guidelines of 2016, the detection of HCV either through core antigen or through anti-HCV can be done on the same instrument and can generate results within 40 minutes.[9] Since PCR requires trained and skilled personnel to perform, is costly and is not widely available, measurement of HCV core antigen is a good alternative to PCR for monitoring of therapeutic efficacy of antiviral treatment. Therefore, we assessed whether the measurement of HCV core antigen has the potential to replace PCR for monitoring of antiviral treatment and what are the comparable cut-off values for monitoring.

METHODOLOGY

This cross-sectional validation study was conducted at the Department of Infectious Diseases, Chughtai Lab, Lahore Pakistan, from June 2017 to March 2018. Approval from the Institutional Ethical Review Board of Chughtai Institute of Pathology was taken

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(Reference number 1015). In addition, informed consent was taken from all the patients whose samples were taken for testing and comparison.

The prevalence of chronic (active) hepatitis C ranges between 5-6% in our country.[10] With a confidence level of 95%, the sample size came out to be 97. Plasma samples from 100 patients of chronic hepatitis C were taken for measurement of HCV RNA by PCR and core antigen on each sample.

Inclusion Criteria: Patients aged 12 to 80 years, with chronic hepatitis C as evidenced by positive HCV RNA were included in the study.

Exclusion Criteria: Patients already treated with antiviral therapy, those with a history of debilitating systemic diseases and patients with malignancy were excluded from the study.

The demographic data of patients and their geographical locations were retrieved from the Nexus pro software installed across all Chughtai Lab collection points in the country.

The PCR-positive samples were divided into three groups based on their viral load; <2000 IU/ml, 2000-10,000 IU/ml and >10000 IU/ml. HCV core antigen value above 3fmol/l was taken as a positive case.

Statistical Package for Social Sciences (SPSS) version 21:00 was used for the data analysis. Continuous variables were analyzed using the One-way ANOVA test to determine whether there was a significant association between HCV core antigen results among the three groups. Specificity, sensitivity, negative predictive value (NPV) and positive predictive value (PPV) of this assay were calculated. The *p*-value of ≤0.05 was set as the cut-off value for significance.

RESULTS

One hundred samples were analyzed, having viral loads between 12 IU/ml to 100,000 IU/ml during this study. Of the 100 patients, 68 were females, and the remaining 32 were males. Age of the patients ranged from 12 to 80 years. Positive tests for HCV core antigen were 73 out of 100. In this study, out of all the positive cases, 68% were females, and 32% were of the male gender. The maximum number of HCV PCR-positive cases were seen in Central Punjab (34%), followed by Sindh (28%) and Southern Punjab (20%). Northern Punjab and KPK showed the minimum number of positive cases (10% and 8%, respectively)

HCV core antigen values in the first group having viral load below 2,000 IU/ml was < 125 IU/ml. The second group's HCV core antigen value was between 125 to 400 IU/ml, while the third group had HCV core antigen results greater than 400 IU/ml (Table-I). Our results showed that the HCV core antigen was concordant with the HCV RNA when the viral load was above 2000 IU/ml. Diagnostic Parameters of HCV core antigen assay were shown in Table-II.

Table-I: HCV Core Antigen Results among All Three Groups (n=100)

Groups	HCV Core Antigen Value	HCV Viral Load
Group 1	<125 IU/ml	< 12 to 2,000 IU/ml
Group 2	125-400 IU/ml	2,000 to 10,000 IU/ml
Group 3	>400 IU/ml	10,000 to 1000,000 IU/ml

Table-II: Diagnostic Parameters of HCV Core Antigen Assay (n=100)

HCV Core Antigen Assay	
Diagnostic Parameters	Values
Sensitivity (Below HCV RNA load of 2000 IU/ml)	94.95%
Specificity (Below HCV RNA load of 2000 IU/ml)	88.89%
Positive Predictive Value	89.52%
Negative Predictive Value	94.62%
Diagnostic Accuracy	
HCV PCR Assay	
Sensitivity	95%
Specificity	99.5%

DISCUSSION

Various studies have shown a correlation between HCV RNA and core antigen assays. Although HCV RNA is the most sensitive assay in diagnosing HCV infection, the core antigen assay can be an alternative in settings where HCV PCR is unavailable and patients cannot afford the cost. In Africa, various studies have concluded that HCV core antigen is a good alternative to PCR in resource-constraint settings and difficult-to-reach areas. In certain situations, HCV core antigen could have an advantage over HCV RNA, especially when the blood samples cannot be tested soon because they have to be shipped to a lab far away.[11] In Hepatitis C endemic areas, it is essential to recognize the individuals with an ongoing active HCV infection. For screening for hepatitis infection, anti-HCV assays are routinely used as the serological markers for the diagnosis of HCV infection. However, most anti-HCV assays do not correlate well with HCV viremia. Therefore, HCV RNA is assessed to validate an active infection after a positive anti-HCV test. The only problem with HCV RNA is that it needs specific equipment and highly trained personnel and usually costs much more than core antigen testing. In contrast,

HCV core Antigen assays are easier to perform, have a short turnaround time and are cost-effective. Alonso *et al*. from Spain compared the sensitivity and specificity of HCV core antigen assay with those of PCR and found that both assays were extremely well correlated (Pearson coefficient=0.951). Although the sensitivity of HCV core antigen is slightly less than HCV RNA, it can be used to monitor HCV infection.[12] Li Cavoli *et al*. conducted a study showing how important the HCV core antigen is in monitoring dialysis patients. Their results showed that this assay has a specificity of 100%, sensitivity of 90%, a positive predictive value of 100%, a negative predictive value of 97%, and an accuracy of 97% (p <0.0159).[13] Descamps *et al*. found a statistically strong correlation between HCV core antigen and HCV RNA quantities (r=0.87, p<0.05) by retrospectively evaluating the sera collected from 22 chronically HCV-infected patients and their liver biopsy specimens and further comparing them with three HCV-negative patients. They concluded that HCV core antigen was detected in every infected liver biopsy specimen and 1pg of HCV core antigen corresponds to the viral RNA load of 9,755 IU/ml, which equals almost 600 IU/ml at the HCV RNA threshold of 3fmol/L.[14] In another study from Italy by Galli *et al*. HCV core antigen measurement was shown as a good alternative to PCR for not only diagnosing active HCV infection but also for monitoring high-risk subjects like injection drug abusers, patients on dialysis and for antiviral therapy monitoring and follow up.[15] Moreover, HCV core antigen assays have been documented to be useful for predicting virologic responses in patients treated with pegylated-interferon/ribavirin therapy. HCV core antigen predicted rapid virologic response (RVR) better than HCV RNA, while sustained virologic response (SVR) prediction was similar to that of HCV RNA.[16]

In another study, Garcia *et al*. compared the detection of the therapeutic failure by PCR versus HCV core antigen in patients undergoing antiviral treatment. HCV core antigen demonstrated comparable sensitivity and specificity for early detection of therapeutic failure in patients undergoing antiviral treatment.[6]

However, there is a difference in correlation and equivalency between HCV RNA and core antigen among numerous other studies. As in our study, the two patients having viral load above 40,000 were negative for HCV core antigen. This may be because complete virions may be associated with only a portion of HCV Ag suggesting that a fraction of circulating HCV Ag may be secreted by the infected cells. Besides this, antigen-antibody complexes formed and measured by the immunoassay are disrupted during the pretreatment procedure, which is a source of HCV Ag. Another possible explanation is that HCV, like HBV, may be present in the bloodstream as complete virions with low or no infectivity. This may explain this small discordance in the correlation of HCV RNA and HCV Ag values.[17] Another likely explanation for this discordance could be due to the presence of HCV core antigen mutants that further needs to be explored. Another advantage is that HCV core antigen assay is not prone to sample carryover contamination compared to that molecular assays.

Buket *et al*,[18] showed that because of the ease of performance, high specificity, positive predictive value and cost-effectiveness, HCV core antigen assay could be used to diagnose chronic Hepatitis C infection. However, it lacks sensitivity and negative predictive value. Buket *et al*. found that all positive HCV core antigen samples were also positive for HCV RNA. However, all negative HCV core antigen samples were not negative with the HCV RNA assay, indicating lower sensitivity of core antigen assays compared to PCR. In these, i.e. false negative core antigen results, core antigen could not detect active infection.[18] Therefore, in a case where the anti-HCV antibody result is positive and the HCV core antigen assay is negative, HCV RNA measurement should be done to diagnose and rule out active HCV infection.[19]

CONCLUSION

HCV core antigen can be used for testing viremia pretreatment. All patients undergoing HCV core antigen testing at SVR12 should have a repeat HCV core antigen 3-6 months later, or alternatively, HCV RNA by PCR should be the recommendation at the SVR12 stage. In patients with a viral load above 2,000 IU/ml, the HCV core Ag assay value can be used as a marker for diagnosing and monitoring antiviral therapy. Real-time PCR should be preferred at later stages of antiviral treatment to confirm viral clearance. In patients with a viral load below 2,000 IU/ml, prognosis should also be considered.

Conflict of Interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

HF & MZI: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

WZT & ORC: Data acquisition, data analysis, drafting the manuscript, critical review, approval of the final version to be published.

AAC & SM: Critical review, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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