

Clinical Utility of Enzyme-Linked Immunosorbent Assay for Hepatitis C Core Antigen Quantification in Diagnosis and Monitoring Patients Treated with Direct-Acting Antivirals in A Resource Limited Setting

Ahmed ElMetwally Ahmed, Wael Ahmed Yousry,

Ghada Kamal Mohamed Abdel-Halim, Walaa Mohammad Hashem*

Gastroenterology and Hepatology Unit, Internal Medicine Department, Faculty of Medicine,
Ain Shams University

*Corresponding author: Walaa Mohammad Hashem, Email: walaa.hashem@med.asu.edu.eg, Mobile: (+2) 01001597662

ABSTRACT

Background: Hepatitis C virus core antigen (HCV-cAg) assay has been proposed as a more economical alternative to HCV RNA detection. **Aim of the work:** To evaluate the clinical utility of ELISA for HCV-cAg quantification in diagnosis and monitoring treatment outcomes in patients treated with direct acting antivirals (DAAs) for chronic hepatitis C. **Patients and Methods:** A prospective study on 40 subjects recruited from hepatology department and outpatient clinic in Ain Shams University Hospitals. Group I included 20 patients with positive anti-HCV antibody. Group II included 20 healthy subjects. The patient group received a combination of sofosbuvir 400 mg and daclatasvir 60 mg once daily for 12 weeks. The levels for both HCV-cAg and HCV RNA were evaluated at baseline and 12 weeks after completion of therapy. **Results:** Baseline HCV-cAg levels showed good correlation with HCV viral load ($r=0.808$, $p<0.001$). A sustained virological response 12 weeks off therapy (SVR12) was achieved in all patients with HCV-cAg levels decreasing significantly at the end of treatment (EOT) (21.5 ± 8.5 vs 5.4 ± 3.63 IU/ml respectively, $p<0.001$). The sensitivity and specificity of HCV-cAg in predicting HCV RNA was 100% and 90% respectively at cut-off value >8 IU/ml. **Conclusion:** ELISA for HCV-cAg can replace the high sensitivity HCV RNA molecular assay to confirm the presence of HCV infection and to monitor treatment outcome.

Keywords: Hepatitis C virus; HCV-core antigen; HCV RNA, Direct acting antiviral agents.

INTRODUCTION

Hepatitis C virus (HCV) infection is one of the major public health problems. The global prevalence of people with HCV antibodies (anti-HCV-positive) are estimated to be 115 million, 80 million of them have active infection (HCV RNA positive) ⁽¹⁾. Most of the HCV-infected individuals remain asymptomatic for decades and 75% of them develop chronic infection. Around 10-20% of chronically infected patients develop cirrhosis and hepatocellular carcinoma (HCC) ⁽²⁾, leading to an estimated 7.2 million deaths between 2015 and 2030 in absence of treatment ⁽³⁾.

In 2016, the World Health Organization (WHO) adopted its first Global Health Sector Strategy on Viral Hepatitis. The strategy defines time-bound targets for eliminating HCV as a public health threat by 2030. One key target is to reduce new HCV cases by 80% and another key target is to reduce HCV-related deaths by 65% ⁽⁴⁾.

In the recent years, the availability of the direct acting antivirals (DAAs) which are potent and highly effective with cure rates above 90-95% will make the achievement of this goal feasible ⁽⁵⁾. In this new scenario, priorities arise for the detection of active HCV infection, treatment monitoring and detection of therapeutic failures.

Currently the diagnosis of HCV infection involves a time consuming and cost ineffective two-step approach. Antibody screening is performed first (anti-HCV), followed by determination of the viral load by real time polymerase chain reaction (RT-PCR) which helps to differentiate between active and past infection ⁽⁶⁾. Despite its high sensitivity and reliability, HCV RNA molecular assay requires skilled laboratory personnel, long detection time and high cost which make it impractical for low-income countries to routinely administer this test ⁽⁷⁾. Thus, a cheaper, rapid and simplified alternative is needed for screening and monitoring of treatment especially in resource-limited settings, where HCV is more prevalent.

HCV core antigen (HCV-cAg) is a highly conserved and antigenic protein of the internal capsid which is released into the blood stream during HCV assembly. It can be detected in the plasma earlier than antibodies and throughout the course of infection. Enzyme-linked immunosorbent assays (ELISAs) have been introduced to test HCV-cAg since 1999. In recent years; assays for HCV-cAg have been developed such as enzyme immunoassays (EIAs) and chemiluminescent immunoassays (CMIA) ⁽⁸⁾.

Several studies showed that the limit of detection of core antigen by CMIA is 3 fmol/L which corresponds to a viral load range of 428-2700 IU/ml. Moreover; there is an excellent correlation between core antigen concentrations and HCV RNA levels.



This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY-SA) license (<http://creativecommons.org/licenses/by/4.0/>)

With this high sensitivity, quantification of HCV-cAg by CMIA was suggested as an alternative to HCV RNA molecular assays in diagnosis and monitoring treatment outcomes in patients with chronic HCV infection⁽⁹⁾. Compared to ELISA, CMIA is expensive and requires costly equipment, so it is rarely used to detect HCV-cAg in developing countries.

The aim of this study is to evaluate the performance of ELISA for HCV-cAg detection in the diagnosis of active HCV infection and monitoring treatment outcomes in patients treated with DAAs in a real world setting.

PATIENTS AND METHODS

Patients:

This was a prospective study conducted in the period between April 2018 and October 2018. It included 40 subjects which were recruited from the hepatology department and outpatient clinic in Ain Sham University Hospitals. Two groups were considered. Group I included 20 patients with positive anti-HCV antibody. Group II included 20 healthy subjects, age and sex matched with no evidence of HCV infection by negative anti-HCV antibody.

Patients with decompensated liver disease, hepatitis B virus (HBV), or human immune-deficiency virus (HIV) co-infection, renal impairment and those with HCC were excluded.

Ethical considerations:

This study was approved by Ain Shams University Ethical Research Committee. Informed written consent was taken from all patients before the start of the study.

Virological and serological testing:

HCV RNA level was measured for the group of patients with positive anti-HCV antibody and those with detectable RNA received a combination of sofosbuvir 400 mg and daclatasvir 60 mg once daily for 12 weeks in accordance with the national protocols. Post-treatment PCR was done three months after completion of therapy with a lower limit of detection of 15 IU/ml. An undetectable HCV RNA level twelve weeks post-treatment was defined as sustained virological response (SVR12). Serum HCV-cAg quantification was measured at the same points as HCV RNA and once in the control group. It is a double antibody ELISA one-step process to analyze the existence of HCV-cAg by comparing the optical density of the samples to the cut-off.

Baseline investigations included complete blood count (CBC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum total and direct bilirubin, serum albumin, and international normalization ratio (INR) were estimated for all patients.

Statistical analysis:

All results were collected, tabulated, and statistically analyzed using computer software (IBM SPSS version 20). Descriptive statistical tests of frequency, percentage, mean, and standard deviation (SD) were used to describe the obtained data. Correlations between HCV RNA and HCV-cAg were analyzed by Pearson correlation coefficient.

The diagnostic performance of core antigen was assessed by calculating the area under the receiver operating characteristics (AUROC) curve. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for HCV-cAg were analyzed versus HCV RNA molecular assay.

RESULTS

Clinical characteristics:

The majority of the patients were females (65%), with age ranging from 25-71 years. All patients were Child A and treatment naïve. The rest of patients' demographics and baseline laboratory investigations are summarized in table (1).

Table (1): Baseline patients' demographic and laboratory data

Characteristics	Frequency (%)/ Mean± SD
Gender	
Male	7 (35%)
Female	13 (65%)
Age (years)	41.5±13.06
TLC (/mm ³)	6.16±1.16
Hb (g/dl)	13.54±1.44
Platelets (10 ³ /mm ³)	207.3±41.68
ALT (IU/L)	34.25±8.02
AST (IU/L)	33.05±5.58
Total bilirubin (mg/dl)	0.89±0.15
Albumin (g/dl)	3.68±0.54
INR	1.1±0.12
HCV-cAg (IU/ml)	21.5±4.5

Correlation between laboratory parameters and HCV-cAg in the patient group:

Before treatment there was a positive correlation between core antigen and total bilirubin and INR and negative correlation with hemoglobin, platelets, and albumin. However, none of these correlations were statistically significant as shown in table (2).

Table (2): Correlation between laboratory parameters of the patient group with HCV-cAg before treatment

	HCV-cAg (Before treatment)	
	r	p value
Hb (g/dl)	-0.327	0.159
Platelets (10 ³ /mm ³)	-0.102	0.668
Total bilirubin (mg/dl)	0.078	0.743
Albumin (g/dl)	-0.132	0.579
INR	0.105	0.658

Analytical performance of HCV-cAg in diagnosis of active HCV infection:

The value of HCV-cAg was analyzed in determining diagnosis by ROC curve. Optimal predictive values were assessed by calculating the AUROC curve. When HCV-cAg was >8 IU/ml as cut-off value, sensitivity, specificity, NPV, and PPV were 100%, 90%, 100%, and 90.9% respectively.

Correlation between HCV-cAg and HCV RNA levels:

Analysis of the 20 anti-HCV positive patients showed a strong positive correlation between HCV-cAg and HCV RNA levels at baseline ($r=0.808$, $p<0.001$) as shown in figure (1). SVR was achieved in the 20 patients (100%) on follow up PCR done three months after the EOT period. Additionally, HCV-cAg level decreased significantly at the EOT (21.5 ± 8.5 vs 5.4 ± 3.63 IU/ml respectively, $p<0.001$).

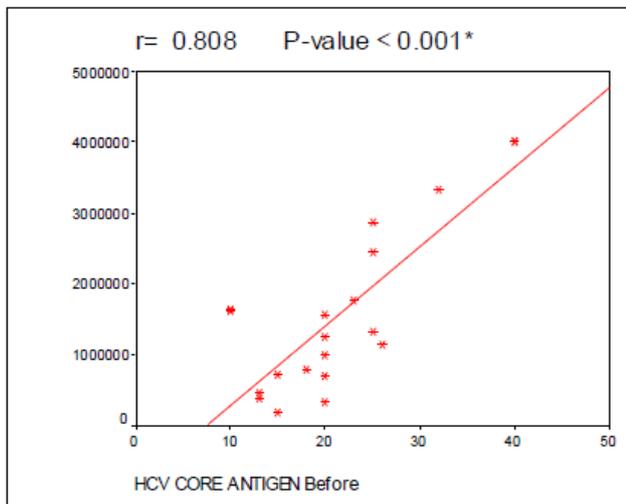


Figure (1): Scatter-plot showing significant positive correlation between baseline HCV-cAg and HCV RNA levels.

DISCUSSION

The diagnosis of HCV infection and efficacy of antiviral therapy is based on testing HCV RNA with sensitive assays. However the most recent recommendations from European Association for the Study of the Liver (EASL) guidelines suggest that measurement of HCV-cAg in serum or plasma can be used instead of HCV RNA to diagnose and monitor treatment efficacy when HCV RNA assays are not available or affordable⁽¹⁰⁾. HCV-cAg assay represent a more cost effective method compared with HCV RNA quantification since it is rapid, affordable, simplified and demands less professional training to operate⁽¹¹⁾.

Our analysis showed that there was a positive correlation between total bilirubin and INR and core antigen and negative correlation with hemoglobin, platelets, and albumin before treatment. Although not reached a significant value, but it goes with the finding that the rise in HCV-cAg level was associated with deterioration of parameters indicating liver function.

The current study showed that when HCV-cAg took the cut-off > 8 IU/ml, sensitivity, specificity, NPV, and PPV were 100%, 90%, 100%, and 90.9% respectively in diagnosing active HCV infection, which is consistent with previous reports⁽¹²⁻¹⁴⁾. **Alves et al.**⁽¹⁵⁾ presented similar results with no false negative or false positive results and sensitivity and specificity of 100%. However; the studied population was chronic hepatitis C patients with chronic kidney disease and patients on hemodialysis. On the other hand, **Wang et al.**⁽¹⁶⁾ reported lower sensitivity and NPV compared to our study (88.96% and 91.3%) using ELISA in detection of the core antigen.

In the present study, the correlation between HCV RNA and HCV-cAg levels was analyzed at baseline showing a significant high correlation coefficient of 0.808 ($p<0.001$). Similar results were reported by other studies. One study conducted by **Wang et al.**⁽¹⁶⁾ found high correlation between HCV RNA and HCV-cAg levels with correlation coefficient of 0.891 using ELISA for detection of HCV-cAg, while other studies presented by **Chang, Lin, Mederacke and their colleagues** reported a good positive correlation coefficient of 0.960, 0.879, 0.75 respectively but CMIA was used in detection of core antigen^(12, 17, 18).

Moreover, another study done on 782 patients with chronic hepatitis C infection enrolled from large multicenter phase IIb and III clinical trials on type Y PegIFN α reported a significant correlation between HCV-cAg and HCV RNA tests ($r>0.950$, $p<0.001$). The samples were further divided into high viral load group and low viral load group at a threshold of 400,000 IU/ml; however the correlation in high viral load group was lower than in lower viral load group (0.517 vs 0.901 respectively)⁽¹⁹⁾.

Several studies address the usefulness of HCV-cAg as an alternative to HCV RNA molecular assays in assessment of treatment outcome in patients treated with interferon-based regimens^(16, 19-22). However, there are few data on the role of core antigen in patients treated with different DAAs. Studies that have evaluated this technique under the new DAA regimens report a good correlation between RT-PCR and HCV-cAg^(13, 23).

The current study showed the ability of HCV-cAg quantification in monitoring patients infected with HCV under treatment with sofosbuvir based regimen. At 12 weeks post-treatment, SVR was achieved in all the 20 patients (100%) and the core antigen level significantly decreased. This agrees with another study by **Lin et al.**⁽¹⁷⁾ on 110 patients who were treated with paritaprevir/ ritonavir/ ombitasvir and dasabuvir with or without ribavirin. SVR was achieved in 108 patients (98%) and core antigen assay identified 99% of these patients with only one false positive case.

Additionally, **García et al.**⁽²⁴⁾ showed that there was only one discrepant case regarding the assessment of SVR at 12 weeks post-treatment (indeterminate core

antigen and negative viremia). However, at 24 weeks post-treatment there was an agreement between HCV-cAg and HCV RNA which confirmed SVR.

Our data suggested that HCV-cAg can be used as an alternative endpoint of DAAs treatment at 12 weeks off therapy, which was against the recent guidelines recommending that core antigen should be assessed at 24 weeks after the EOT to determine the outcome ⁽¹⁰⁾.

CONCLUSION

Fast and immediate results, lower costs of the technique are some of the main advantages of HCV-cAg. Therefore, ELISA for HCV-cAg can replace the high sensitivity HCV RNA molecular assay to confirm the presence of HCV infection and to monitor treatment outcome especially in low-income countries with limited resources. However, this study was limited by the small number of patients, absence of patients with decompensated liver disease, HBV or HIV co-infection, HCC, and absence of patients who didn't respond to treatment. More studies are needed to address such aspects in the future.

Declarations of interest: None.

Acknowledgements: None.

REFERENCES

1. **WHO (2016):** Guidelines for the screening, care and treatment of persons with chronic hepatitis C infection. www.who.int/publications/hepatitis-c-guidelines-2016
2. **Ponziani F, Mangiola F, Blinda C et al. (2017):** Future of liver disease in the era of direct acting antivirals for the treatment of hepatitis C. *World J Hepatol.*, 9(7):352-367.
3. **WHO (2016):** Combating hepatitis B and C to reach elimination by 2030. Geneva: World Health Organization 2016. <https://www.who.int/hepatitis/publications/hep-elimination-by-2030-brief/en/>
4. **WHO (2016):** Global health sector strategy on viral hepatitis, 2016-2021: Towards ending viral hepatitis. Geneva: World Health Organization 2016. <https://apps.who.int/iris/handle/10665/246177>
5. **Wang J, Chen C, Chang C et al. (2020):** Hepatitis C virus core antigen is cost-effective in community based screening of active hepatitis C infection in Taiwan. *Journal of the Formosan Medical Association*, 119:504-508.
6. **Freiman J, Tran T, Schumacher S et al. (2016):** Hepatitis C core antigen testing for diagnosis of hepatitis C virus infection: a systematic review and meta-analysis. *Ann Intern Med.*, 165: 345-355.
7. **Hu K, Cui W (2016):** A highly specific and sensitive hepatitis C virus antigen enzyme immunoassay for one-step diagnosis of viremic hepatitis C virus infection. *Hepatology*, 64: 415-424.
8. **Kuo Y, Chang K, Wang J et al. (2010):** Is hepatitis C virus core antigen an adequate marker for community screening? *J Clin Microbiol.*, 50: 1989-1993.
9. **Ottiger C, Gygli N, Huber A (2013):** Detection limit of architect hepatitis C core antigen assay in correlation with HCV RNA, and renewed confirmation algorithm for reactive anti-HCV samples. *J Clin Virol.*, 58: 535-540.
10. **European Association for the Study of the Liver (2018):** EASL Recommendations on Treatment of Hepatitis C 2018. *J Hepatol.*, 69: 461-511.
11. **Gupta E, Bajpai M, Choudhary A (2014):** Hepatitis C virus: screening, diagnosis, and interpretation of laboratory assays. *Asian J Transfus Sci.*, 8: 19-25.
12. **Chang C, Hung C, Wang J et al. (2018):** Hepatitis C core antigen highly correlated to HCV RNA. *Kaohsiung Journal of Medical Sciences*, 34: 684-688.
13. **Alonso R, García F, Ampuero D et al. (2017):** New direct-acting antivirals for patients with chronic HCV infection: can we monitor treatment using an HCV core antigen assay? *Diagnostic Microbiology and Infectious Disease*, 87: 243-246.
14. **Kuo Y, Chang K, Wang J et al. (2012):** Is hepatitis C virus core antigen an adequate marker for community screening? *J Clin Microbiol.*, 50: 1989-1993.
15. **Alves R, Goncalves H, Goncalves M et al. (2019):** Hepatitis C virus core antigen test: an HCV screening alternative in end-stage renal disease and hemodialysis? *Port J Nephrol Hypert.*, 33(2): 87-90.
16. **Wang L, Chen W, Xi W et al. (2016):** Utility of enzyme-linked immunosorbent assays to test core antigen in the diagnosis and antiviral therapy management of hepatitis C virus infection. *J Med Virol.*, 9999: 1-6.
17. **Lin S, Tung S, Wei K et al. (2020):** Clinical utility of hepatitis C virus core antigen assay in the monitoring of direct-acting antivirals for chronic hepatitis C. <https://doi.org/10.1371/journal.pone.0229994>
18. **Mederacke I, Wedemeyer H, Ciesek S et al. (2008):** Performance and clinical utility of a novel fully automated quantitative HCV-core antigen assay. *J Clin Virol.*, 46: 210-215.
19. **Feng B, Yang R, Jiang H et al. (2020):** Correlation analysis of hepatitis C virus core antigen and low viral loads: Can core antigen replace nucleic acid test? *Clinical and Experimental Medicine*, 20: 131-141.
20. **Fan Z, Liu J, Wang F et al. (2019):** HCV core antigen is a useful predictor during pegylated-interferon/ribavirin therapy in patients with hepatitis C virus genotype 1b. *Medicine*, 98: 10-13.
21. **Moscato G, Giannelli G, Grandi B et al. (2011):** Quantitative determination of hepatitis C core antigen in therapy monitoring for chronic hepatitis C. *Intervirology*, 54 (2) 61-65.
22. **Tedder R, Tuke P, Wallis N et al. (2013):** Therapy-induced clearance of HCV core antigen from plasma predicts an end of treatment viral response. *J Viral Hepat.*, 20 (1): 65-71.
23. **Arboledas J, Guerrero I, Rodríguez M et al. (2017):** Hepatitis C virus core antigen in the management of patients treated with new direct-acting antivirals. *Diagnostic Microbiology and Infectious Disease*, 89: 29-34.
24. **García A, Aguinaga A, Navascués A et al. (2019):** Hepatitis C core antigen: Diagnosis and monitoring of patients infected with hepatitis C virus. *International Journal of Infectious Diseases*, 89: 131-136