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The Utility of Automated HCV Core Antigen Assay as an Alternative to PCR in Chronic HCV Egyptian Patients

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Abstract

Hepatitis C is a disease with a significant global impact. Although the detection and quantitation of HCV RNA have been the standard method for the diagnosis and assessing individual patient response to the antiviral drug regimen, it is very sensitive testing, expensive, labor intensive, and requires technical skill.

Since the HCV core Ag assay is easy to perform in an immunoassay format, cheap, and less prone to sample carryover contamination compared to nucleic acid tests, There is an increasing interest to use HCV core antigen as a reflex test for seropositive individual to identify the active HCV infection also can be used to detect the early HCV infection.

Aim of the work: To compare the diagnostic accuracy of HCV core antigen with HCV-PCR in chronic HCV Egyptian patients.

Patients and methods: HCV core Ag was measured by a fully automated chemiluminescent immunoassay (CLIA) ABBOTT diagnostics in 57 patients proven to be chronic hepatitis C from those 32 were treated with pegylated interferon and ribavirin therpay and achieved HCV RNA negative six monthes after stoppage of treatment (successful SVR) and 25 chronic HCV patients not considered for treatment . Viral load was quantified with branched DNA (bDNA, Versant, Siemens) also sera were tested with the Architect HCV Ag test (Abbott Laboratories). Statistical analysis was performed on logarithmically transformed values.

Results: HCV core antigen was detectable in 19/25 and grey zone in 4/25 HCV RNA positive sera while 2 sera were negative for HCV Ag. HCV-Ag was undetectable in all 32 HCV RNA negative samples. The sample with the lowest viral load that tested positive for HCV-Ag contained 1200 IU/mL HCV RNA. The levels of HCV core antigen showed a good correlation with those from the HCV RNA quantification ($r = 0.907$) HCV core Ag levels were correlated significantly with ALT levels ($r = 0.516$; $P < 0.0001$)

Conclusion: In conclusion, the Architect HCV antigen assay is highly sensitive, (>90%) reliable, easy to perform, cost-effective, and applicable as a screening, supplemental, and preconfirmatory test for anti-HCV assays used in laboratory procedures for the diagnosis of hepatitis C virus infection.

Keywords: HCV infection; Immunoassay; Hepatitis C; Diagnosis; Virological marker

Introduction

HCV usually suspected by the detection of HCV antibodies. Anti-HCV assays have several disadvantages as the lack of sensitivity of detection in the early window period after infection, the inability to distinguish between different stages of infection. Recombinant immunoblot assays, also have several disadvantages, such as being difficult to perform and having a high percentage of indeterminate results and a high cost [1]. The HCV RNA assay is a reliable method but needs technical skill and may also result in false positivity because of contamination; it is time intensive and more expensive [2].

HCV like other memberes of flavivirdiae family produce an excess of protiens that from the diagnostic point of view may provide usefull marker of infection HCV core antigen is another direct virological marker which has been investigated in last year's [3]. HCV core antigen assay is as simple as the HCV antibodies assay and can detect HCV infection only 1 day delay compared to the HCV RNA assay. HCV core antigen (Ag) tests have been introduced to supplement anti-HCV tests over the last decade and these quantitative HCV Ag assays could be used for monitoring of antiviral therapy as well as for diagnosis of HCV infection [2]. However, most of the past studies detecting HCV Ag utilized enzyme-linked immunosorbent assays (ELISA) or enzyme immunoassays (EIA) which may need considerable time and skills [3]. In previous years, manual HCV-Ag ELISA tests were available, but they demonstrated significant limitations, mainly in terms of sensitivity [3]. Recently, An HCV core Ag automated chemiluminescent immunoassay (CLIA) has been recently developed with higher sensitivity and throughput was developed to overcome shortcomings of the conventional core Ag assays [4,5].

Thus, we evaluated the newly introduced HCV Ag assay and compared it with a quantitative RNA assay to verify the utility of this automated Ag assay as an alternative to HCV RNA-PCR.

Patients and Methods

This study was carried out on 57 patients who were chosen from

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100 chronic HCV patients attending tropical medicine outpatients clinic, EL-Minia university hospital.

They diagnosed as chronic hepatitis C by the presence of HCV Abs by third generation ELISA and detectable HCV RNA in addition to histological evidence of chronic hepatitis C in liver biopsy specimen obtained within the preceding year. Patients who previously received immune-modulatory agents within the last 6 monthes, showed evidence of other liver diseases, had decompansated liver cirrhosis, co infected with shistosomiasis or had pulmonary or cardiac diseases were excluded from the study. The included patients were classified into two groups

Group A: included 32 patients (20 males and 12 females with mean age 47.12 ± 6.89) treated with pegylated interferon and ribavirin therapy for 48weeks and obtained SVR

Group B: included 25 HCV RNA positive patients (14 males and 11 females with mean age 48.23 ± 5.6) not considered for treatment All the patients were subjected to

1. Medical history
2. Stressing upon: possible risk factors of HCV transmission-
3. History of bilharziasis and its treatment
4. History of alcohol intake
5. History of drug use
6. Associated medical diseases etc.
7. Clinical examination
8. General examination: particulary for detection of stigmata of chronic liver disease e.g. jaundice, palmar erythema etc.
9. Abdominal examination to assess the condition of liver (e.g. size, consistency), spleen as well as other abdominal organs
10. Ultra sonographic imaging: to assess the liver size, border, echognicity pattern, presence of focal lesion, spleen, gall bladder, portal vein (diameter and thrombosis) and detection of ascites etc.
11. Laboratory investigation including AST, ALT and HCV RNA with a sensitive PCR assay, the Cobas Amplicor v2.0 test HCV RNA PCR (Roche Molecular Systems, Inc., Pleasanton, CA) according to the manufacturer's recommendations [5]. In HCV RNA positive sera, viral load was quantified by bDNA (Versant 2.0; Siemens Healthcare Diagnostics, Deerfield, IL), an HCV RNA signal amplification method, with detection limit of 15 IU/mL [6-8].

HCV Ag Detection and Quantification

Sera, stored at -70°C, were thawed once and tested with the Architect HCV-Ag assay. Samples were processed automatically, first treated on-board to dissociate antigen-antibody complexes and release the antigen from the viral particles. HCV core Ag is detected with a cut-off value of 3.0 fmol/L, a grey zone of 3-11 fmol/L and an upper limit of 180,000 fmol/L (1.0 fmol/L of HCV Ag equals to 0.02 pg/mL) [9].

Ethical approval of the study

Ethical approval for the study was obtained from EL-Minia university Ethics committee in accordance with the 1975 Declaration of Helsinki.

Statistical analysis

A statistical analysis was performed by a statistical analysis software version 9.1.3(SAS Institute, Inc., NC, USA.) GraphPad PRISM software and MedCalc statistical software.

The correlation coefficients between HCV-Ag and HCV RNA were calculated by Spearman's rank test and a comparison between the groups was carried out using the Mann-Whitney U test. P value of less than 0.05 was considered statistically significant.

Results

Table 1 shows the demographic, clinical and laboratory data of the studied groups. there are no significant statistical difference was seen apart from the mean level ± SD of AST and ALT 45.44 ± 4.77)and (47.45 + 3.72) p value (<0.1, <0.2) respectively.

While, Table 2 showed that HCV core Ag was undetected in two patients with HCV RNA was 3.06 and 3.29Log IU, four sera were found in grey zone with value between 6 and 10.6 fmol/L or in Logs 0.84 Log 10 their respective HCV RNA level were between 3.07 and 4.9Log HCV core Ag was clearly positive in 19 patients with HCV RNA levels between 3.12 and 6.87 log10 IU/mL (median 5.55) the correlation coefficient (r) of the logarithmic values of HCV RNA and HCV-Ag, in these cases, was 0.89 (y = 0.8465 x -1.5343) and it was statistically significant (P < 0.001). Figure 1 also the correlation coefficient (r) of the logarithmic values of HCV RNA and ALT was statistically significant a (r = 0.205; P < 0.04) as shown in Figure 2.

Discussion

Since 1999, an ELISA assay has been introduced for serum HCV-Ag detection and quantification however this method showed marked differences in HCV RNA/core Ag ratios and did not demonstrate the required sensitivity in order to be clinically applied as a substitute for HCV RNA measurements that had become widely used [10]. The employment of chemiluminescent magnetic particle- based technology and immune complex-dissociating reaction increases the sensitivity of the studied Architect HCV-Ag test 8 to 21 fold from previous HCV core Ag ELISA assay [11].

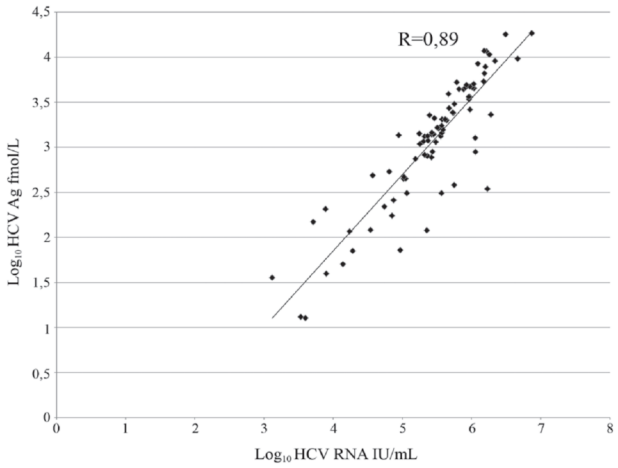
In our study we demonstrated sensitivity in clinical specimen's

Parameter	Group A Treated patients N:32	Group B Non treated patients N:25	P-Value
Age[mean+SD]	47.12 ± 6.89	48.23 ± 5.6	0.1
Male n(%)	20(62.5%)	14(56%)	0.2
Female n(%)	12(37.5%)	11(44%)	0.3
Jaundice n(%)	0	0	
Hepatomegly n(%)	23(71.8%)	20(80.0%)	0.2
Splenomegaly n(%)	2(6.25%)	5(20.0%)	0.06
AST	45.44 ± 4.77	110.38 ± 19.83	0.0001
ALT	47.45 ± 3.72	114.38 ± 33.01	0.0001

Table 1: Shows demographic, clinical and laboratory data of the studied group.

HCV Ag	HCV RNA + sample	HCV Ag values (fmol/L) Median (range)	HCV RNA values (IU/mL) Median (range)
Positive	19	3.15 log10 (1.10 - 4.27 log10)	5.55 log10 (3.12 - 6.87 log10)
Grey zone	4	0.94 log10 (0.84 - 1.02 log10)	3.39 log10 (3.07 - 4.9 log10)
Negative	2	0	3.175 log10 (3.06 – 3.29 log10)
Total	25	3.13 log10 (0 - 4.27 log10)	5.48 log10 (3.06 – 6.87 log10)

Table 2: Shows HCV Ag in relation to HCV RNA.



The correlation coefficient (R) indicates agreement between logarithmically transformed results.

Figure 1: Correlation between hepatitis C virus (HCV) core antigen (HCV-Ag) and HCV-RNA.

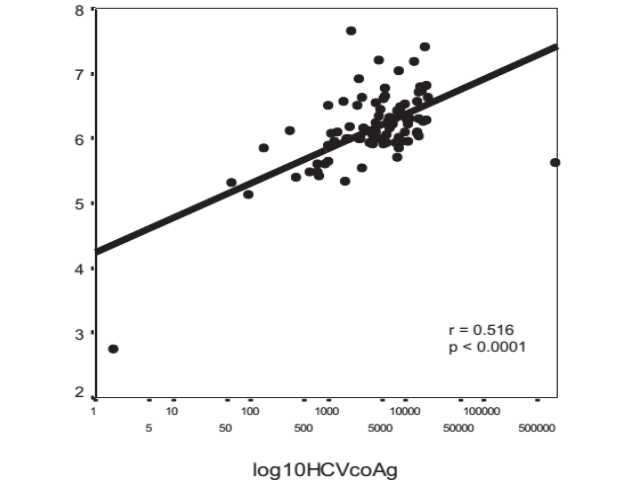


Figure 2: Correlation between hepatitis C virus (HCV) core antigens (HCV-Ag) measured by Architect and ALT level.

equivalent to 1200 IU/mL, corresponding to the serum with the lowest HCV RNA that tested positive for HCV-Ag. This is clear evidence of the enhanced ability of the new HCV core Ag test compared to older ELISA, to detect low level viremia and this in accordance with Medici et al. [12], who studied 193 HCV RNA low positive samples (<3.9 log10 IU/mL), HCV-Ag was detected in low levels (median 16 fmol/L) in 81.9% of studied samples and the sensitivity of HCV-Ag corresponded to 1000 IU/mL of HCV RNA and also in agreement with Emilia et al. [13]. Who demonstrated sensitivity in clinical specimen's equivalent to 1200 IU/mL, corresponding to the serum with the lowest HCV RNA that tested positive for HCV-Ag. These results significantly correlated with HCV RNA measurements by bDNA with a correlation coefficient of 0.89. Higher correlation was reported in a previous study using a more sensitive Cobas-TaqMan RT-PCR assay (r=0.95).

On the other hand Medici et al. [12]. Concluded that the correlation coefficient between HCV-Ag and bDNA was 0.713 and this could be explained by that these results were based on calculations with absolute and not logarithmically transformed values. We used logarithmic transformation of HCV RNA values as wide range of the previous studies used it which is also common practice in the clinical assessment of chronic hepatitis

HCV core Ag levels were correlated also with ALT levels (0.205; P < 0.04) and this in agreement with Durante-Mangoni et al. [14]. who studied in a prospective cohort of 114 patients with chronic hepatitis whoever this study included genotype 1,2 and 3 only not in genotype 4 as in our study.

Data emerging from this study suggest that, HCV Ag positivity is clear proof of viremia and active HCV infection related to liver disease activity as measured by levels of ALT, The Architect HCV Ag assay potentially offers a cost-effective alternative to HCV RNA quantification. It is operationally easier than molecular assays, being available on a fully automated random access platform. Specimen transit time is short with a time of 36 min to the first result, and its low cost per assay

Suggesting that it is likely to be established in the clinical laboratories as a routine used tool to aid in early diagnosis of HCV infection. More studies, with concomitant consideration of the financial cost of HCV viral load testing, need to be conducted in order to pinpoint the proper applications of HCV Ag testing.

References

1. Chevaliez S (2011) Virological tools to diagnose and monitor hepatitis C virus infection. Clin Microbiol Infect 17: 116-121.
2. Bouvier Alias M, Patel K, Dahari H, Beaucourt S, Larderie P, et al. (2002) Clinical utility of total HCV core antigen quantification: a new indirect marker of HCV replication. Hepatology 36: 211-218.
3. Morota K, Fujinami R, Kinukawa H, Machida T, Ohno K et al. (2009) A new sensitive and automated chemiluminescent microparticle immunoassay for quantitative determination of hepatitis C virus core antigen J Virol Methods 157: 8-14.
4. Mederacke I, Wedemeyer H, Ciesek S, Steinmann E, Raupach R et al. (2009) Performance and clinical utility of a novel fully automated quantitative HCV-core antigen assay. J Clin Virol 46: 210-215.
5. Tanaka E, Ohue C, Aoyagi K, Yamaguchi K, Yagi S, et al. (2000) Evaluation of a new enzyme immu- noassay for hepatitis C virus (HCV) core antigen with clinical sensitivity approximating that of genomic amplification of HCV RNA Hepatology 32: 388-393.
6. Lee SC, Antony A, Lee N, Jeff Leibow, Yang JQ et al.(2000) Improved version 2.0 qualitative and quantitative AMPLICOR reverse transcription-PCR tests for hepatitis C virus RNA: calibration to international units, enhanced genotype reactivity, and performance characteristics. J Clin Microbiol 38: 4171-4179.
7. Alter HJ, Sanchez-Pescador R, Urdea MS, Wilber JC, Lagier RJ, et al. (1995) Evaluation of branched DNA signal amplification for the detection of hepatitis C virus RNA J Viral Hepat 2: 121-132.
8. Beld M, Sentjens R, Rebers S, Weegink C, Weel J, et al. (2002) Performance of the New Bayer VERSANT HCV RNA 3.0 assay for quantitation of hepatitis C virus RNA in plasma and serum: conversion to international units and comparison with the Roche COBAS Amplicor HCV Monitor, Version 2.0, Assay. J Clin Microbiol 40: 788-793.
9. Park Y, Lee JH, Kim BS, Kim do Y, Han KH, et al. (2010) New automated hepatitis C virus (HCV) core antigen assay as an alternative to real-time PCR for HCV RNA quantification. J Clin Microbiol 48: 2253-2256.
10. Veillon P, Payan C, Picchio G, Maniez-Montreuil M, Guntz P, et al. Comparative evaluation of the total hepatitis C virus core antigen, branched-DNA, and amplicor monitor assays in determining viremia for patients with chronic hepatitis C during interferon plus ribavirin combination therapy. J Clin Microbiol 41: 3212-3220.
11. Ross RS, Viazov S, Salloum S, Hilgard P, Gerken G, et al. (2010) Analytical performance characteristics and clinical utility of a novel assay for total hepatitis C virus core antigen quantification. J Clin Microbiol 48: 1161-1168.

12. Medici MC, Furlini G, Rodella A, Fuertes A, Monachetti A, et al. (2011) Hepatitis C virus core antigen: Analytical performances, correlation with viremia and potential applications of a qquantitative automated immunoassay. J Clin Virol 51: 264-269.

13. Hadziyannis E, Minopetrou M, Georgiou A, Spanou F, Koskinas J (2013) Is HCV core antigen a reliable marker of viral load? An evaluation of HCV core antigen automated immunoassay. Annals of Gastroenterology 26: 1-4.

14. Durante-Mangoni E, Vallefuoco L, Sorrentino R, Iossa D, Perna E, et al. (2013) Clinico-pathological significance of hepatitis C virus core antigen levels in chronic infection. J Med Virol 85: 1913-1918.