The Egyptian Journal of Hospital Medicine (July 2018) Vol. 72 (2), Page 4034-4038

Role of Anti-Hepatitis C Virus (HCV) Signal to Cut-off Ratio (S/CO) in Following-up Patients Received Anti-HCV Therapy in Egypt

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ABSTRACT

Background: Hepatitis C Virus (HCV) infection is diagnosed by antibody and RNA based methods. Anti HCV-RNA testing based methods are introduced to confirm viremia in seropositive samples. This study aimed to evaluate the relationship between quantitative anti-HCV (S/CO ratio) and HCV-RNA by PCR as a diagnostic test to identify viremic from non-viremic HCV patients received anti-HCV therapy in Egypt. **Subjects and Methods:** Patients serum samples used in this study were collected from Al Hussein University hospitals after they had completed their anti-HCV therapy. A total of 172 patients were included in this study 82 were positive RNA, 90 of them were negative RNA their serum samples were assessed for the presence of antibodies to HCV using ELISA method. **Results:** The results were expressed as the ratio between the signal detected on the sample and the cutoff value of the run (S/CO). Patients with HCV-positive RNA were considered viremic. Receiver operator characteristic (ROC) curve was constructed by plotting sensitivity versus 1 − specificity, using HCV RNA and the S/CO ratio results respectively. Of the 172 patients with HCV infection the mean age was 51.9 ± 7.2 years ranging 35-67 years, 111 (64.5%) were males while 61(35.5%) were females. In the present study there was significant difference in S/CO ratio between viremic and non-viremic subjects. The sensitivity, specificity, negative predictive value, and positive predictive value were 98.78%, 71.11%, 98.46%, and 75.70%, respectively in the S/CO ratio of 8. Area under ROC curve was estimated to be 0.982 (95% confidence interval 0.967 – 0.997). **Conclusion:** by establishing 8 as cutoff value of the S/CO, it is possible to distinguish between viremic and non-viremic patients without need to use Anti HCV-RNA testing as a confirmatory test.

Key Words: Hepatitis C, Hepatitis C antibody. Enzyme Linked Immunosorbent Assay. Signal-to-cut-off ratio.

INTRODUCTION

HCV is a roughly spherical, enveloped, positive-strand RNA virus approximately 55 nm in diameter. It is a member of the family Flaviviridae, yet it is sufficiently distinct from the type genus Flavivirus (e.g., yellow fever virus and dengue virus) to merit classification within a separate genus, Hepacivirus, which also include Pestivirus (e.g., bovine viral diarrhea virus and classical swine fever virus) and Pegivirus [(1)]. It is estimated that more than 80 million people are chronically infected worldwide, with 3–4 million new infections and 350 000 deaths occurring each year because of HCV-related complications [(2)]. Egypt is the country with the highest HCV prevalence in the world; in 2015, a national Egyptian health issue survey was conducted to describe the prevalence of HCV infection. The prevalence of HCV antibody in Egyptian population was found to be 10.0% [(3)]. Transmission is mainly associated with infected blood products, intravenous drug abuse, accidental needle sticks or perinatal infection although other less common routes such as vertical or sexual transmission are reported. Laboratory assays that are available for the diagnosis and management of HCV infection include: serologic tests to detect HCV antibodies, molecular tests to detect and quantitate HCV-RNA and genotyping techniques in addition to assays to detect and quantify HCV core antigen [(4)]. The diagnosis of HCV infection is based on the detection of anti-HCV antibodies in serum by means of enzyme-linked immunosorbent assays (ELISA). Anti-HCV positive result might be one of three possible conditions: Current active infection, past infection and false positive result. Detection of HCV-RNA by polymerase chain reaction (PCR) is considered the gold standard to confirm the diagnosis of HCV infection and for assessing viremia in patients during and following antiviral therapy [(5)]. The Centers for Disease Control and Prevention (CDC) published recommendations for laboratory testing and reporting of anti-HCV results, with the option to classify the positive antibodies as low or high, and then choose supplementary testing based on the signal-to-cut-off (S/CO) ratio. The CDC guide also proposed that cases which could be classified as having true positive results with a high level of Anti-HCV should be confirmed by (Immunoblot) IMB [(6)]. Recently, the CDC guidelines were updated to use a second anti-HCV antibody assay to distinguish between true positive and false positive HCV antibody [(7)]. The decision to use a specific supplemental testing strategy has economic implications for society. In health care systems with limited budgets, the recommendation always to use Anti HCV-RNA testing might not be feasible and requires a sophisticated molecular laboratory. The aim of this study is to evaluate the relationship between quantitative anti-HCV (S/CO

Received:28/4/2018
Accepted:7/5/2018

ratio) and HCV-RNA testing and to determine a specific S/CO to identify viremic from non-viremic anti-HCV positive patients received anti-HCV therapy in Egypt.

SUBJECTS AND METHODS

A total of one hundred seventy-two serum samples used in this study were collected from patient received anti-HCV therapy at Al Hussein University hospitals, 82 of them with HCV-positive RNA and 90 of them with HCV-negative RNA. This study was approved by the Ethical Committee of Al-Azhar University and written consent was taken from each patient before blood sample was collected. All serum samples were assessed for the presence of antibodies to HCV using Murex anti-HCV-version 4.0 provided by Diasorin the diagnostic specialist, Inc., South Africa. The results were expressed as the ratio between the signal detected on the sample and the cutoff value of the run (S/CO). In the anti-HCV test, S/CO ≥1 is considered reactive. Samples with positive HCV-RNA testing were considered viremic. Samples with negative HCV-RNA testing were considered non-viremic.

Statistical analysis

ROC curve was constructed by plotting sensitivity versus 1 − specificity, using Anti HCV-RNA testing and the S/CO ratio results respectively. We determined the diagnostic sensitivity, diagnostic specificity, positive predictive value (PPV), negative predictive value (NPV), and their respective exact 95% confidence interval (CI) to predict HCV viremia and ELISA status at S/CO ratios of 3.0, 8.0 and 20.0. Optimal S/CO ratios were identified from the analysis of ROC curve. We performed ROC analysis using IBM SPSS Statistics version 24.0.

RESULTS

A total of 172 patients with previous HCV infection were assessed for the presence of antibodies to HCV in their blood, 82 (47.7%) of them were positive to Anti HCV-RNA testing results (viremic) and 90 (52.3%) with negative Anti HCV-RNA testing results (non-viremic) (Table 1) after they had received their anti-HCV treatment. Their mean age was 51.9 ± 7.2, 111 (64.5%) of them were males and 61 (35.5%) of them were females (Table 2). Their samples were assessed for the presence of antibodies to HCV using anti-HCV antibody ELISA test, 170 out of 172 (98.8%) demonstrated reactive results (S/CO ≥1). The area under the ROC curve (Figure 1) was estimated to be 0.982 (95% confidence interval 0.967 – 0.997).

Table (1): Results of ELISA HCV antibody S/CO ≥1 compared to PCR results.

S/CO ratio	Viremic	Non-viremic	Number (Percent)
<1	0	2	2 (1.2%)
≥ 1	82	88	170 (98.8%)
Total numbers	82	90	172
McNemar's test P value was <0.05			

Table (2): Sex distribution.

Sex	Number	Percent
Male	111	64.5%
Female	61	35.5%
Total	172	100%

Table (2): Profile of HCV antibody S/CO at 3, 8 and 20.

S/CO ratio	≥3	≥8.0	≥20
Diagnostic sensitivity, %	100 (95.6-100)	98.78(93.39-9.97)	85.37(75.83-2.20)
Diagnostic specificity, %	58.89(48.2-69.16)	71.11(60.60-0.18)	100(95.98-100)
NPV, %	100	98.46(90.08-9.78)	88.24(81.64-2.67)
PPV, %	68.91(63.38-73.94)	75.70(69.24-1.17)	100
Values in parentheses are the limits of the 95% CI.			

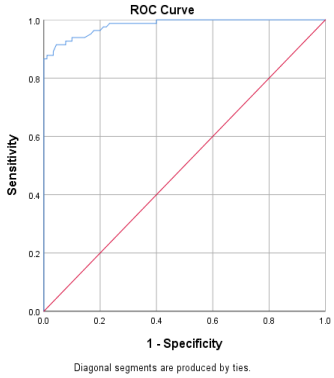

Figure (1): ROC analysis of data from 172 samples. Cut-off, sensitivity and specificity of S/CO ratio for anti-HCV were calculated using PCR as a reference. The area under the ROC (AUROC) was estimated to be 0.982 (95% confidence interval 0.967 – 0.997). The maximum Youden index was determined to be at the point of 8 (sensitivity 98.7%, specificity 71.1%).

DISCUSSION

In several published studies, different S/CO values ranging from 2.7 to 34 were determined in the third generation of anti-HCV assays[8]. In this study serum samples from 172 patients with previous HCV infection, 82 (47.7%) of them with HCV-positive RNA and (viremic) 90 (52.3%) with HCV- Negative RNA (non-viremic) after they had received their anti-HCV treatment, their mean age was 51.9 ± 7.2 and 111 (64.5%) of them were males, 61 (35.5%) were females. their samples were assessed for the presence of antibodies to HCV 170 out of 172 (98.8%) demonstrated reactive results (S/CO ≥1) (Table 1). Using the ROC curve S/CO ration of 8 can distinguish viremic from non-viremic patients, the area under the ROC curve was estimated to be 0.982 (95% confidence interval 0.967 – 0.997). Fahimeh et al. examined 265 patients with HCV infection, 204 (77%) were male and the mean age was 43.53 ± 13.17 years, ranging 1 - 81 years. No correlation was found between S/CO ratios and HCV-RNA levels. There was significant difference in S/CO ratio between viremic and non-viremic subjects. The sensitivity, specificity, negative predictive value, and positive predictive value were 100%, 81.4%, 100%, and 77.2%, respectively in the S/CO ratio of 2.7[8]. Marco et al. reviewed results from 12,800 samples tested for hepatitis C virus (HCV) antibody by screening (Ortho Chemiluminescence immunoassay [CIA]) and supplemental tests (Chiron recombinant Immunoblot assay [RIBA]) and found that a signal-to-cutoff (S/Co) ratio of 10.3 was the most efficient cutoff point to improve the diagnostic algorithm of HCV infection[9]. ***Seo et al.*** examined serum samples of 487 patients with positive anti-HCV enzyme immunoassay their mean age was 56 ± 16 the S/CO that distinguish viremic from non-viremic patients was 10.9[10]. ***Balk et al.*** examined 124 anti-HCV positive patients, S/CO values for anti-HCV were correlated with the quantitative values of HCV-RNA and found that S/CO ratio > 25.9 could distinguish viremic from non-viremic patients[11]. ***Dufour et al.*** retrospectively reviewed 17,418 consecutive anti-HCV results from a screening program for high-risk veterans. RIBA was performed in 263 patients with low-positive anti-HCV; results were negative in 86%, indeterminate in 12%, and positive in 2%. Only 16 of 140 individuals (11%) with low-positive anti-HCV values were Anti HCV-RNA testing positive, whereas Anti HCV-RNA testing was positive in 90% of 1435 individuals with high-positive anti-HCV values ($P < 0.0001$). S/CO ratio ≥ 3.7 could distinguish viremic from non-viremic results[12]. Lai et al. reported that an S/Co ratio of 3.0 determined by the VITROS anti-HCV assay was the highest value associated with a diagnostic sensitivity of 100% and NPV of 100%, using either PCR or RIBA as gold standards. No positive RIBA or PCR test results were found in samples with an S/CO ratio <3.0 in their analyses. Therefore, it was suggested that supplemental testing was not necessary for patient samples with S/CO ratio <3.0. In the present study S/CO ratio of 3.0 was the highest value associated with a diagnostic sensitivity of 100% and NPV of 100%, using PCR as gold standards. Interestingly, the ROC curve analysis in our study demonstrated that an S/CO ratio of 20.0 was not an optimal cutoff, as has been suggested in previous studies as in Lai et al. and ***Oethinger et al.*** studies [13,14]. The differences in the findings could be attributed to the following reasons. First, the study populations were different. Lai et al. proposed an algorithm for HCV testing based on the results in a population of veteran[13]. Oethinger et al conducted the study using blood donor samples. However, the population in our study came from hospitals after they had received their anti-HCV treatment. The difference in the prevalence of anti-HCV antibodies in the various study populations might account for the differences in optimal S/CO ratio cutoffs. Second, the distribution of the predominant HCV genotype varies regionally; for example, HCV-4 is the most common genotypes in Egypt. This might have caused the varying results between the studies for the S/CO ratios with different assays. Finally the difference in anti-HCV assays may account for the difference in the S/CO ratios between our study and previous studies. In the current study by comparing sensitivity, specificity, NPV and PPV for different S/CO ratios (Table 2) found that at S/CO ratio of 3.0, sensitivity, specificity, NPV and PPV were 100, 58.89, 100 & 68.91 respectively. For S/CO of 8.0, sensitivity, specificity, NPV and PPV were 98.78, 71.11, 98.46 and 75.70 respectively. For S/CO of 20.0, sensitivity, specificity, NPV and PPV were 85.37, 100, 88.24 and 100 respectively. The sensitivity decreases with the increase of S/CO ratios and this could be attributed to that at different level of viremia there were different levels of antibodies and there were no relationship between viral load and antibody state. In the current study we compared results from PCR with results of EIA and found that all positive samples with PCR (viremic) were

positive with EIA (positive antibody), however negative PCR samples were positive except in two samples (2/90, 0.02%) this could be attributed to the nature of each test. PCR detect viremic state while EIA detect antibody state that remains positive although complete eradication of the virus from the body. In the current study stratifying the data by sex found that there was no significant difference between males and females ($p>0.05$), however by comparing data from patients aged ≥60 with patients <60 years old there was a significant difference ($p<0.05$) and this may be due to failure of anti-HCV therapy for patients aged more than 60 years old[14]. ***Kuo et al.*** compared results from CIA, EIA, RIBA and Anti-HCV RNA of 1,017 samples[15]. ***Fahimeh et al.*** examined 265 patients with HCV infection using EIA and PCR[8]. Abdelaziz examined serum results of 183 Egyptian patients using CIA in comparison with IMB [16].

In the current study we compared results of 172 patients using EIA and PCR. HCV-RNA testing is expensive and time consuming, requires a sophisticated molecular laboratory, and may not be always readily available in underdeveloped parts of the world, where the greatest numbers of HCV infected patients are found. Although false positive EIA results are a problem in low prevalence settings, the accuracy of the third –generation test is very good in high–prevalence populations, and therefore, HCV-RNA testing may not be necessary in high-risk patients with positive anti-HCV results. In conclusion, by establishing 8 as cutoff value of the S/CO it is possible to distinguish between viremic and non-viremic patients. Based on our results, subjects with S/CO values < 8 were more likely to be cases of past infection or of nonspecific reaction. Most of the subjects with S/CO >8 could represent current or persistent infection. Our data demonstrate that viremic HCV patients had higher S/CO values in the ELISA test in comparison with non viremic patients. Based on our results, the cutoff value of the S/CO was 8 in differentiating viremic from non-viremic patients. Thus, a threshold set at 8 S/CO needs no supplemental confirmation by PCR for following up patients received their anti-HCV therapy. HCV-RNA testing is more expensive than ELISA and time consuming, requires a complicated molecular laboratory, and may not be always readily available in underdeveloped parts of the world, where the greatest numbers of HCV infected patients are found. Although false positive ELISA results are a problem in low prevalence settings, the accuracy of the third –generation test is very good in high–prevalence populations, and therefore, HCV-RNA testing may not be necessary in high-risk patients with positive anti-HCV results. Additional studies are helpful to predict practically viremia by using anti-HCV S/CO values.

LIMITATIONS

Although recent CDC guidelines stated that a second anti-HCV antibody assay is needed to confirm the results of the first anti-HCV assay, but our study aimed to reduce the cost in following-up patients receiving their anti-HCV therapy by establishing a cut-off at which no need for HCV-RNA testing which is mandatory for treatment monitoring.

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