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HEPATITIS C VIRUS (HCV) CORE ANTIGEN AS AN ALTERNATIVE TO PCR FOR HCV, RNA QUANTIFICATION TO EARLY DIAGNOSIS OF HCV INFECTION AMONG BLOOD DONORS

By

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ABSTRACT

Background: Safety blood transfusion is based on reliable donor screening for transmissible infections such as the hepatitis C virus (HCV) infection. HCV-RNA by polymerase chain reaction (PCR) test is a gold standard test for HCV infection but with three major limitations: liability of RNA molecules, higher costs, and longer turnaround time as compared with HCV core antigen (HCVcAg) testing. **Objective**: To evaluate HCV core Ag as an alternative to PCR for HCV RNA to early diagnosis of HCV infection among blood donors. **Patients and Methods**: 4222 blood donors collected between March 2015 and July 2016 at Blood Bank Center of Najran Hospitals, Southwestern Saudia Arabia. Anti-HCV, HBsAg, syphilis and HIV were screened by third generation ELISA. Those reactive to HbsAg, syphilis and/or HIV were excluded. HCV core Ag and HCV RNA by RT-PCR were performed on 76 positive HCV-Ab sera. **Results**: Among total 4222 blood donors (3837 males (90.88%) and 385 females (9.12%)), 76 (1.8%) were positive for HCV-Ab (72 males; 94.74% and 4 females; 5.26%). HCV core Ag was ≥ 11 fmol/L in 65 cases (85.53%) of HCV Ab positive patients (61 males; 93.85% and 4 females; 6.15%), 3 cases with HCVcAg levels in grey zone (3-11 fmol/L) and 8 cases with HCVcAg ≤ 3 fmol/L all of both were males. HCV RNA was detected in 71/76 (93.42%) of donors with positive HCV-Ab and in 3 of 8 (37.5%) of those with positive HCV-ab and negative HCVcAg. HCVcAg has sensitivity, specificity, PPV and NPV of 97.14, 100, 100 and 99.76 values respectively at a cutoff 2.82 fmol/L compared to HCV RNA test. **Conclusion**: Higher sensitivity and specificity of HCVcAg with rapid turn around time of the results, and its lower price suggested that can be used alternatively to HCV RNA test in early diagnosis of HCV infection.

Keywords: HCV infection, HCV core Ag, Immunoassay, Virological marker.

INTRODUCTION

Hepatitis C virus (HCV) is a global pathogen, infecting more than 185 million individuals, with global seroprevalence estimated at 2.8% (2.6 to 3.1%), and more than 1 million new cases reported annually **(Mastro et al., 2016).**

Detection of blood borne viruses by conventional serology tests depend upon levels of viral specific antibodies, virus antigens and sensitivity and specificity of serology method used. During early infection, the virus is present in the blood of the infected individual and may be transmitted to the recipient of this infected blood even though the conventional

serological test is negative **(Elbjeirami et al., 2015)**.

Diagnosis of HCV infection requires the detection of anti-HCV virus antibodies with confirmation of positive results using polymerase chain reaction (PCR). HCV RNA by PCR can detect HCV virus as early as 15-20 days post exposure and at a very low level of viremia. It is a reliable method but needs technical skill. It is time intensive and more expensive, and may result in false positivity because of contamination **(Abd El-Reheem et al., 2016).**

HCV core Ag (HCVcAg) tests have been shown to detect HCV infection 45 days earlier than HCV-Ab screening test, and 1-2 days following the detection of HCV RNA in the serum. Moreover, it has been reported that detection of HCVcAg can decrease the window period interval to 23.9 days **(Zhang et al., 2007).**

HCVcAg estimation by automated chemiluminescent immunoassay has developed with higher sensitivity to overcome shortcomings of the HCVcAg ELISA assays **(Mederacke et al., 2009; Morota et al., 2009** and **Abd El-Reheem et al., 2016).**

The present study was designed to assess whether the HCVcAg test by chemiluminescent immunoassay is a good alternative to PCR for the early diagnosis of HCV infection, particularly in the acute phase of the illness and during which the infection can be missed in a substantial number of blood donors, if anti-HCV Ab testing is employed alone.

PATIENTS AND METHODS

This observational study was carried out on a cross sectional sample of voluntary 4222 blood donors (3837 males (90.88%) and 385 females (9.12%) collected between March 2015 and July 2016 at Blood Bank Center of Najran Hospitals, Southwestern Saudia Arabia. Of these donors, 76 (1.8%) were positive for HCV-Ab (72 males; 94.74% and 4 females; 5.26%).

A written consent was obtained for blood donation and permission to perform relevant tests on blood. After initial interview and mini-medical examination, demographic data were recorded, and donors were accepted for blood donation. Blood samples were withdrawn from each blood unit during donation under strict aseptic conditions for blood grouping, cross-match, anti-body screening, syphilis serology, malaria slide examination, anti-HCV, HBsAg, and HIV were taken. Anti-HCV, HBsAg and HIV were screened by third generation ELISA. Those reactive to HBsAg, HIV, syphilis serology or malaria were excluded.

Three ml from each positive HCV-Ab blood unit was added to a sterile vacutainer tube and then separated by centrifugation in PCR unit under strict sterile conditions and serum was stored at -70 °C to be used for PCR and HCVcAg testing.

Anti-HCV antibody screening

Screening for anti-HCV antibody was performed using Murex anti-HCV (version 4.0), (DiaSorin S.p.A., Dartford, UK) ELISA kits on Evolis system (Bio-Rad® Technology INC. USA).

Anti-HCV antibody test results of ≥ 1.00 signal-to cut- off (s/co) were considered reactive, results of <0.90 s/co were considered non-reactive, and results

of ≥ 0.90 s/co and <1.00 s/co were considered equivocal (gray zone) according to the manufacturers' instructions, and were confirmed by repeating the sample in duplicate using the same kit, and then testing it using HCVcAg and PCR-HCV RNA tests.

HCVcAg determination:

HCVcAg was measured using Abbott Architect HCV Chemiluminescenat Microparticle Immunoassay (CMIA) on Architect i2000 system (Abbott Laboratories, Abbott Park, IL, USA).

HCVcAg test results of <3.00 femtomole/liter (fmol/L) were considered nonreactive. Values between ≥3.00 fmol/L and ≤11.00 fmol/L were considered grey zone, and results of ≥11.00 fmol/L were considered reactive (1.0 fmol/L of HCVcAg equals to 0.02 pg/mL) according to the manufacturers' instructions.

HCV RNA by PCR determination:

Viral RNA was extracted from all serum HCV-Ab positive samples using AmpliPrep/COBAS® TaqMan® HCV Test Kit on Mx3000P QPCR System (Agilent Technologies, California, USA).

The lower limit of detection of this assay was 12 IU/ml, with a linear range between 43 x 10^6 and 69 x 10^6 IU/ml. Each specimen was analyzed in duplicate and the mean value was reported as the HCV RNA level in the serum. A known quantity of internal quality control serum was included in each preparation of RT-PCR. The cycle conditions of RT-PCR included 95 ˚C for 10 min, followed by a further 40 cycles at 95 ˚C for 10s, 58 ˚C for 15s, and 72 ˚C for 10s. According to the manufacturers' instructions, HCV RNA measures of <10^1 International Unit/mililiter (IU/ml) were considered as low-level viremia, and values of >10^1 IU/ml were considered as positive.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 20.0 (SPSS Inc., Chicago, IL, United States). Descriptive variables were presented as numbers, percentages mean±standard deviation (SD). Sensitivity and specificity as well as receiver operating characteristic (ROC) curve analysis were applied to HCV Ag value compared to HCV RNA. Correlations between different parameters calculated by the Spearman test. $p<0.05$ was considered statistically significant.

RESULTS

Among total 4222 blood donors (3837 males (90.88%) and 385 females (9.12%), 76 (1.8%) were positive for HCV-Ab (72 males; 94.74% and 4 females; 5.26%), with age between 26 and 50 years (mean 37.01±5.32 years). HCVcAg varied between 0.23-2072 fmol/L with mean 473.78±539.89 and HCV RNA ranged between 0.0-6.94 log10 IU/mL with mean 6.12±6.30 log 10 (Table 1).

As regard to HCVcAg, it was positively determined (≥ 11 fmol/L) in 65 cases (85.53%) of HCV Ab positive patients. There were 3 males with HCVcAg levels in grey zone (3-11 fmol/L), and 8 cases with HCV Ab positive donors were also negative to HCVcAg (≤ 3 fmol/L). HCV RNA level was successfully determined in all cases of HCVcAg in positive and grey zone (68 cases, 100%) and 3 of 8 cases in which HCVcAg was negative (37.5%) (Table 2).

ROC curve analysis revealed cutoff of HCV Ab, HCVcAg and HCV RNA was

1.67 index, 2.82 fmol/L and 455 IU/mL respectively. HCVcAg has area under curve (AUC), sensitivity, specificity, positive and negative predictive values were 0.981, 97.14, 100, 100 and 99.76 respectively, with p<0.0001 when compared to values of HCV RNA test (Table 3).

Among cases with positive of both HCVcAg and HCV RNA, there was statistical significant link between HCVcAg and HCV RNA levels (r=0.759; p <0.001 - Figure 1).

Table (1): Demographic and virological markers in studied donors

Parameters	Values
Total donors No (%)	4222 (100)
Males / females: No %	3837/385 90.88/9.12
Positive HCV-Ab No (%)	76 (1.80)
Males / females No (%)	72/4 (94.74/5.26)
Age years :mean±SD Range	37.01±5.32 (26.0-50.0)
HCV core Ag fmol/L : mean±SD Range	473.78±539.89 (0.23 - 2072)
HCV-RNA Log10 IU/mL : mean±SD Range	6.12±6.30 (0.0 - 6.94)

Table (2): Comparative results of HCV core Ag and HCV RNA in HCV-Ab positive sera

HCV / Parameters	HCVAntibodies positive sample No (%)	HCV Ag values (fmol/L) Mean (range)	No of HCV RNA positive sample
Positive (> 11 fmol/L)	65 (85.53)	560.5 (17.0 - 2072.0)	65
Gray zone (3 - 11 fmol/L)	3 (3.94)	6.28 (4.33-8.32)	3
Negative (<3 fmol/L)	8 (10.53)	1.74 (0.18-2.82)	3

Table (3): ROC curve results of HCV-Ab, HCVcAg and HCV RNA at a cutoff 455 IU/mL

Test / Parameters	HCV Ab test	HCVcAg test
AUC	0.939	0.981
Cut off	1.67	2.82
Sensitivity	81.43	97.14
Specificity	100	100
PPV	100	100
NPV	98.48	99.76
P value	0.0001	0.0001

AUC= Area under curve; PPV= positive predictive value; NPV= negative predictive value.

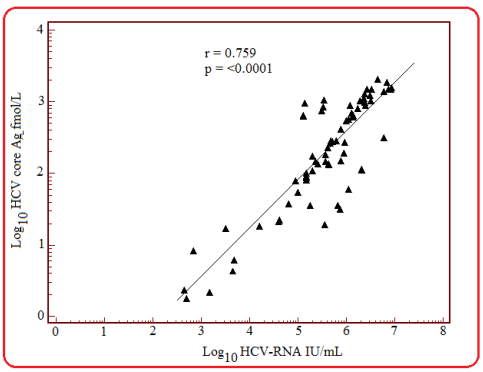

Figure (1): Correlation between HCV core Ag and HCV-RNA

DISCUSSION

Hepatitis C virus (HCV) has been reported to be on the decline over the past decade, although it remains a major public health concern in Saudi Arabia. In current study, blood samples from 4222 subjects (90.88% males and 9.12% females) were randomly collected, and analyzed. The results indicated that 76 (1.80%) cases were positive for anti-HCV antibody only (94.74% males and 5.26% females) with a mean age 37.01±5.32 years. However, in Saudi Arabia, the HCV prevalence in blood donors ranges from 0.4% to 1.9% **(El-Beltagy et al., 2008; Abdo et al., 2012; Abdullah, 2013 and Alhetheel & El-Hazmi, 2014).**

The prevalence of HCV infection in blood donors among American was 0.072% **(Zou et al., 2009),** in North Europeans 0.01% - 0.02% and in South Europeans was 1-1.5% **(Khodabandehloo et al., 2013)**.

HCV RNA detection remains gold standard for the early diagnosis and confirmation of active HCV infection **(Waldenstr?m et al., 2013)**, but high costs, time consumption, requirement of sophisticated equipment, and skilled manpower of HCV RNA have turned it not to be frequently used in the developing countries of Africa, Asia and Latin America **(Zhang et al., 2007)**. Therefore, during past decades, researchers have put enormous effort to develop HCVcAg, an otherwise economic and easy to perform assay, as a potential alternative to HCV RNA tests **(Chakravarti et al., 2013)**.

Compared to PCR technique, HCVcAg assay is simple as HCV antibodies assay and can detect HCV infection only 1 day delay compared to the HCV RNA assay. HCVcAg tests have been introduced for monitoring of antiviral therapy as well as for diagnosis of HCV infection **(Nguyen et al., 2016)**.

Among HCV-antibody positive specimens, 93.42% were positive by HCV RNA-PCR while 85.53% were positive by HCVcAg test, 3.94% was borderline (grey zone) and 10.53% were negative. In the present study, viral load was in the range of 700–4900 IU/mL in the three HCV RNA positive HCVcAg borderline subjects and in the range of 455–1500 IU/mL in HCV RNA positive/HCVcAg negative specimens.

The sample with the lowest serum level of HCV RNA (455 IU/mL), which was tested positive for HCVcAg (2.82 fmol/L) may be accepted as the lower limit of range for analytical sensitivity for HCVcAg assay, from our study. This correlated with the study performed by **Hassan et al. (2014)** who revealed that 3 fmol/L core antigen is equivalent to 263 IU/mL HCV RNA. **Abd El-Reheem et al. (2016)** demonstrated sensitivity in clinical specimen's equivalent to 1200 IU/mL HCV RNA. Other studies suggesting it is around 7000 IU/mL **(Zhang et al. 2007** and **Abed Al-Gani, 2011)**. Furthermore, **Feng et al. (2014)** indicated that enzyme immunoassay tests for HCVcAg do not detect it when HCV RNA is below 15,000 IU/ml, which limits their use in clinical practice.

In this study, the sensitivity, specificity, PPV, NPV of the HCVcAg assay to detect HCV infection, compared to HCV RNA in studied donors were 97.14%, 100%, 100% and 99.76% respectively. Our

results were higher than those reported by **Park et al. (2010)** (90.2%), and **Feng et al. (2014)** (95.2%). Similar specificity was reported by **Morota et al. (2009)** (99.98%), **Miedouge et al. (2010)** (99.2%), **Ross et al. (2010)** (100%) and **Medici et al. (2011)** (100%).

Koroglu et al. (2012) found that sensitivity, specificity and positive and negative predictive values were 96.9%, 100%, 100% and 99.1%, respectively. **Kesli et al. (2011)** found that sensitivity, specificity and positive and negative predictive values were 96.3%, 100%, 100% and 89.7%, respectively. In addition, **Park et al. (2010)** obtained similar results comparing HCV RNA with HCVcAg. Sensitivity, specificity and positive and negative predictive values were determined to be 90.2%, 100%, 100% and 86,4%, respectively.

The optimum cutoff of the HCVcAg assay in our study was 2.82 fmol/l (i.e. 0.06 pg/ml). This assay (Architect HCV core antigen assay) is therefore more sensitive than similar assays utilized in a study of **Bucket et al. (2014)** i.e. 3.44 fmol/l. In addition, the ROC curve analysis showed a closer sensitivity and specificity rates with HCV RNA results if HCVcAg is ≥2.82 fmol/L. This finding was close to the manufacturer's cut-off (3 fmol/L). However, we considered that the small difference might be due to the low number of negative patients.

In our study there was a statistical significant positive link between HCVcAg and HCV RNA levels. Numerous studies have explored the correlation between HCV RNA values and HCVcAg **(Morota et al., 2009; Miedouge et al., 2010; Murayama et al., 2012; Waldenstr?m et al., 2013; Bucket et al., 2014** and **Tillmann, 2014)**.

This would indicate that detection of HCVcAg assay in serum or plasma is useful as an indirect marker of HCV replication due to the good correlation between HCVcAg and HCV RNA levels, as well as HCVcAg assay is highly sensitive compared to HCV RNA tests. Such studies explored the option to shorten the window of HCV seroconversion when HCVcAg would be used vs anti-HCV testing, and compared the performance of the HCVcAg to that of HCV RNA testing, and in common HCVcAg leads to earlier detection compared to anti-HCV.

However, since we conducted this study with anti-HCV antibody positive serum samples, we did not have any sample with a result like HCVcAg -reactive and anti-HCV antibody negative. So, we were not able to consider whether the early HCV infection without antibodies could be detected using the HCVcAg assay. But high specificity and the simplicity of HCVcAg, the rapid turnaround time of the results and its lower price suggest that it could be cost effectively applied in clinical practice.

CONCLUSION

The Architect HCVcAg assay potentially offers a good alternative to HCV RNA quantification. It is operationally easier with low time-assay and low cost per assay, suggesting that it is likely to be established in the blood banks as a tool to aid in early diagnosis of HCV infection.

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إمكانية إستخدام مستضد لب فيروس سي كبديل لتفاعل البلمرة التسلسلي في التشخيص المبكر لفيروس سي لدي المتبرعين بالدم

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خلفية البحث: يعتمد نقل الدم بطريقة آمنة علي المسح المناسب للعدوي المتنقلة خاصة فيروس سي الكبدي للمتبرعين بالدم. ويعتبر إختبار البلمرة التسلسلي لفيروس سي هو أهم الإختبارات في تشخيص فيروس سي ولكن هناك صعوبة في عمل هذا الإختبار وزيادة تكلفته المادية و طول وقت إجرائه مقارنة بتحليل مستضد لب فيروس سي.

الهدف من البحث: تقييم تحليل مستضد لب فيروس سي كبديل لتفاعل البلمرة التسلسلي في الكشف المبكر عن فيروس سي في المتبرعين بالدم.

المرضي و أدوات البحث: أجريت الدراسة الحالية علي 4222 متبرع بالدم (3837 من الذكور و385 من الاناث) ببنك الدم الرئيسي - جامعة نجران - السعودية العربية في الفترة من شهر مارس 2015 وحتي شهر يونيو 2016. وتم الكشف عن الفيروس الكبدي بي و سي و الزهري و فيروس الإيدز، وإستبعاد كل ما هو موجب تجاه الفيروس الكبدي بي والزهري و فيروس الإيدز، وتم عمل إختبار تفاعل البلمرة التسلسلي و تحليل مستضد لب فيروس سي علي وحدات الدم موجبة مضادات فيروس سي و التي بلغت 76 وحدة دم : 72 من الذكور و 4 من الإناث.

النتائج : كان تحليل مستضد لب فيروس سي إيجابي في 65 حالة ، وكان مستواه في المساحة الرمادية في 3 حالات ، و سلبي في 8 حالات من إجمالي 76 حالة موجبة مضادات فيروس سي. بينما كانت نتائج تحليل تفاعل البلمرة التسلسلي إيجابي في 71 حالة بنسبة 93.42% ، وإيجابي في ثلاث من ثمان حالات موجبة مضادات فيروس سي وسالبة مستضد لب فيروس سي

الإستنتاج: إرتفاع نسبة حساسية وخصوصية تحليل مستضد لب فيروس سي بنسب قريبة من تفاعل البلمرة التسلسلي ، مع قصر مدة إجراء التحليل و قلة تكلفته المادية تجعل إمكانية لإستخدام تحليل مستضد لب فيروس سي كبديل لتفاعل البلمرة التسلسلي في الكشف المبكر عن فيروس سي في المتبرعين بالدم