

Detection of Hepatitis C Virus 5' Noncoding RNA in Serum Samples

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Objective: To evaluate the level of 5' noncoding region (copies/ml) of Hepatitis C Virus and correlate that with the clinical and laboratory parameters.

Design: Experimental controlled trial.

Setting: National Hospital, Riyadh, Saudi Arabia.

Method: Sixty-four patients were included in this study, 47 patients were sero-positive for anti-HCV antibodies by third generation enzyme immunometric assay (EIA). Quantitative and qualitative HCV 5' noncoding RNA from serum samples was detected by nested PCR. Another four serum patients' samples were investigated for hepatitis C virus genotyping. In addition to 13 normal control subjects were included in this study.

Result: Alanine transaminase (ALT) levels were raised during the acute infection (mean 83.51). The clinical features were varied from 23 (45%) asymptomatic patients to 17 (33%) who had jaundice, 46 (90%) complained from fatigue and 34 (66.6%) had nausea and vomiting.

Among the 51 HCV-RNA positive samples obtained from patients, 12 samples had below 2,000 copies of HCV-RNA/ml, 4 had between 2,001 and 15,000 copies, 3 had between 15,001 and 50,000 copies, 4 had between 50,001 and 100,000 copies, 9 had between 100,001 and 300,000 copies, and 19 had over 300,000 copies of HCV-RNA/mL.

Conclusion: 5' noncoding region of HCV could completely distinguish between genotype and subtype of HCV and these could be important for the initiation of treatment.

Bahrain Med Bull 2010; 32(4):

HCV is a member of the genus *Hepacivirus* and of the family Flaviviridae. It is an enveloped virus with an RNA genome of approximately 9,400 bp. Most of the genome forms a single open-reading frame that encodes three structural (core, E1, E2) and seven non-structural (p7, NS2-NS5) proteins. Comparison of sub genomic regions has allowed variants to be classified

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into six genotypes. Short untranslated regions (UTRs) at each end of the genome are required for replication of the genome, a process that has recently been found to require a cis-acting replication element in the coding sequence of NS5B¹.

Variability is distributed throughout the genome with the non-structural gene of different genotypes shown 30-50% nucleotide sequence disparity². Some patients have a persistently normal ALT; therefore, the disease could be detected by checking HCV antibodies.

One hundred and seventy million individuals are estimated to be infected with hepatitis C virus (HCV) worldwide; therefore, hepatitis C has a strong impact on public health. A vaccine protecting against infection is not available, due to rapid change in the enveloping proteins. Antiviral therapy offered is characterized by limited efficacy, high costs and substantial side effects^{3,4}. Studies on the natural history of hepatitis C indicate that 55% to 85% of patients who develop acute disease remain infected and develop chronic hepatitis. Among these individuals, 5% to 20% are at risk of developing cirrhosis within a period of 20 to 25 years^{5,6}. Most patients will not be diagnosed until they present later with an evidence of abnormal transferase values at health checks or with chronic liver disease.

The aim of this study is to evaluate the level of 5' noncoding region (copies/ml) of HCV and correlate that with the clinical and laboratory parameters.

METHOD

Sixty-four patients were included in this study, 47 patients were sero-positive for anti HCV antibodies. The patients were anti hepatitis B surface antigen negative and HIV I & II antibodies negative. No patients had previously received any antiviral or immunosuppressive therapy nor did they have any potential cause of liver disease, such as cancer, cirrhosis, alcohol intake or autoimmune disease affecting the liver. Thirteen serum samples from normal individuals served as controls. The hospital ethical committee had approved the study.

Serum samples were removed from each erythrocyte clot 1 to 4 hour after venipuncture and were allocated into five tubes and stored at -70°C until being thawed for testing.

The detection of HCV was carried out by using Roche Amplicor HCV Monitor v 2.0 kits assays as per the manufactures instructions. Roche assays were based on reverse transcription and amplification of HCV-RNA with primers for 5' noncoding region in presence of internal control which shared primers with HCV. Primary calculations of IU/ml were performed by SoftMax Pro v 3.0 (Molecular Devices, Inc.) using the formulas described in the package insert.

Statistical Analysis was performed using the Instat (Instat Biostatistics, GraphPad Package USA).

RESULT

Twenty-three (45.1%) patients were asymptomatic, 17 (33.3%) had jaundice, 46 (90.2%) complained from fatigue, 34 (66.7%) had nausea and 18 (35.3%) had been vomiting, see table 1.

Table 1: Demographical and Biochemical Features of HCV Patients

	Number and percentage (n=51)
Asymptomatic	23 (45.1)
Jaundice	17 (33.3)
Nausea	34 (66.7)
Fatigue	46 (90.2)
Vomiting	18 (35.3)
Cirrhosis	20 (39.2)
Anorexia	41 (80.4)
AST Mean \pm SD	53.74 \pm 26.43
ALT Mean \pm SD	83.51 \pm 37.75

Among those who suffered from jaundice, the peak levels were usually less than 2 mg/dL bilirubin. Fulminant hepatic failure with primary HCV infection was seen in 3 (5.9%). Five individuals (9.8%) had symptoms before the sero conversion to anti HCV occurred.

During acute infection, person's ALT levels rose to 200-600 IU/L. There was often epidemic fluctuating pattern of ALT levels during the course of disease, in others ALT levels were getting normal.

None of the thirteen normal controls (negative for hepatitis C) showed any detectable 5' noncoding region (copies/ml) of HCV.

Fifty-one HCV-RNA positive samples obtained from patients, 12 samples had below 2,000 copies of HCV-RNA/ml, 4 had between 2,001 and 15,000 copies, 3 had between 15,001 and 50,000 copies, 4 had between 50,001 and 100,000 copies, 9 had between 100,001 and 300,000 copies, and 19 had over 300,000 copies of HCV-RNA/mL, as determined by the AMPLICOR HCV Monitor assay, see table 2. According to the manufacturer's recommendations, all samples which tested positive by the AMPLICOR HCV Microwell plate assay and negative by AMPLICOR HCV Monitor were defined as having below 2,000 copies of HCV-RNA/mL.

Table 2: mRNA Levels (copies /mL) in Serum Samples of HCV Patients

mRNA copies/mL	Number and percentage
Less or equal 2000	12 (23.5)
2001-15000	4 (7.8)
15001-50000	3 (5.9)
50001-100000	4 (7.8)
100001-300000	9 (17.6)
Over 300000	19 (37.2)

Four patients decided to go for treatment and investigated for hepatitis C virus genotyping, which was performed by hybridization with sequence-specific oligonucleotides, the result as follow: three had genotype 4, one subtype 4a/4c/4d and the remaining three no subtype differentiated, the fourth patient showed genotype 3 (subtype 3).

DISCUSSION

Patients with chronic hepatitis C infection are usually asymptomatic, the disease being discovered only following a routine biochemical test when mild elevation in the ALT are

noticed^{7,8}. Some patients have a persistently normal ALT and the disease could be detected by checking HCV antibodies. Fatigue is the commonest symptom (90.2%); anorexia (80.4%), nausea (66.7%) and weight loss, the latter does not correlate with disease activity.

Generally diagnosis of HCV infection by convention relies on detection by serologic assays, such as ELISA and confirmed by recombinant immunosorbent assay, which are in their third generation and have improved sensitivity and specificity compared with those of previous generations⁹.

Researcher found that nearly all methods are designed to distinguish sequences either in the 5'NCR or in the nearby^{10,11}.

5'-UTR assay is designed for the detection of HCV; it provides a sensitive, standardized amplification protocol specifically intended for large volume testing and commonly used in HCV genotyping.

Suspected cases of acute hepatitis C advised to be tested for both anti-HCV antibodies by EIA and HCV-RNA; the lower limit for detection is 50 IU/mL or less¹². The knowledge and skill to detect and manage HCV infection have improved. The incidence of HCV infections has declined, but the complications of those infected are predicted to increase¹³.

As new therapeutic options evolve and laboratory assays change, the clinical relevance and use of laboratory testing for HCV will require frequent reassessments^{14,15}.

The HCV strains can be classified into at least 6 major genotypes, and a large number of subtypes within each genotype. Genotype I is by far the most frequent genotype in chronically infected patients worldwide, with subtypes Ia and Ib representing the vast majority of circulating strains¹.

HCV genotyping and quantitative evaluation of HCV-RNA could be helpful in managing chronic HCV and interferon treatment.

5' noncoding HCV-RNA assays are advised to predict the response to the full course of therapy¹⁵.

For patients receiving antiviral therapy, it is recommended the cessation of treatment for patients with detectable HCV-RNA after three months of therapy.

To the best of our knowledge, this is the first study of its kind performed in ethnic region of Saudi Arabian, while most studies focused directly on genotyping of chronic HCV, the present study stressed on the importance of different HCV 5' noncoding RNA because consistent data are lacking in this country^{16,17}.

Similar studies have been performed using primers specific for 3' noncoding region, and 5' noncoding region of HCV¹⁸. Others used saliva sample for HCV while Japanese used peripheral blood^{19,20}.

Several studies have reported the presence of HCV core protein in 80-90% of patients while others 90-95% in patients with HCV- RNA²¹. The present study provides additional evidence

of the HCV 5' noncoding RNA in Saudi Arabian population which is different than reported from other Arab countries, but it has similar pattern to Europe and United States²².

In a nutshell, it is more appropriate to use HCV-RNA based assays for the detection of acute liver diseases and advise therapy according to HCV-RNA load.

Farther molecular study for 5' noncoding RNA (copies/ml) and genotype studies with large sample are essential for clinical and therapeutic purposes.

CONCLUSION

5' noncoding region of HCV could distinguish between genotype and subtype of HCV. Therefore, HCV 5' noncoding region genotyping methods give sufficient information for clinical purposes. More studies are warranted before guidelines could be established for the routine use of genotyping.

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