ORIGINAL RESEARCH PAPER

Microbiology

HEPATITIS C VIRUS AND ITS RAPID DIAGNOSIS-UPDATES

KEY WORDS: Hepatitis C Virus, Cirrhosis, Hepatocellular carcinoma, Enzyme immunoassay, Direct-acting antiviral (DAA), Ribavirin.

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INTRODUCTION

Hepatitis C virus (HCV) is a major blood borne disease. According to WHO, HCV is responsible for affecting 58 million people worldwide, with approximately 1.5 million new HCV infections annually [7]. It is a systemic disease with primary inflammation of the liver. In case of acute infection, it often progresses into chronic hepatitis C and puts the patient at a high risk of developing chronic complications such as cirrhosis and hepatocellular carcinoma. It is also responsible for causing portal hypertension and hepatic decompensation with encephalopathy. HCV is a small virus, 56-65 nm diameter, icosahedral in shape, enveloped and carries positive-single stranded RNA. It is enclosed within a core that is about 45nm in diameter and carries glycoprotein spikes.

DISCOVERY

In 1970's, clinicians closely observed cases of transfusion associated with hepatitis that often progressed into a chronic disease that could neither be attributed to Hepatitis A (HAV) nor Hepatitis B (HBV) and it was named as non- A, non- B hepatitis (NANBH). This newly discovered disease was found to be responsible for causing 90% of the post transfusion hepatitis. The presence of the transmissible disease-causing agent was confirmed in the year 1978 as HCV.

The two important studies done by Alter et al.[1] and Tabor et al.[6] demonstrated that on injecting the plasma or/and serum of a patient with NANBH in healthy chimpanzees induced clinically apparent hepatitis, with an onset time of 2-10 weeks, which was similar to the incubation period seen in humans with NANBH.

Very quickly it became obvious that the disease transmission was not limited to blood transfusions but also via intravenous immunoglobulin transfusion and an increased number of cases were documented among patients with hemophilia. 50-80% of people with NANBH developed chronic progressive liver disease on long- term observation. A major breakthrough was achieved when Choo et al.[4] from Michael Houghton's group at Chiron, USA published the discovery of HCV in 1989. They believed that the past attempts failed due to very low viral concentration. Thus, they designed a complementary DNA (cDNA) library using randomly created primers, reverse transcriptase and plasma from an infected chimpanzee with high titre of the presumed infectious agent. The cDNA was cloned by inserting into a viral cloning vector, phage Lambda-gt11 expressed in Escherichia coli that induces the production of the cDNA encoded polypeptides. Using serum from a patient with chronic NANBH, around 1 million clones were screened to identify the viral proteins, until finally the cDNA clone 5-1-1 was identified. After confirming the reactivity of the cDNA clone 5-1-1 with the sera of seven different patients with NANBH and by conducting other experiments, enough proof was established that the discovered infectious agent was indeed an RNA virus, which was later termed as HCV.

ETIOLOGY

HCV is one of the six hepatitis viruses known i.e., hepatitis A, B, C, D, E and G. According to a study conducted in CMC hospital, Ludhiana by Berry et al.[2] during 2005 to 2009, HCV had the highest prevalence of 37.11% among all the five types of hepatitis viruses. It belongs to the genus Hepacivirus under family Flaviviridae. The single stranded, positive- sense RNA encodes for a polyprotein that gets further processed into various proteins. Three of these proteins are "structural" proteins namely, the nucleocapsid proteins, core (C) and envelope proteins. The envelope proteins have 2 subtypes E1 and E2. The two proteins p7 and NS2 essential for virion production. The five nonstructural proteins are essential for virion replication complexes (NS3, NS4A, NS4B, NS5A, and NS5B).

A closely related but diverse viral variant called "quasispecies" is produced in the HCV patients as a result of high-level virion turnover by the NS5B (nonstructural protein 5B) RNA polymerase with an absence of proofreading. 1%-5% variation in the nucleotide sequence is seen in a single HCV patient. This accumulation of nucleotide substitution has resulted in the various subtypes classified into eleven genotypes, which differ in their nucleotide sequence by 30%-50%. Among these the first seven genotypes are the major ones, with genotype 1 being the most widespread one worldwide (60%-70%) [3].

EPIDEMIOLOGY

HCV can be transmitted through various ways. HCV is mostly transmitted by needle stick injuries, transfusion of infected blood and blood products, use of contaminated needles and syringes and through intravenous drug abuse. HCV can also be acquired through sexual intercourse, which is rarely seen. Vertical transmission of HCV has also been reported. HCV RNA can be detected from blood (including serum and plasma), seminal fluid, saliva, tears, cerebrospinal fluid and ascitic fluid. The virus can be detected in the plasma from a few days to 1-4 weeks following exposure. The viral count in the blood peaks in the first 8 to 12 weeks of the infection and then drops to undetectable levels in some patients but in the majority (50% to 80%) of the patients it persists.

LABORATORY DIAGNOSIS

The patient's blood (serum/ plasma) sample is being collected for the detection of the hepatitis C viral agent.

Screening Tests:

One of the various serological tests done for the detection of HCV includes antigen-antibody reactions (HCV TRI-DOT), an enzyme immunoassay (EIA). This rapid card assay is a qualitative test.

Development of enzyme immunoassay:

In the beginning, the first-generation immunoassay used the recombinant c100-3 epitope from the NS4 region for the detection of HCV in patients. However, the window period from the infection to the detection of protein was 16 weeks to 24 weeks. The first-generation lacked enough specificity and sensitivity. To overcome the drawbacks in the first-generation, the second-generation assay was developed in 1992. The

second-generation assay used epitopes c22, c33c, c200 and HC-31 from the HCV core, NS3, NS4, and NS4 regions respectively. In the second-generation the window period was 10 to 24 weeks which is almost parallel to the first-generation assay. But the second-generation assay had a higher sensitivity compared to the first generation, making it applicable in clinical settings but it was associated with a high rate of false positives and false negatives.

The third-generation assay was developed in 1996 with a basic principle to detect anti- HCV antibodies in plasma /serum against several HCV protein epitopes. The third-generation assay used a multi target format that included detection of greater range of antigens from the core (c22p), NS3 (c33c), NS4 (C100-3, 5-1-1p), and NS5 regions. Third-generation assay was more effective in reducing the window period to 7 to 8 weeks compared to the previous assays. However, the drawback in the third-generation assay was that the low positive predictive values resulted in a low prevalence of HCV infection.

This led to the development of the fourth-generation assay, which simultaneously detects the HCV antigen and antibody. This assay is commonly known as the antigen–antibody combo assay. The fourth-generation assay is advantageous as it provides a single platform where the two HCV markers-antigens and antibodies are identified in the same test. This makes the assay highly applicable in resource-constrained settings. The fourth-generation assay is very sensitive as the window period is reduced to an all-time low of 26 days. The fourth-generation assay was reported to increase reactivity for the detection of antigens derived from the core, NS3, NS4A, NS4B and NS5A regions for the detection of HCV genotypes 1a and 1b. This results in improved detection of NS3 and NS4 antigens and allows highly sensitive detection of HCV genotypes 2 and 3a.

Table no. 1

Type Of Test Generation / Year	Components Detected
1st Generation (1989)	Ÿ NS4 (C100-3) Ÿ Window Period: 16-14
2nd Generation (1992)	Core (c22-3) NS3 (c33c) NS4(C200, HC-31) Window Period: 10-24 weeks
3rd Generation (1996)	Core (c22p) NS3 (c33c) NS4 (C100-3, 5-1-1p) NS5 Window Period: 7-8 weeks
4th Generation (2003)	Core(c22) NS3(c200) NS4(c200) NS5 Window Period: 26 days

TREATMENT

Hepatitis C is treated with antiviral medicines. Hepatitis C is treated using direct-acting antiviral (DAA) drugs, which are the safest and the most effective medicine for treating hepatitis C[5]. These are highly effective at clearing the infection in more than 90% of the patients when taken for 8 to 12 weeks depending on the type of HCV being treated. Some types of hepatitis C can be treated using more than 1 type of DAA. NHS (National Health Service) approved medicines used to treat HCV include:

- Sofosbuvir.
- Combination of ledipasvir and sofosbuvir.
- Combination of elbasvir and grazoprevir.
- Combination of velpatasvir and sofosbuvir.
- Ribavirin.

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