REVIEW ARTICLE

Hepatitis C-Clinical Outcome and Diagnosis

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Introduction

Nearly 170 million people worldwide have been infected with Hepatitis-C Virus (HCV). About 85% of these develop persistent infection and are at risk of long term complications like liver cirrhosis and hepatocellular carcinoma-HCC (1,2). HCV has been classified into 1-6 genotypes on the basis of the identification of their different genomic sequences (3). The main role of HCV genotyping is to predict the likelihood to maintenance of long-term response to therapy, as the genotypes are associated with susceptibility to antiviral therapy (4).

The genome of hepatitis C virus (HCV) is identified as a separate entity by using a recombinent complimentary DNA (cDNA) approach (5,6). Hepatitis-C virus (HCV) belongs to the family Flaviviridae. It is a spherical, 30-60nm in diameter, enveloped, single stranded RNA virus. With the elimination of HBsAg positive blood, HCV became the commonest cause of post transfusion hepatitis.

The clinical course of untreated HCV infection is highly variable with the majority of patients experiencing a slow fluctuating disease that may take 20 years or more for full expression. The incubation period is about 7 weeks with a range of 2 to 26 weeks. After initial exposure HCV-RNA can be detected in serum by PCR within a few weeks. Most patients develop liver cell injury within a few months as indicated by elevated alanine aminotransferase. A large percentage of patients are physically asymptomatic and anicteric. A “silent period” may ensue for weeks to month after initial infection, during which viral titres are low and antibody responses are not detectable. Seroconversion to HCV antibody positive status occurs within 3 months in the majority of the exposed patients, but in some it may take upto 6 months. Fulminant hepatitis C is extremely rare. Approximately half of HCV patients develop chronic active hepatitis and this may progress to liver cirrhosis and hepatocellular carcinoma-HCC (2).

Chronic HC means the presence of disease for longer for more than 6 months. Chronic HCV infection follows an insidious course involving episodes of elevated serum aminotransferases and hepatocellular injury, often associated with fluctuations or increases in HCV titre. Nonspecific physical symptoms such as weight loss, fatigue, muscle or joint pains, irritability, nausea, malaise, anorexia and pain in hepatic region develop in about 20% of the cases.

The development of severe fibrosis and necro-inflammatory changes in liver indicate poor prognosis. Hepatitis C has become an increasingly important public health problem in recent years (7). Chronic hepatitis C accounts for 40% of the deaths from chronic liver disease. The development of cirrhosis worsens prognosis and enhances the risk of hepatocellular carcinoma.

Chronic hepatitis C varies greatly in its course and outcome. At one end of the spectrum are patients who have no signs or symptoms of liver disease and have normal levels of serum liver enzymes. Liver biopsy usually shows some degree of chronic hepatitis, but the degree of injury is usually mild, and the overall prognosis may be good. At the other end of the spectrum are patients with severe hepatitis C who have symptoms, HCV-RNA in serum, elevated serum liver enzymes and who ultimately develop cirrhosis and end-stage liver disease. In the middle of the spectrum are many patients who have few or no symptoms, mild to moderate elevations in liver enzymes, and an uncertain prognosis. Chronic hepatitis C can cause cirrhosis, liver failure and liver cancer. Researchers estimate that approximately 20 percent of patients with chronic hepatitis C develop cirrhosis, a process that takes at least 10 to 20 years. After 20 to 40 years, a smaller percentage of patients with chronic disease develop liver cancer.

Hepatitis C is the cause of about half of cases of the primary liver cancer in the developed world. Men, alcoholics, patients with cirrhosis, people over 40 years of age, and those infected for 20 to 40 years are more likely to develop HCV related liver cancer.

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Risk Factors, Transmission and Epidemiological Features

HCV is spread primarily by contact with blood and blood products. With the introduction in 1991 of routine blood screening for HCV antibody and improvements in the test in the mid-1992, transfusion-related hepatitis C has virtually disappeared. At present, injection drug use is the most common risk factor for contracting the disease.

Pooled plasma or its derivatives have a high risk of transmission eg clotting factors used for hemophiliacs. Hemodyialysis patients have a seropositivity rate of 10% to 40%. Sexual exposure accounts for 10% to 20% of new cases. The risk of HCV infection from an HCV positive partner in a long term monogamous relationship is as low as 1.5%. The risk of HCV transmission after a single exposure is negligible. The other risk factors besides hemodialysis are occupational, household and perinatal exposures, which combined, account for 10% of all HCV infections. Viral transmission to infants from HCV infected mothers is approximately 5%. The rate increases when the mother is coinfected with HIV. Breast feeding is not important in transmission of HCV, hence it is not contraindicated. Because of the high prevalence of this disease, transmission prevention counselling is an important measure to be framed up.

The incidence is reduced in developed countries where blood banks are employing tests for presence of HCV antibodies or HCV RNA before collection of blood. But so far this is woefully neglected in many developing countries including India (8). HCV testing of suspected asymptomatic persons or items should be carried out in injection drug abusers either current or former, recipients of untested blood or blood products, hemodialysis patients, persons with persistently elevated alanine aminotransferase concentration, blood donors, organ donors, children born to HCV positive women and health care workers with percutaneous or mucosal exposure to HCV positive blood. Nosocomial HCV transmission during dialysis, bronchoscopy and surgery has also been reported (9).

The effective screening of blood donations by intensive questionnaire and the use of specific 3rd generation HCV antibody testing can greatly reduce the risk of transmission from blood to negligible levels. PCR-RNA test would pick up any possible infection during the early months of infection (window period).

Sporadic Transmission: Sporadic transmission, when the source of infection is unknown, occurs in about 10 percent of acute hepatitis C cases and in 30 percent of chronic hepatitis C cases. These infections may have come from exposure to the virus from cuts, wounds, injections and medical procedures. The rate of HCV seroconvension among health care workers after a needlestick injury is 0 to 7% (10). In the United States, multiple-use vials are a frequent culprit in leading to nosocomial spread of hepatitis C.

HCV infection is present worldwide and the varying prevalence of the disease depends on the geographical area. It also varies with age. The highest prevalence of HC is found in the age group of 30-40 years.

Of the 170 million people worldwide who have evidence of HCV infection about 66% are chronically infected. Chronic HCV infection leads to 8000 to 10,000 deaths annually (2). The frequency of patients with complications of HCV is expected to triple within the next 20 years resulting in a large increase in incidence of cirrhosis and hepatocellular carcinoma and an increased demand for liver transplantation. This will throw a heavy burden on treatment for the disease all over the world. HCV is now known to be responsible for most of the transfusion associated hepatitis.

The global prevalence of HCV carriers is estimated to average 3% ranging from 0.1 to 10%. There is high prevalence in Mongolia, Vietnam, Myanmar, China, Central Africa and Egypt. The highest prevalence of 10% or more is seen in Mongolia, Egypt, Tanzania, Ceisinea and Cameroon (2).

Clinical Symptoms and Signs

Many people with chronic hepatitis C have no symptoms of liver disease. If symptoms are present, they are usually mild, nonspecific, and intermittent. They may include fatigue, mild right-upper-quadrant discomfort or tenderness, nausea, poor appetite as well as muscle and joint pains. Similarly, the physical examination is likely to be normal or show only mild enlargement of the liver or tenderness and vascular spiders or palmer erythema.

Once a patient develops cirrhosis or if the patient has severe disease, symptoms and signs like muscle weakness, poor appetite, nausea, weight loss, itching, dark urine, fluid retention, and abdominal swelling are commonly seen. Physical findings of cirrhosis may include enlarged liver, enlarged spleen, jaundice, muscle wasting, excoriations, ascites and ankle swelling.

Complications that do not involve the liver develop in 1 to 2 percent of people with hepatitis C, termed as extrahepatic manifestations. The most common is cryoglobulinemia, which is marked by skin rashes (purpura, vasculitis, or urticaria), muscle and joint aches, kidney

disease, neuropathy, cryoglobulins, rheumatoid factor, and low complement levels in serum. Other complications are glomerulonephritis, porphyria cutanea tarda, seronegative arthritis, keratoconjunctivitis sicca (Sjogren's syndrome), non-Hodgkin's type B-cell lymphomas, fibromyalgia and lichen planus.

Diagnosis of Hepatitis C

In chronic hepatitis C increases in the alanine and asparate aminotransferases range from 1 to 20 times (but usually less than 5 times) the upper limit of normal. Alanine aminotransferase (ALT) levels are usually higher than aspartate aminotransferase (AST) levels, but that finding may be reversed in patients who have cirrhosis. Alkaline phosphatase and gamma glutamyl transpeptidase are usually normal. If elevated, they may indicate cirrhosis.

Rheumatoid factor and low platelet and white blood cell counts are frequent in patients with severe fibrosis or cirrhosis, providing clues to the presence of advanced disease.

The enzymes lactate dehydrogenase and creatine kinase are usually normal. Albumin levels and prothrombin time are normal until late-stage disease. Iron and ferritin levels may be slightly elevated. The presence of HCV RNA indicates that the patient has ongoing viral infection despite normal ALT levels.

Acute hepatitis C is diagnosed on the basis of symptoms such as jaundice, fatigue and nausea, along with marked increase in serum ALT (usually greater than 10 fold elevation), and presence of anti-HCV or de novo development of anti-HCV.

In 30 to 40 percent of patients, anti-HCV is not detected until 2 to 8 weeks after onset of symptoms. In this situation, testing for HCV RNA is helpful, as this marker is present even before the onset of symptoms and lasts through the acute illness. Another approach to diagnosis of acute hepatitis C is to repeat the anti-HCV testing a month after onset of illness. Almost all patients with chronic infection will have the viral genome detectable in serum by PCR.

Diagnosis is problematic in patients who cannot produce anti-HCV because of immunosupression. Thus, HCV RNA testing may be required for patients who have a solid organ transplant, are on dialysis, are taking corticosteroids, or have agammaglob ilinemia. Diagnosis is also difficult is patients with anti-HCV who have another form of liver disease that might be responsible for the liver injury, such as alcoholism, iron overload, or autoimmunity. In these situations, the anti-HCV may represent a false-positive reaction, previous HCV infection, or mild hepatitis C occurring on top of another liver condition. HCV RNA testing in these situations helps confirm that hepatitis C is contributing to the liver problem.

Screening tests for hepatitis C include ELISA for anti HCV and PCR for HCV-RNA (11,12). First generation EIAs (EIAs 1.0) used the c100.3 epitope of an NS protein (NS4) (13). The sensitivity of these EIAs were low for a high prevalence population (approximately 80%) and the fraction of positive results that were false positive as high as 70% for a low-prevalence population (blood donors) (14). This led to the development of more sensitive and specific second generation EIAs. Second generation assays detect HCV antibodies in 20% more patients with acute non A-non B hepatitis and in 10% more patients with chronic infection much earlier (15). The third-generation test (EIA-3) used today is more sensitive and specific than previous ones. However, as with all enzyme immunoassays, false-positive results are occasionally a problem with a EIA-3, additional or confirmatory testing is often helpful. Fig.1 depicts HCV genome and recombinant proteins.

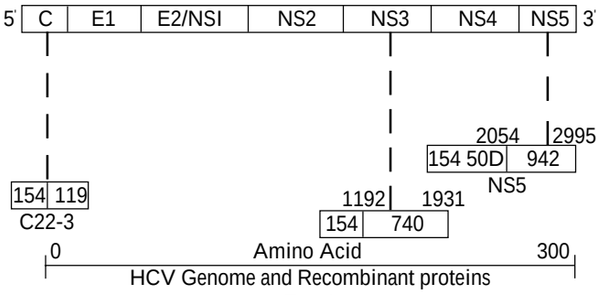

Fig. 1

Immunoblot assays can be used to confirm anti-HCV reactivity as well. These tests are also called "Western blots". In some clinical situations, confirmatory testing by immunoblotting is helpful, such as for the person with anti-HCV detected by EIA who tests negative for HCV RNA. The EIA anti-HCV reactivity could represent a false-positive reaction, recovery from hepatitis C, or continued virus infection with levels of virus too low to be detected (the last occurs only rarely when sensitive PCR or TMA assays are used. If the immunoblot test for anti-HCV is positive, the patient has most likely recovered from hepatitis C and has persistent antibody. If the immunoblot test is negative, the EIA result was probably a false positive. Immunoblot assays are highly specific and valuable in verifying anti HCV reactivity. PCR and TMA amplification can detect low levels of HCV RNA in serum.

Antigen detection

An accurate and specific ELISA for the detection and quantitation of HCV core antigen has recently been developed. The assay has better sensitivity than an earlier version because of an immune complex dissociation step prior to antigen detection with monoclonal antibody. The performance of the assay correlates well with those of molecular HCV RNA detection methods, but the lower level of detection (20,000 IU/ml) is significantly higher. A more sensitive assay is under development.

Recently the 4th generation assay for testing of anti-HCV has been established. The 4th generation HCV TRI-DOT utilizes a unique combination of modified HCV antigens from the putative core, NS3, NS4 and NS5 regions of the virus to selectively identify all subtypes of hepatitis C virus in human serum plasma with a high degree of sensitivity and specificity. The antigens used are chemically treated and unfolded in a special way to make them more reactive and specific to different epitopes of the core and NS3 region thereby minimizing the chances of cross reactivity and enhancing the specificity. Also, the superior sensitivity of the test allows for the significantly earlier detection of antibodies during seroconversion following HCV infection, thereby reducing the incidence of post transfusion hepatitis and providing a safer blood supply.

4th generation HCV tri-dot has been developed and designed using modified HCV antigens representing the immunodominant regions of HCV antigen. The device (an immuno-filtration membrane) includes two test dots 'T_1" and "T_2" and a "Built in Quality Control Dot". The control dot will always develop colour during the test, thereby confirming proper functioning of the device, reagent and correct procedural application.

Of the 25 to 35% of patients with acute infection who develop symptoms, only 50 to 70% will have detectable antibodies at that time, but 0% will have measurable antibodies after 3 months (16). Serologic assays detect HCV antibodies that indicate present or previous infection (0-3 months) (16) but they cannot discriminate acute from chronic or resolved infection. Anti-HCV IgM antibodies can be detected in 50 to 93% of patients with acute HCV infections and 50 to 70% of patients with chronic cases, so they are not a reliable indicator of acute infection (17). Occasionally immunocompromised patients, patients undergoing hemodialysis and patients with mixed cryoglobulinemia have false negative serology results and may require HCV-RNA listing for diagnosis (18).

Laboratories detect HCV RNA with commercially available assay kits or by in house home-brewed methods. Because of the limited amount of HCV RNA in infected individuals, a target or signal amplification step is needed. Reverse transcriptase (RT) PCR (RT-PCR) and transcription-mediated amplification (TMA) are target amplification methods. The branched DNA (bDNA) assay is a signal application technique (19).

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