



Case Study

Hepatitis C Viruses-A Case Study

M. Ram Mohan Rao^{1*} and Syam D Gopal²

Department of Microbiology, SRMC, Akathumuri, Varkala, TVM, India
Department of General Medicine, SRMC, Akathumuri, Varkala, TVM, India

*Corresponding author

ABSTRACT

Many viruses can produce infectious damage to the Liver. EBV and CMV occasionally cause symptomatic hepatitis, as part of the mononucleosis syndrome. The primary hepatitis viruses are diverse group. The “alphebit – soup” of hepatitis is summarised below. Several other viruses have been described as possible agents of hepatitis e.g. G.Virus (or G B variant of Hepatitis C virus, a member of the family Flaviviridae, as well as TT virus and SEN virus, both are the members of the family Circoviridae). Here prevalence of HCV and CHC (Chronic Hepatitis C) are increasing due to aging of the infected population and progression of liver fibrosis. History of repeated blood transfusions in a case of abortions in females, major trauma, alcoholism, biliary stenosis, parasitic infections and secondary liver infections, jaundice and needle stick injuries are chosen for study. Recent reports show that extra hepatic immunologic manifestation, such as cryoglobulinemia and rheumatoid factors are a prominent part of Hepatitis C infections and they are for Hepatitis B also. Seventy five patients are screened for various diseases. All serum samples collected and stored at -20°C freezer. ELISA was conducted with positive and negative controls. All the serum samples collected from date 1st October 2015 to 30th October 2015 at Sivagiri Sree Narayana Medical Mission Hospital, Varkala, Trivandrum after obtaining permission from the authorities. Out of 75 patient’s sample, 24 males and 51 females and most of them are middle age females groups having HCV infection.

Keywords

Hepatitis C
Virus,
Flaviviridae,
Cryoglobuli-
nemia,
biliary
stenosis

Introduction

After diagnostic reagents for hepatitis A and B viruses become available it became obvious that there were other causes of transfusion-associated hepatitis. The unknown virus or viruses were dubbed non-A, non-B hepatitis. Thanks to the sophisticated technology of molecular biology we now recognize hepatitis C as the cause of most cases of transfusion-

associated non-A, non-B hepatitis. The virus, which has not been cultivated, was eventually characterized by molecular techniques, one of the early triumphs of molecular biology. Surprisingly, the newly characterized agent was found to be most closely related to a group of viruses that are transmitted by arthropods; it is now a genus (Hepacivirus) in the family Flaviviridae.

Concepts of genetic relationships among hepatitis C virus strains are evolving. At least 11 genotypes have been described, but other investigations as sign the genotypes to six clades. Patients who are infected with strains of clades 1a and 1b (corresponding to genotypes 1a and 1b) respond less well to interferon treatment and progress more rapidly to chronic liver disease than do individuals who have been infected with other strains. Otherwise, there appear to be few phenotypic differences among genetically dissimilar strains (Alberti *et al.*, 1997).

The epidemiology of hepatitis C infections has many similarities to that of hepatitis B, but some significant differences are apparent. The most common route of spread is through blood product, including immune globulin, surgery, and intravenous-drug abuse. Screening of blood product of hepatitis C virus has substantially reduced the risk of transmission by this route. Sexual transmission occurs, but the frequency is much less than in hepatitis B infection. The prevalence of antibody to hepatitis C among healthcare workers in Baltimore was similar to that in the general population. Suggesting that workers are exposed to low levels of virus or that transmissibility is not great.

Acute hepatitis C virus infection is often less severe than that of hepatitis B, but the frequency of chronic hepatitis C disease is high. The subject has been reviewed by Iwarson and colleagues (1995).

The long-term mortality of patients who are infected with hepatitis C virus is similar to that of controls, although infected patients have a slightly increased risk of dying from liver disease. Once a patient has documented chronic infection, however, the outlook is much worse. Cirrhosis is a significant complication of chronic infection. Hepatitis

C infection is an independent risk factor for hepatocellular carcinoma after the development of cirrhosis. Extrahepatic immunologic manifestations, such as cryoglobulinemia and rheumatoid factor, are a prominent part of hepatitis C infection, as they are for hepatitis B.

Recognition of the disease

Viral hepatitis had been separated into two distinct diseases termed infectious hepatitis and homologous serum hepatitis during World War II. Later, the terms hepatitis A (HA) and hepatitis B (HB), respectively, were applied, and the infectious agents associated with these diseases were named hepatitis A virus (HAV) and hepatitis B virus (HBV). Until sensitive and accurate tests became available to distinguish patients who were infected by these viruses, however, the diagnosis was based on the clinical and epidemiologic features of the individual patient. With the discovery of Australia antigen, later termed hepatitis B surface antigen (HBs Ag), in 1965 and its association with hepatitis B in 1968, a clearer picture of viral hepatitis began to emerge. In 1971, a radioimmuno-precipitation test for antibody to HBsAg was developed that reached a sensitivity sufficient to identify virtually all patients who had previously been infected with HBV (Ling and Overby, 1972). Soon after, highly sensitive radioimmunoassay's (RIAs) for HBsAg become available as well as a test for antibody to the hepatitis B core antigen (HBcAg). With these three tests, virtually all patients infected by HBV in the present or past could be identified and their current clinical status determined.

Surprisingly, using these assays, it was found that most patients with transfusion-associated hepatitis did not have hepatitis B (Ali and Siddiqui, 1997). Because the only

other known hepatitis agent was HAV, which did not fit well with the epidemiologic or clinical picture of post transfusion hepatitis, there began to emerge the belief that there may be other, yet to be identified, hepatitis agents. In 1973, the hepatitis A virus was identified by immune electron microscopy (IEM) (Feinstone *et al.*, 1973). Although this assay was cumbersome, not only is capable of detecting HAV in some clinical samples but also it was reasonably sensitive for detecting antibody to HAV. To determine the role of HAV in post transfusion hepatitis, a study was undertaken in which very well characterized patients from the National Institutes of Health (NIH) Clinical Center prospective trials of post transfusion hepatitis were examined for the development of antibody to HAV after their acute hepatitis. Of 22 patients whose pretransfusion and posthepatitis sera were studied, none developed new antibody to HAV or rising levels of preexisting antibody as measured by IEM. A similar group of patients was characterized by Prince and colleagues, who also concluded that these cases were not hepatitis A based on clinical characteristics (Prince *et al.*, 2010). Finally, in a study of 13 patients with a total of 30 distinct multiple bouts of community-acquired hepatitis, only 2 cases could be diagnosed as hepatitis A, 12 were hepatitis B, and the remaining 16 were termed non-A, non-B hepatitis (NANBH) (Alter, 1999). From these studies, the concept of NANBH becomes accepted, and the search began for the causative agent.

Identification of the virus

During the ensuing 15 years a large number of reports appeared claiming to have discovered the virus, a viral antigen, or a specific antibody associated with NANBH. None of these reports proved accurate.

Nevertheless, many things were learned about the etiologic agent, the disease, and the epidemiology of NANBH. The agent was passed to chimpanzees (Alter, 1999). It was clearly shown that NANBH caused chronic infections, as would be predicted by the fact that it is frequently transmitted by blood transfusion from presumably chronic carriers. It was shown that NANBH could be a very serious disease that could result in chronic liver disease and cirrhosis, whereas the acuter disease was often relatively mild compared with that of transfusion-associated hepatitis due to HBC. The pathology was described at both the light and electron microscopic levels. Several viral characteristics were also described using the chimpanzee model for the infectivity readout. It was shown that the virus could be inactivated by lipid solvents, suggesting that the virus was enveloped. The virus was also shown to be able to pass through a 50-nm filter. Thus, a picture of a small enveloped virus emerged.

With the development during the 1980s of increasingly powerful and sensitive molecular biologic techniques, the detection of previously unrecognized infectious agents became possible. Using a large volume of plasma that had been collected over time from a chronically infected chimpanzee and that had a relatively titer of infectious virus (40), a group of scientists at the Chiron Corporation, led by Michael Houghton, made a lambda phage complementary DNA (cDNA) expression library from the nucleic acid extracted from the plasma (64). This library was screened with serum from a patient with chronic NANBH as a likely source of antibody to the virus. They identified a clone termed 5-1-1 that expressed an antigen that was shown to be specific for NANBH. They were able to show that the origin of the clone was a single stranded RNA molecule of about

10,000 nucleotides in length and that the RNA had one continuous long open reading frame (ORF). The expression product of clone 5-1-1 becomes the basis for the first serologic assay for antibody to the NANBH agent, which was now termed hepatitis C virus (HCV). The specificity of the clone and the antibody assay was determined by testing sera from patients who had previously been used to transmit NANBH to chimpanzees implicated blood donors, and transfusion recipients who developed NANBH. They found that at least 80% of patients with post transfusion NANBH developed antibody to 5-1-1, and 58% of patients with Chronic NANBH without a known exposure to blood had antibody. The 5-1-1 antigen remains a part of the commercial multiantigen test for HCV antibody in use today.

Later, sequence analysis of clones representing nearly the complete HCV genome showed that the virus most closely resembled the Flaviviridae. Since the discovery of HCV by the technology of modern molecular biology, many of the fine details of this virus have been determined, even though it still has not been grown to useful levels *in vitro*.

Infectious agent

Classification

HCV has a similar genomic organization and polyprotein hydrophobicity profile as the pestiviruses and flaviviruses and has been classified as a separate genus in the family Flaviviridae. The HCV viral particle is about 50nm in diameter and consists of an envelope derived from host membranes into which are inserted the virally encoded glycoprotein (E1 and E2) surrounding a nucleocapsid and a positive –sense, single-stranded RNA genome of about 9500

nucleotides. The genome contains highly conserved untranslated regions (UTRs) at both the 5' and 3' termini, which flank a single ORF encoding a polyprotein of 3000 amino acids. This is processed cotranslationally and posttranslationally by cellular and viral proteases to produce the specific viral gene products outlined in figure 1. The structural proteins, core, E1 and E2, are located in the N-terminal quarter, with the nonstructural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) in the remaining portion of the polyprotein.

Virion morphology

The genome of HCV was sequenced and characterized long before virus-like particles were described. It was shown early on that HCV is inactivated by chloroform (Feinstone *et al.*, 1983), indicating that particles are enveloped, and it is shown through filtration that the diameter is between 30 and 60 nm. Low levels of virus in plasma samples and problems of *in vitro* cultivation have made visualization of this virus difficult; however, virus-like particles have been identified using electron microscopy. Figure 2 shows spherical particles about 50 nm in diameter that specifically reacted to HCV antibodies, observed in thin-section electron microscopy of an *in vitro* infected cell line.

Sucrose gradient analyses of HCV positive sera and plasma have isolated both Low-density (1.06 to 1.13 g/mL) and high-density (1.17 to 1.25g/mL) fractions containing HCV RNA. It has yet to be established whether these fractions represent intact virions or forms of subviral HCV RNA-containing material. It is probable that association with low-density lipoproteins (LDL) is responsible for the low-density fraction. A density of 1.06 g/mL is

unusually low, even for an enveloped RNA virus such as HCV, and is close to that of plasma LDL (Gautier *et al.*, 2000), whereas the higher-density fractions (1.1 to 1.18 g/mL) possibly represent free virus or particles complexed with immunoglobulin (Ig) (Chatterjee and Sparks, 2011). Association with LDL could confer two advantages to the virus: (a) particles may be protected from antibody-mediated neutralization, and (b) virions may gain entry into cells through the cellular LDL receptor (Agnello *et al.*, 1999). These data argue in favor of a protective association between HCV virions and LDL, and recent reports suggest that the LDL receptor can mediate uptake of HCV, but it is unclear whether this leads to productive infection.

Replication

One of the major hurdles in testing both neutralizing antibodies and antiviral drugs to combat HCV infection is the lack of an effective cell culture system for the virus. The data regarding the functions of HCV genes and replication of the virus have been derived using established mammalian cell expression systems or the chimpanzee animal model. Infectious RNA transcripts used in chimpanzees have demonstrated requirements for 3'-UTR elements and functional enzymatic activities in HCV replication. This model, however, is too expensive for extensive analyses such as these. Unfortunately, a tissue culture system reliable enough to support HCV growth to the extent that experiments on replication can be carried out is yet to be developed.

Tissue culture

There have been several reports of mammalian cells able to support the growth of HCV. The cell lines used include MT2, peripheral blood mononuclear cells,

lymphocyte cell lines, and hepatocytes from humans and chimpanzees (Yanagi *et al.*, 1997). An enhancement of HCV replication of MT2 cells by Epstein-Barr virus was recently reported; in particular, the EBNA1 gene was thought to be involved. These systems rely on the use of reverse transcription-polymerase chain reaction (RT-PCR) for the detection of virus and, in particular, strand-specific RT-PCR as evidence of virus replication. Data indicating the presence of negative-stranded RNA need to be interpreted with care because specificity and sensitivity can vary depending on the choice of primers and reaction conditions. Data indicate, however, that there exist limited cell culture systems for HCV; with further development, these may provide an effective *in vitro* study system for the virus.

Alternative approaches to studying virus assembly or the role of viral antigens in replication have involved the production of virus-like particles or pseudotype vesicular stomatitis virus (VSV) particles containing E1 or E2. These systems provide a potential means of studying envelope glycoprotein interactions, essential elements required for particle assembly, and neutralizing antibody activity. A subgenomic replicon containing most of the nonstructural proteins of HCV was shown to replicate to high levels in a hepatoma cell line (206); radiolabeling of viral RNA and proteins was possible. Systems such as this may provide the basis for defining functional HCV replication units and testing of antiviral drugs.

Pathogenesis

Immunobiology of infection

Viral entry into the host

Although the primary source of HCV

infection has not been determined in up to 40% of cases, HCV infection is generally thought to occur through parenteral routes. HCV virions spread through the bloodstream, and as early as 2 days after intravenous infection of chimpanzees, HCV RNA becomes detectable in the liver, the primary site of HCV replication.

A candidate protein for the cellular HCV receptor is human CD81, a transmembrane molecule that is expressed on most human cells except red blood cells and platelets. Binding of CD81 coated on beads to HCV E2 domain and to HCV RNA containing particles in chimpanzee infectious plasma is species specific and can be inhibited by anti-E2 antibodies and by serum from chimpanzees vaccinated with E2 protein.

Another candidate receptor is the low-density lipoprotein receptor (LDLR). Endocytosis of the HCV receptor complex, as well as its inhibition by anti-LDLR antibody, has recently been demonstrated *in vitro*. It was yet to be shown whether either of these receptors provides a route of entry that leads to productive infection in cells.

Cell and tissue tropism

As the primary site of HCV replication, the liver contains the highest levels of HCV RNA, ranging from 10^8 to 10^{11} copies per gram of tissue. However, only a small percentage of hepatocytes (5% to 19%) are HCV RNA positive by *in situ* hybridization. Accordingly expression of HCV antigens is rather low (1% to 10%). In addition, only 1% to 5% of mononuclear cells, and an even smaller proportion of biliary epithelial or sinusoidal lining cells, express HCV antigens. Most infected cells display little or no hepatocellular damage, which may suggest weak stimulation of the immune system due to low antigen expression.

RT-PCR techniques with a high specificity for the negative-stranded HCV RNA also commonly detected RNA in lymph nodes and pancreas and less frequently on adrenal glands, bone marrow, thyroid tissue, and spleen. Whether HCV also replicates in PBMC is controversial. Indirect evidence for extra hepatic replication of HCV in PBMC is derived from the observation that HCV HVRI sequences isolated from PBMC of HCV-infected patients displayed considerable differences in the complexity of quasi-species as compared with HVRI sequences isolated from serum and liver of the same individual's. The predominant sequences from each source were mutually different in 23% of patients.

Humoral immune response

Although individuals vary in both specificity and timing of anti-HCV seroconversion, HCV-specific antibodies are generally detectable 7 to 31 weeks after infection. The humoral immune response is usually multispecific and targeted against epitopes within the HCV core, envelope, NS3, and NS4 proteins (Alexander *et al.*, 1998).

A major problem in assessing the relevance of these antibody responses is the lack of a convenient neutralization assay for HCV. As in other viral infections, antibody binding could interfere with HCV entry into host cells or viral replication, and it could opsonize virions for elimination by macrophages.

Evidence for a protective role of antibodies stems from limited studies in which chimpanzee or tissue culture infectious HCV was neutralized *in vitro* by incubation with antibody (Farci *et al.*, 1994). Thus far, the HVRI of the HCV E2 protein has been identified as the major target for neutralizing antibodies (Farci *et al.*, 1994; Shimizu *et al.*,

1994). This N-terminal sequences of 30 amino acids exhibits high sequence variability. Sequence variability in HVRI, however, does not appear to evolve by random drift, because virologic data from a single-source infection showed that amino acid replacements either are limited to particular sets of amino acids with shared biochemical properties or are completely conserved. Thus, amino acid substitutions appear to be influenced by both negative and positive selection, presumably by the immune response of the host.

Cellular immune response

The cellular immune response is thought to play a particularly important role in the host's defense against infections with noncytopathic viruses such as HCV because of its ability to recognize viral antigens in and to eliminate virus from infected cells. Although it is known that the liver is significantly enriched for cells of the innate immune response, such as natural killer cells, as well as for T-cell receptor- γ - and δ -positive cells and Va24-positive T-cells, the role of the innate and antigen-nonspecific immune response of HCV has not yet been sufficiently studied. Most analyses have concentrated on the induction, effector function, and maintenance of HCV-specific CD4+helper T cells and CD8+cytotoxic T cells.

Clinical features

Acute hepatitis

The incubation period for hepatitis averages about 7 weeks, with a range of 2 to 26 weeks, after exposure as measured by prospective studies of transfusion-associated hepatitis and needle sticks (Shepard *et al.*, 2005). The clinical picture of acute hepatitis C resembles other forms of acute viral

hepatitis. However, symptoms of malaise, nausea, and right upper quadrant pain followed by dark urine and jaundice appear in only about one third of patients. Biochemical evidences of hepatitis, such as elevated levels of serum ALT, are observed in more than 80% of cases, with levels typically 10 times normal or higher. ALT elevations usually coincide with symptoms, and both tend to resolve in 2 to 12 weeks. Hepatitis C RNA can be detected in the serum early after exposure, usually within the first 3 weeks. In acute resolved cases, the RNA disappears as the disease resolves. HCV is rarely associated with fulminant hepatitis, but it has been reported (Liang *et al.*, 1993). There are no recognized sequelae in patients who resolve hepatitis C infections. However, patients in whom HCV resolves do not in general appear to be protected from reinfection. Multiple bouts of hepatitis C have been reported in patients repeatedly treated with blood or plasma products (CDC, 1991). Liver biopsy is not recommended for the diagnosis of acute viral hepatitis, but in case in which it has been performed, the histologic changes in addition to lymphocytic infiltrates include distinctive eosinophilic clumping of hepatocyte cytoplasm, acidophilic bodies, micro vesicular steatosis, and activation of the sinusoidal cells.

Chronic hepatitis

The single most important feature of HCV infection is the propensity of the virus to cause persistent infection. About 85% of patients infected remain so for more than 6 months, most for the remainder of their lives. These chronically infected patients are the source of almost all new infections and themselves are at increased risk for the development of significant chronic liver disease, cirrhosis, and HCC. Many chronically infected patients do not have

symptoms and would not be detected unless tested by their physicians or if they donated blood. Others may develop severe chronic liver disease over only a few years or, more commonly, over several decades. Patients with chronic HCV infection may experience increased fatigue as their only symptoms. Other may have overt symptomatic liver disease with anorexia, nausea, right upper quadrant pain, dark urine, and pruritus. ALT levels frequently fluctuate over time and may be normal or significantly elevated ALT levels. A summary was published of 10 reports in which 233 patients with chronic HCV infection but with normal ALT levels were studied by liver biopsy; it revealed that 12% had normal liver biopsies, 25% had only nonspecific changes, 41% had histologic findings of chronic persistent hepatitis, 22% had chronic active hepatitis, and only 0.5% had cirrhosis. Therefore, normal ALT levels do not always correlate perfectly with benign disease.

Outcomes of chronic hepatitis C infections in individual patients are difficult to predict. Hepatitis C patients presenting with significant chronic liver disease develop serious sequelae. For instance, of 100 Japanese patients presenting with chronic hepatitis C and liver disease and followed an average of 11 years earlier, 42 developed cirrhosis and 19 HCC (Takahashi *et al.*, 1993). When patients are followed prospectively from infection, however, the picture is quite different. The National Heart Lung and Blood Institute conducted a series of five studies on post transfusion hepatitis between 1968 and 1980 in which 8% to 18% of blood recipients developed hepatitis; about 90% of these cases were designated NANBH, almost all due to HCV. Follow-up studies of the 568 cases of NANBH an average of 18 years after transfusion as well as of 984 matched controls who were transfused but did not develop hepatitis

revealed that death from all cases occurred in 51% in the hepatitis groups and 52% in the controls. The mortality rate from liver disease was 3.2% among the hepatitis patients and 1.5% among controls, which was a small but significant difference. About one third of the patients who underwent liver biopsy after 18 to 20 years had cirrhosis, but presumably only the sicker patients had biopsies.

A group of children in Germany who had undergone cardiac surgery before 1991 when HCV screening of blood donors was begun were studied a mean of 19.8 years after their first surgery. Sixty-seven of 458 (14.6%) patients studied have anti-HCV, but only 37 of these (55%) have detectable HCV RNA. Only one HCV RNA-positive patient had elevated ALT levels, and this patient had right-sided heart failure. Seventeen HCV RNA-positive patients underwent liver biopsy. There was periportal fibrosis in 10 patients, both of whom had congestive heart failure and micro nodular cirrhosis in another. The remaining 14 patients had minimal lymphocytic periportal infiltrates. Therefore, in this group of patients infected as children and evaluated nearly 20 years later, the infection had cleared in 45% and was following a relatively benign course in the others. It is possible that in subsequent decades, these patients will begin to develop significant liver disease.

In contrast to the prospective studies reviewed previously, studies of patients with hepatitis C referred for evaluation of chronic liver disease show a rather different picture. Liver biopsies were performed in 101 patients in the United States with transfusion associated chronic liver disease due to HCV. An additional 30 patients were studied but did not undergo biopsy because of abnormal coagulation tests. The mean interval from

transfusion to diagnosis in this group was 19.5 years. This study showed the progressive nature of HCV-associated chronic liver disease. The mean interval from transfusion to diagnosis for patients with chronic hepatitis was 13.7 years, for chronic active hepatitis was 18.4 years, for cirrhosis was 20.6 years, and for HCC was 28.3 years. During the follow-up, which averaged about 4 years, 8 patients died from cirrhosis. And 11 died from HCC. These results were very similar to those reported earlier from Japan, in which the mean intervals to chronic hepatitis, cirrhosis, and HCC were 10.0, 21.2 and 29 years, respectively. Thus, chronic liver disease due to HCV infection generally follows an indolent but progressive course.

Hepatocellular carcinoma

The most serious late outcome of chronic HCV infection is HCC. Soon after specific tests become available to detect patients with HCV infection, the association of chronic HCV infection with HCC was made. Worldwide, most HCC cases are related to either chronic HBV or HCV infection, and the prevalence of HCC and the proportion of causes related to either virus is directly reflected by the prevalence of each virus in that population. Chronic infection with either virus results in chronic inflammation, necrosis, regeneration, and cirrhosis, which are all related to increased risk for oncocarcinogenesis. Cirrhosis of almost any cause carries an increased risk for HCC, and about 80% of patients with HCC have cirrhosis. HBV is a DNA virus that can integrate into the host genome, and it expresses the X protein, which is a transactivator, interacts with p53, and has other properties that may contribute to tumorigenesis. On the potential for its genome to be reverse-transcribed by a viral polymerase into DNA, the question of whether any of the HCV gene products have

an effect on cellular metabolism that could lead to oncogenic transformation is under investigation. For instance, studies have shown that the HCV core protein can enter the nucleus and protect the cells from apoptosis mediated by anti-Fas or TNF- α , probably through activation of nuclear factor kappa B. In addition, NS3 has been reported to transform 3T3 cells in which it is expressed, and NS5a contains a potential transcriptional transactivator domain near its C terminus. In addition, transgenic mice expressing the core protein developed primary liver cancer resembling HCC after 16 months of age. Either of these phenomena could be related to carcinogenesis. Interaction of HCV proteins with cellular regulatory factors may exert only small effects on the cell. However, because it typically takes 20 to 30 years for tumors to appear in persistently infected patients, it is possible that even such a small effect when exerted over a long time could be responsible for hepatocarcinogenesis.

Extrahepatic manifestations of infection

A variety of clinical syndromes that may be either autoimmune or mediated by immune complexes have been associated with chronic HCV infections. The diseases most closely associated with chronic HCV infections are essential mixed cryoglobulinemia, membranoproliferative glomerulonephritis, and porphyria cutanea tarda. Other diseases that have been related to HCV include keratoconjunctivitis sicca, lichen planus, autoimmune thyroiditis, Mooren corneal ulcer, idiopathic pulmonary fibrosis and diabetes mellitus. Type II or essential mixed cryoglobulinemia (MC) (Agnello, 1997) or characterized by vasculitis with palpable purpura, arthralgias, and weakness and may involve the kidneys or central nervous system. This association has been reported from many areas of the

world but appears to be particularly strong in Italy. A prospective study of MC in patients with chronic liver disease revealed that 46% of patients with chronic liver disease revealed that 46% of patients with chronic HCV infections had cryoglobulinemia, and about half of these patients had the clinical syndrome. The cryoglobulins have been shown to be composed of HCV. Anti-HCV Ig, rheumatoid factor, and complement and the cutaneous vascular lesions contain HCV, rheumatoid factor, and IgG. Successful treatment of HCV with IFN generally improves the MC. There is an additional association among HCV<MC> and lymphoproliferative disorders. Among 25 patients with MC, 7 had B-cell non-Hodgkins lymphoma. Serum anti-HCV was detected in 88% of the patients with MC and in all the patients with lymphoma (Zignego *et al.*, 1997). It is possible that the putative replication of HCV in lymphoid cells contributes to the pathogenesis of MC and the associated lymphoproliferative disorders. Membranoproliferative glomerulonephritis has also been linked to HCV infection and is most often seen together with MC. However, immune complex desposition, including HCV core protein, in the absence of cryoglobulins has also been reported.

Porphyria cutaneatarda also has a strong association with chronic HCV infection, although there appears to be a marked geographic variation in the rate of HCV positivity in patients with porphyria. A recent study in the United States found that 16 of 17 porphyria patients tested positive for anti-HCV, whereas only 17% in Northern Europe, 20% in Australia and New Zealand, and 65% in Southern Europe tested positive. Iron overload is often improved by frequent phlebotomy to deplete the excess iron stores; antiviral therapy also appears to

have a beneficial effect.

Diagnosis

The common tests used for diagnosis of HCV infection were designed primarily for screening of blood donors. These assays are based on detection of serum antibody to various HCV antigens because these antibodies are nearly universally present in patients who are chronically infected with HCV. Acute HCV infections are relatively rare among blood donors, but the antibody tests often fail to detect these patients in the window period between the time of infection and the time of appearance of antibody detectable by the assay. Therefore, the antibody assays also have limited utility for diagnosis of patients with acute HCV infections. Tests for HCV RNA genome detection based on the PCR or other highly sensitive RNA detection systems have been used for the diagnosis of acute hepatitis.

Case Study

175 serum samples are subjected to ELISA Test, out of 175 samples 24 males and 51 females no children only total zero positives are 7 only one male and six female patients or proved zero positive cases. Females are more in number they are middle aged person.

1. ELISA Kit manufacturer name TRANSASIA
2. KIT name is ERBA ELISA
3. Negative control value = +0.3
4. Cut off value = 0.395

Results and Discussion

HCV Results in a Tabular form including Age wise, sexwise, children wise and Lab investigations conducted (Table.1). From 01/10/2015 to 30/10/2015

Table.1 Hepatitis positive samples

Age Group	M	F	Children	Total cases	Positive each in group	DISEASES, Blood Transfusions occurred	ELISA test done (reactive)
10 TO 20	2	6		8	1F +VE	Patient-1, F/18yrs ELISA reactive (abortion +blood transfusion)	And cut off valves (2.636)
21 to 30	4	25		29	1M +VE 1F +VE	Patient-2, M/ 29Yrs, reactive RA(rheumatoid arthritis) gout Patient-3, F/ 28 Yrs. Repeated abortion +Blood transfusion	1.315 0.889
31 to 40	3	8		11	-	--	-
41 to 50	4	4		8	2 F +VE	Patient-4, F/48Yrs, Pain Abdomen +Jaundice(2 month) Patient-5,F/41Yrs, Hysterectomy+ Blood Transfusion (10Yrs ago)	2.415 0.550
51 to 60	5	4		9	1 F +VE	Patient-6,F/55Yrs, Rheumatoid arthritis + pain abdomen right costal area liver enlarged (10Yrs)	++, scanning abdomen +Elise (1.018)
61 to 75	6	3		9	1 F +VE	Patient-7,F/79Yrs # BB Lower leg + Blood transfusion (20Yrs ago)	1.212
Total	24	51	75		-	--	-

HCV presents a nearly perfect target for an antiviral drug approach to therapy. Hepatitis C is a chronic disease that progresses slowly over many years. Therefore, there is a long period of time in which antiviral therapy could be initiated as well as a long treatment window when therapy could be initiated as well as a long treatment window when therapy might be effective. The viral genome code for several enzymatic activities is vital for replication and is quite distinct from human analogs. These include proteases, a helicase, and the RNA-dependent RNA polymerase, which all could be targeted by inhibitors.

One of the most promising approaches to anti-HCV drug discovery is the development of inhibitors of the virally encoded protease NS3. This chymotrypsin-like serine protease is essential for the maturation of the viral

polyprotein, and efficient enzymatic activity requires complex formation between NS3 and its cofactor, NS4A. Targets for inhibition could include the NS3-NS4A interaction, zinc binding in the NS3 domain, and direct protease inhibitors. Of these, inhibitors of protease activity appear the most realistic.

Prevention of hepatitis C at present is based on prevention of exposure to contaminated blood by screening of blood and plasma donors, identification of carriers by testing high-risk individuals, and public health measures developed to prevent HCV infection. In developed countries, screening of blood donors has virtually eliminated transmission of HCV by transfusion. Even before the specific screening tests were available, HCV transmission by blood had been significantly reduced by elimination of

paid donors, ridged donor screening implemented to reduce HIV transmission, and possibly the use of surrogate tests for hepatitis C infection (Alter *et al.*, 1997).

Source plasma for manufacture into various plasma products, such as clotting factors, is now all screened by the specific assays. Because these products are all made from pools containing plasma from thousands of donors, most of these pools continue to have low levels of HCV. Presently, these plasma-derived products all must undergo some specific process designed to eliminate or inactivate viruses, especially lipid-enveloped viruses that include HCV, HBV, and HIV. At the present time, plasma products are all considered to be free of infectious HCV.

Some recent studies indicate that the passive immune prophylaxis of HCV infections may be possible. Pooled Ig with a high titer of anti-HCV was mixed with HCV *in vitro*, and this mixture was shown to be non-infectious when injected into a chimpanzee. Using the same Ig preparation, chimpanzees were inoculated with HCV and then given the Ig weekly for 13 weeks. Relative to the untreated control chimpanzees, the treated animals had a short, low-level viremia with minimal disease. When the treatment was stopped, however, the HCV viremia reappeared with acute disease. These two experiments show that antibody alone can have a profound effect on HCV infection and can neutralize the virus under limited conditions. The place for passive immunization with high tittered Ig preparations in the prevention of HCV infection in humans has not been determined.

HCV itself presents numerous problems that complicate the development of an effective vaccine. The lack of a suitable *in vitro* culture system makes production of vaccine

quantities of whole virus impractical. The genetic diversity of the virus and the high level of mutability also complicate vaccine development. A vaccine based on the E1 and E2 glycoproteins was tested in chimpanzees. The vaccine produced an antibody response to E1 and E2, but it was short lived and required frequent boosting. Animals that were challenged intravenously with a very low dose of HCV were protected if the challenge virus was the same as the virus that was used to make the vaccine. Although protection against infection was not produced against other strains of HCV or higher levels of challenge virus, infections in the vaccinated chimps tended to be mild and short lasting. It is possible that a vaccine that only prevents chronic infections would be useful. Such a vaccine would prevent the most serious problems associated with chronic HCV and would greatly reduce the chance of transmitting the virus to others. Phase 1 clinical trials are in progress with a related vaccine based on the E2 glycoprotein only.

The challenges to the development of an effective HCV vaccine may require non classic approaches. A strong T- cell response, including both helper T cells and cytotoxic T lymphocytes, may be very important for both clearing and preventing infection. It has been suggested that specifically inducing CTL responses to well-conserved epitopes included in non-structural proteins may be important for a prophylactic vaccine. Several laboratories are now studying this possibility in the chimpanzee model.

Update on the management of chronic hepatitis C

Since the last update of the CASL management guidelines for chronic hepatitis C (CHC) in 2012, major advances have

occurred including: the approval of novel direct-acting antiviral agents (DAAs) used with pegylated interferon (PEG-IFN) that have improved efficacy and tolerability compared with first-generation DAAs and/or standard PEG-IFN based therapy; and the approval of all-oral, IFN-free, DAA combination therapies with markedly improved efficacy and tolerability and activity beyond just HCV genotype 1.

Reference

- Agnello, V. 1997. The etiology and pathophysiology of mixed cryoglobulinemia secondary to hepatitis C virus infection. *Springer Semin. Immunopathol.*, 19: 111–129.
- Agnello, V., Abel, G., Elfahal, M., *et al.* 1999. Hepatitis C virus and other Flaviviridae viruses enter cells via low density lipoprotein receptor. *Proc. Natl. Acad. Sci. USA*, 96: 12766–12771.
- Alberti, A., Chemello, L., Noventa, F., *et al.* 1997. Therapy of hepatitis C: Re-treatment with alpha interferon. *Hepatology*, 26: 1378–1342S.
- Alexander, J., Del Guercio, M.F., Fikes, J.D., *et al.* 1998. Recognition of a novel naturally processed, A2 restricted, HCV-NS4 epitope triggers IFN-gamma release in absence of detectable cytopathicity. *Hum. Immunol.*, 59: 776–782.
- Ali, N., Siddiqui, A. 1997. The La antigen binds 5' noncoding region of the hepatitis C virus RNA in the context of the initiator AUG codon and stimulates internal ribosome entry site-mediated translation. *Proc. Natl. Acad. Sci. USA*, 94: 2249–2254
- Alter, H.J. 1999. Discovery of non-A, non-B hepatitis and identification of its etiology. *Am. J. Med.*, 107(6B): 16S–20S.
- Alter, H.J., Conry-Cantilena, C., Melpolder, J., *et al.* 1997. Hepatitis C in asymptomatic blood donors. *Hepatology*, 26: 29S–33S.
- American Heart Association http://my.americanheart.org/idc/groups/ahamapublic/@wcm/@sop/documents/downloadable/ucm_319826.pdf
- CDC, 1991. Public Health Service inter-agency guidelines for screening donors of blood, plasma, organs, tissues, and semen for evidence of hepatitis B and hepatitis C. *MMWR*, 40(No. RR-4): 1–17.
- Chatterjee, C., Sparks, D.L. 2011. Hepatic lipase, high density lipoproteins, and hypertriglyceridemia. *Am. J. Pathol.*, 178: 1429–1433.
- Farci, P., Alter, H.J., Wong, D.C., Miller, R.H., Govindarajan, S., Engle, R., Shapiro, M., Purcell, R.H. 1994. Prevention of hepatitis C virus infection in chimpanzees after antibody-mediated in vitro neutralization. *Proc. Natl. Acad. Sci. USA*, 91: 7792–7796.
- Farci, P., Alter, H.J., Wong, D.C., Miller, R.H., Govindarajan, S., Engle, R., Shapiro, M., Purcell, R.H. 1994. Prevention of hepatitis C virus infection in chimpanzees after antibody-mediated in vitro neutralization. *Proc. Natl. Acad. Sci. USA*, 91(16): 7792–6.
- Feinstone, S.M., Kapikian, A.Z., Purcell, R.H. 1973. Hepatitis A: detection by immune electron microscopy of a viruslike antigen associated with acute illness. *Science*, 182(4116): 1026–8.
- Feinstone, S.M., Mihalik, K.B., Kamimura, T., Alter, H.J., London, W.T., Purcell, R.H. 1983. Inactivation of hepatitis B virus and non-A, non-B

- hepatitis by chloroform. *Infect. Immun.*, 41: 816–821.
- Gautier, T., Masson, D., de Barros, J.P., Athias, A., Gambert, P., Aunis, D., Metz-Boutigue, M.H., Lagrost, L. 2000. Human apolipoprotein C-I accounts for the ability of plasma high density lipoproteins to inhibit the cholesteryl ester transfer protein activity. *J. Biol. Chem.*, 275: 37504–37509.
- Iwarson, S., Norkrans, G., Wejsal, R. 1995. Hepatitis C: natural history of a unique infection. *Clin. Infect. Dis.*, 20: 1361–70.
- Liang, T.J., Jeffers, L., Reddy, R.K., *et al.* 1993. Fulminant or subfulminant non-A, non-B viral hepatitis: the role of hepatitis C and E viruses. *Gastroenterology*, 104: 556–62.
- Ling, C.M., Overby, L.R. 1972. Prevalence of hepatitis B virus antigen as revealed by direct radioimmune assay with 125 I-antibody. *J. Immunol.*, 10: 834–841.
- Prince, M.I., Ducker, S.J., James, O.F.W. 2010. Case-control studies of risk factors for primary biliary cirrhosis in two United Kingdom populations. *Gut*, 59(4): 508–512.
- Ralf Bartenschlager, Volker Lohmann, Francois Penin, 2013. The molecular and structural basis of advanced antiviral therapy for hepatitis C virus infection. *Nature Rev. Microbiol.*, 11: 482–496.
- Shepard, C.W., Finelli, L., Alter, M.J. 2005. Global epidemiology of hepatitis C virus infection. *Lancet Infect. Dis.*, 5(9): 558–67.
- Shiffman, R.N., Shekelle, P., Overhage, J.M., *et al.* 2003. Standardized reporting of clinical practice guidelines: a proposal from the Conference on Guideline Standardization. *Ann. Intern. Med.*, 139: 493–8
- Shimizu, Y.K., Hijikata, M., Iwamoto, A., Alter, H.J., Purcell, R.H., Yoshikura, H. 1994. Neutralizing antibodies against hepatitis C virus and the emergence of neutralization escape mutant viruses. *J. Virol.*, 68(3): 1494–500.
- Takahashi, M., Yamada, G., Miyamoto, R., *et al.* 1993. Natural history of chronic hepatitis C. *Am. J. Gastroenterol.*, 88: 240–243.
- Yanagi, M., Purcell, R.H., Emerson, S.U., Bukh, J. 1997. Transcripts from a single full-length cDNA clone of hepatitis C virus are infectious when directly transfected into the liver of a chimpanzee. *Proc. Natl. Acad. Sci. U.S.A.*, 94: 8738–8743.
- Zignego, A.L., Ferri, C., Giannini, C., La Civita, L., Careccia, G., Longombardo, G., Bellesi, G., Caracciolo, F., Thiers, V., Gentilini, P. 1997. Hepatitis C virus infection in mixed cryoglobulinemia and B-cell non-Hodgkin's lymphoma: evidence for a pathogenetic role. *Arch. Virol.*, 142(3): 545–55.