California Association for Medical Laboratory Technology

VIRAL HEPATITIS 2015: CAUSES, DIAGNOSIS, AND TREATMENT

Course # DL-004

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Approved for 2.0 CE
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Level of Difficulty: Intermediate

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**COURSE NAME: VIRAL HEPATITIS 2015: CAUSES, DIAGNOSIS, AND TREATMENT**

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   5 4 3 2 1

2. The objectives of this Distance Learning course were met.
   
   5 4 3 2 1

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   5 4 3 2 1

4. I will use what I learned from this Distance Learning course.
   
   5 4 3 2 1

5. The time to complete this Distance Learning course was: __________ hours

6. Please comment on this Distance Learning course on the back of this sheet. What did you like or dislike?
OBJECTIVES:
After completing this course the participant will be able to:
1. List the viral causes of hepatitis.
2. Summarize the history of the hepatitis viruses.
3. Describe the extent of the toll of the viral hepatitides world-wide and in the U.S.
4. Outline the causative agents, transmission, symptoms, sequelae, diagnosis, treatment, and prevention of the five viral hepatitides.
5. Discuss the laboratory testing for each type of hepatitis.

INTRODUCTION:
Hepatitis has been known since ancient times. Hippocrates characterized its signs, including jaundice. It was recognized as a disease affecting the liver, causing the skin to turn yellow. By the eighth century some cases were found to be infectious. Epidemics of jaundice have been reported since the 5th century BC, with major epidemics documented in Europe during the 17th and 18th centuries. Epidemics were so frequent among armies that the disease was termed campaign jaundice. In the 19th century the cause was thought to be obstruction of the common bile duct by a mucus plug and was called acute catarrhal jaundice. However growing evidence for the infectious nature of the disease culminated in 1923 when Blumer concluded that infectious hepatitis was the epidemic form of catarrhal jaundice.

Hepatitis is inflammation of the liver, usually producing swelling and, in many cases, permanent damage to liver tissues. A number of agents can cause hepatitis, including infectious diseases, chemical poisons, drugs, and alcohol. In the 1940s doctors began to suspect that many hepatitis cases were caused by a virus that was carried in human blood. In 1965 Dr. Baruch Blumberg discovered the Australia antigen, which later would be known as the hepatitis B surface antigen or HBsAg. The hepatitis A agent was discovered about 10 years later. Around this time Harvey J. Alter, Chief of the Infectious Disease Section at the National Institute of Health, and his research team noted many hepatitis cases that were not hepatitis B virus (HBV) or hepatitis A virus (HVA). This virus was called non-A, non-B hepatitis (NANBH) and was confirmed by Alter in a panel of NANBH specimens in 1988. In 1989 the discovery of the causative virus was published in The Journal of Science and was renamed Hepatitis C (HCV). Hepatitis delta virus (HDV) was identified in the 1970s, but it was not until 1990 that it was found to exist only in the presence of HBV. Hepatitis E (HEV) virus was discovered in 1990 and Hepatitis G virus in 1995.
Viral hepatitis is the most common type of liver disease worldwide, including in the United States. Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D, and Hepatitis E are all found in the U.S.

The liver, which is located in the upper right quadrant of the abdomen, performs many vital functions. These functions include: purifying the blood by converting harmful chemicals into harmless ones; producing proteins such as albumin and blood clotting factors; storing sugars, fats, and vitamins until they are needed; manufacturing necessary hormones and enzymes; and forming and secreting bile. If the liver is extensively damaged and these functions are impaired, the result is liver disease that may cause the following:

- jaundice from accumulation of bilirubin in the blood
- bleeding or easy bruising from lack of clotting factors
- swelling of the legs due to decrease in serum albumin

Laboratory tests for hepatitis include routine tests CBC, albumin, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin. Serologic tests include immunoassays for antibodies and antigens to detect the presence of infection, the immune status, the disease status and need for treatment. Nucleic acid-based tests are used to detect viremia and measure viral load. Genotyping tests are used to determine the hepatitis genotype.

This paper will discuss each of the major types of the viral hepatic infections, with extensive discussion of hepatitis C. It will elaborate on each of the viral causes of the hepatitides including causative agent, methods of transmission, symptoms, sequelae, diagnosis, treatment, prevention, and laboratory diagnosis.

**HEPATITIS A**

Hepatitis A, caused by the Hepatitis A virus (HAV), is also known as infectious hepatitis or short-incubation hepatitis. It is the most common form of viral hepatitis worldwide. It accounts for about 1,700 of the approximately 7,000 new cases of hepatitis reported in the United States each year. Hepatitis A is an acute form of hepatitis that never becomes chronic. However, 10-15% of patients may experience a relapse of symptoms during six months after acute illness.

HAV is primarily spread through the fecal-oral route. HAV is excreted in the bile and then shed in the feces from two weeks before to one week after the onset of clinical illness. It is commonly ingested through contaminated food or water, and frequently spread among household members due to poor hand washing or by close contact. It can also be spread by sexual practices that involve oral-fecal contact. Blood-borne transmission of HAV is uncommon.

The incubation period of HAV is about 28 days with a range of 2-6 weeks. The length of the incubation period depends on the number of infectious particles consumed. The patient presents with symptoms of fever, malaise, loss of appetite, nausea, abdominal discomfort, dark urine, and jaundice. Symptoms seldom last for more than three weeks. Symptoms occur more frequently in adults; children may be asymptomatic or have a mild, unrecognized infection. Only in rare cases can HAV develop into fulminant liver failure.

Although hepatitis A was recognized as separate from other types of hepatitis during World War II, the virus wasn’t isolated until 1973, by Robert Purcell. HAV is an RNA virus in the Picornavirus family. It is non-enveloped and contains a single-stranded RNA in a protein shell.
Diagnosis of Hepatitis A

Preliminary diagnosis of HAV is through the classical liver enzyme analysis. Today serological antibody detection methods are used for identifying clinical markers that diagnose and follow the progression of HAV infection. The serologic antibodies used as markers follow this progression: immunoglobulin M (IgM) antibodies to HAV are detectable when symptoms first present and decline after 3 to 6 months; next immunoglobulin G (IgG) antibodies appear and may remain for years after infection. The presence of IgG anti-HAV without IgM indicates a past infection and the presence of IgM anti-HAV indicates an acute infection. Another way to detect acute infection is by testing for the viral antigen, HAVAg, but this antigen is no longer present after liver enzymes have reached their peak.

Hepatitis A virus infection can also be detected using reverse transcription-polymerase chain reaction (RT-PCR) to amplify the viral RNA. This type of HAV detection is useful in epidemiological testing of samples from different origins, such as food, environment, and clinical specimens.

Hepatitis A Treatment and Prognosis

There are no specific medications to cure or treat hepatitis A virus. Most treatments are focused on ameliorating the symptoms of the disease, such as resting frequently, eating small meals in order to reduce the nausea, and elimination of alcoholic beverages. However those who have been exposed to an infected person may be given hepatitis A immunoglobulin, which may prevent infection. Hepatitis A symptoms usually subside within 2-6 weeks.

Hepatitis A Prevention

A vaccine to protect against acquiring HAV was created by Maurice Hilleman, a scientist from Merck, in the 1990s. The vaccine is 95% effective in protecting against the virus. The vaccine works for about 10 years and should be given to anyone who risks infection, such as:

- children over the age of 1
- travelers to countries where the virus is common
- those working with the virus
- people with chronic liver disease
- people with high-risk sexual behavior

The use of the Hepatitis A vaccine has dramatically decreased the incidence of HAV in the United States.

HEPATITIS B

Hepatitis B is referred to as “long incubation hepatitis” and occurs as both an acute and chronic form. Today there are about 20,000 new cases in the United States each year. This is a sharp decrease of about 82% since 1991 when a national strategy to eliminate HBV infection was implemented in the United States. Approximately 1.25 million are infected (new and older cases) in the United States; globally, however, 2 billion people are infected, with 240 million of them being chronic carriers.

In 1965 the Hepatitis B Virus (HBV) surface antigen was isolated from the blood of aborigines in Australia and was characterized by 1979. Hepatitis B is a hepadnavirus. Hepa- coming from hepatropic and dna because it is a DNA virus. Hepatitis B interferes with the function of the liver by replicating in the liver cells called hepatocytes. A person with chronic HBV will have ground-glass hepatocytes.

Hepatitis B is spread through contact with infected bodily fluids such as blood, semen,
and vaginal fluid. Although the virus is found in every bodily secretion, it is not transferred through casual contact. People who are at an increased risk of being infected with the hepatitis B virus include those who:
  • have sex with a person infected with Hepatitis B virus
  • have multiple sex partners especially if a condom is not used
  • have other sexually transmitted diseases
  • inject drugs with shared needles
  • receive transfusions of blood or blood products
  • undergo dialysis with for kidney disease
  • are men who have sex with men

**Hepatitis B Diagnosis**

Serological markers are used to diagnose and monitor Hepatitis B virus infection. The first antigen present is the HB surface antigen (HBsAg) and is the only antigen present during the first 3-5 weeks in newly infected individuals. HBsAg is also useful because it is detectable before clinical symptoms appear. People who chronically carry HBsAg can be considered infectious.

The body normally produces antibodies to HBsAg as part of the normal immune response. Individuals who have anti-HBs antibody have had a past infection and are not susceptible to reinfection. Anti-HBs also develops in those who have been successfully vaccinated against Hepatitis B.

The hepatitis B core antigen (HBcAg) is serologically significant because it is present in the nuclei of hepatocytes only during an acute infection. In an acute infection, the antibody to HBc, anti-HBc, develops before anti-Hbs. Anti-HBc appears at the onset of symptoms and can persist for life. The presence of anti-HBc indicates previous or ongoing infection with HBV in an undefined timeframe. However, the IgM antibody to hepatitis B core antigen positively indicates recent infection with HBV (< 6 months). Its presence indicates an acute infection.

The hepatitis B e antigen or HBeAg is the secreted product of the nucleocapsid gene of HBV that is found in serum during acute and chronic hepatitis B. Its presence indicates that the virus is replicating and the infected person has high levels of HBV and is highly infectious. The presence of HBeAg and HBsAg generally indicates a poor prognosis and is indicative of a chronic infection with chronic liver disease. Alternatively the presence of anti-HBe means recovery and a very low infectivity. Conversion from e antigen to e antibody is a predictor of long-term clearance of HBV in patients undergoing antiviral therapy and indicates lower levels of HBV (see Figure 1).

HBV antigens and antibodies are an excellent way to determine the status of the individual, as shown in the following table:
### Interpretation of Hepatitis B Serologic Test Results*

<table>
<thead>
<tr>
<th>Tests</th>
<th>Results</th>
<th>Interpretation</th>
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<tr>
<td>HbsAg</td>
<td>negative</td>
<td><strong>Susceptible</strong></td>
</tr>
<tr>
<td>anti-HBc</td>
<td>negative</td>
<td></td>
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<tr>
<td>anti-HBs</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>HbsAg</td>
<td>negative</td>
<td>Immune due to natural infection</td>
</tr>
<tr>
<td>anti-HBc</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>anti-HBs</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>HbsAg</td>
<td>negative</td>
<td>Immune due to Hepatitis B vaccination</td>
</tr>
<tr>
<td>anti-HBc</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>anti-HBs</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>HbsAg</td>
<td>positive</td>
<td>Acutely infected</td>
</tr>
<tr>
<td>anti-HBc</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>IgM anti-HBc</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>anti-HBs</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>HbsAg</td>
<td>positive</td>
<td>Chronically infected</td>
</tr>
<tr>
<td>anti-HBc</td>
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<td></td>
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<tr>
<td>IgM anti-HBc</td>
<td>negative</td>
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<td>anti-HBs</td>
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Another way to measure disease progression is by the use of polymerase chain reaction (PCR) technique to isolate specific HBV DNA. PCR is highly specific and can be used to monitor effectiveness of antiviral therapy. Other ways to measure disease progression are liver function tests, blood electrolytes, CT of the liver to look for extent of damage, and liver biopsy.

**Hepatitis B Treatment**

Acute HBV infection will subside on its own and does not require medications. If patients have severe vomiting or diarrhea, they can be treated by restoring fluids. Alternatively chronic HBV infection can be treated with antiviral medication. The decision to treat a patient with antiviral medication is determined by the extent of liver damage. The liver is damaged in HBV by the virus’s multiplying rapidly in the liver. This can be tested by monitoring the HBV DNA in the blood, liver function tests, and biopsy of the liver. Antiviral medications work by preventing the virus from multiplying and are thought to help reduce the incidence of cirrhosis and liver failure, but they do not work on everyone. Currently there are several preferred medications approved for use in the United States for chronic hepatitis B. These are Peginterferon (Interferon-alpha 2b), Entecavir, and Tenofovir. Antiviral medication is not a cure for HBV; it is a way to allow the liver to heal for a period of time.

**Hepatitis B Prognosis**
Only about half of new HBV infections in adults are symptomatic. The majority of those showing symptoms recover rapidly. Some will have a prolonged disease course with symptoms persisting for a long period of time. A small fraction, about 1%, has rapid progression of the disease in the acute stage and develops fulminant hepatitis. Fulminant hepatitis is a severe form of acute hepatitis that can be life-threatening if not treated immediately. The symptoms of fulminant hepatitis develop very suddenly and may include: mental disturbances such as confusion, lethargy, extreme sleepiness or hallucinations, hepatic encephalopathy, sudden collapse with fatigue, jaundice, and swelling of the abdomen.

The risk for chronic HBV infection is inversely related to age: about 90% of infants and 30% of young children develop chronic infection. Only 2-6% of adults become chronically infected. Chronic HBV infection can cause severe liver damage including cirrhosis, liver cancer, liver failure, and ultimately death. Premature death from cirrhosis or hepatocellular carcinoma is 15-25% of those with chronic infection.

**Hepatitis B Prevention**

Fortunately there is a vaccine for hepatitis B virus. The first vaccine became available in 1981 and consists of the viral envelope of the hepatitis B surface antigen, HBsAg, produced in yeast cells. This is done by injecting the DNA of HBsAg into yeast where it is grown, harvested, and purified. The vaccine is given in three separate injections. The second injection is given 1 month after the first injection and the third injection is given 6 months after the first injection. After the 3 injections the body has an immune response and creates an antibody to HBsAg called anti-HBsAg. The person who received the vaccine is now immune to HBV. The following groups should be vaccinated for hepatitis B:

- all children younger than 19 years, including all newborns - especially those born to mothers who are infected with HBV
- all health care and public safety workers who may be exposed to blood
- people who have hemophilia or other blood clotting disorders and receive transfusions of human clotting factors
- people who have end-stage renal disease including those who require hemodialysis for kidney disease
- travelers to countries where HBV infection is common
- prisoners
- intravenous (IV) drug users
- people with chronic liver disease such as Hepatitis C
- people who have multiple sex partners or have ever had a sexually transmitted disease
- household contacts of persons who are carriers of HBV
- anyone who wants to be vaccinated, regardless of risk factors

The HBV vaccine is effective. If a person has been exposed to someone with HBV and they are not vaccinated, they can be given hepatitis B immunoglobulin along with the HBV vaccine. The chance of NOT becoming infected is 80-90% with the combination of immunoglobulin and vaccine.

**HEPATITIS C**

Hepatitis C virus (HCV) is the most common chronic blood borne infection in the United States. About 3.2 million people (1.5% of the population) are chronically infected. Worldwide the estimate of infection is 130-150 million. Prevalence varies from 0.3–2.5% in Western Europe
and North America, 3–7% in much of Asia and South America, to over 10% in parts of Africa, with major variations occurring within these populations.

In the 1970s it was realized that there were a number of cases of hepatitis, particularly from blood transfusions, that were not due to hepatitis A or hepatitis B. The causative agent was called “non-A, non-B (NANB) hepatitis.” This term was regarded as a diagnosis of exclusion because of the absence of specific serologic markers and unknown viral origin. It wasn’t until 1987 that D.W. Bradley of CDC and Michael Houghton, et al., of Chiron Corp., using a novel molecular cloning approach, identified the virus and developed a diagnostic test. Homology does not exist among hepatitis A, hepatitis B, hepatitis D, or hepatitis E viruses and HCV.

Since HCV was not explicitly identified until 1989, and blood tests were not available until 1991, its emergence as a global health issue went largely undetected before that time and the analysis of the pandemic is largely retrospective. Hepatitis C had originally been considered limited to transfusion recipients. Now HCV is recognized in many other epidemiologic settings and as a major cause of chronic hepatitis worldwide.

The hepatitis C virus is one of the most important causes of chronic liver disease in the United States. It accounts for about 15% of acute viral hepatitis, 60 to 70% of chronic hepatitis, up to 50% of cirrhosis, one-third of liver cancer, and significant numbers of end-stage liver disease. See Chart I. It is the number one cause of death in those infected with HIV. Of the U.S. population, 1.6%, or an estimated 4.1 million Americans, have antibodies to HCV (anti-HCV), indicating ongoing or previous infection with the virus. Hepatitis C causes an estimated 17,000 to 20,000 deaths annually in the United States. Following a peak in 1992, incidence of acute hepatitis C declined; however, since 2009 rates have increased from 16,000 in 2009 to almost 30,000 in 2013 (latest statistics available).

Virus Characteristics

Hepatitis C virus is a small, enveloped, single-stranded RNA virus. HCV is closely related to flaviviruses and pestiviruses. Its genetic organization and protein products classify it in the flaviviridae family; its diversity is great enough for it to be classified as a separate genus. HCV is not related to any of the other known hepatitis viruses; however, the recently described hepatitis G virus is a distant relative. There are six known genotypes of the HCV virus, numbered 1-6. Approximately 80% of the hepatitis C patients in the United States have genotype 1; genotype 4 is more common in the Middle East and Africa. Type 1 is the hardest to treat.

The HCV genome is a positive-sense RNA molecule of approximately 9,500 nucleotides. There are highly conserved 5' and 3' untranslated regions flanking an approximately 9,000 nucleotide single open reading frame that encodes a large polyprotein of about 3,000 amino acids. This protein undergoes posttranslational processing by host and viral enzymes to form the structural and nonstructural proteins and enzymes of the virus.

The 5' terminus of the viral RNA is an untranslated region (5' UTR) known to be essential for replication; it contains elements thought to coordinate viral protein synthesis. It is not surprising that this region is highly conserved, and therefore serves as a useful target for amplification in diagnostic assays.

The polymerase enzyme of RNA viruses such as HCV lacks proof-reading ability and is therefore unable to correct copying errors made during viral replication. Many of these nucleotide changes result in a nonfunctional genome or a replication incompetent virus (lethal mutants). However, others persist and account for the tremendous viral diversity that is characteristic of HCV. This heterogeneity is extremely important in the diagnosis of infection, pathogenesis of disease, and the response to treatment. It prevents the development of
conventional vaccines, allows the virus to escape eradication by the host's immune system, and affects the completeness of the response to antiviral therapies such as interferon.

**Viral transmission and damages**

The presence of HCV in the liver triggers the human immune system, which leads to inflammation. Over time (usually decades) prolonged inflammation may cause scarring. Extensive scarring in the liver is called cirrhosis. When the liver becomes cirrhotic, the liver fails to perform its normal functions, leading to serious complications and even death. Cirrhotic livers also are more prone to become cancerous.

Hepatitis C is believed to be transmitted only by blood. However, unlike many other blood borne viruses (like HIV) virtually any source of blood or blood products seems to be capable of carrying the virus, even if the source is indirect – like a used razor, for example. This makes hepatitis C far more transmissible than most other blood borne viruses – including HIV.

Many hepatitis C victims contracted the disease through blood transfusions in the 1970s and 1980s. Rates of post-transfusion hepatitis during this period were determined to have been between 8% and 10%. Effective blood screening for the virus was developed and implemented by 1990, which lowered the rates of post-transfusion hepatitis to less than 5% from 1990-1993. Since then, improved testing has led to drastic reductions in risk, down to less than 1% after 1993. However, anyone who had a blood transfusion prior to that time is at risk for having been infected. Incidence of hepatitis C infection among hemophiliacs remained high through 1993 because plasma used as a source of clotting factors to treat hemophilia was often a mixture from many different donors. However, incidence of new infection among hemophiliacs has rapidly approached zero as better methods for producing coagulation factors have been employed.

The practice of intravenous (IV) drug use provided the greatest opportunity for large-scale HCV transmission. It is responsible for about 30-40% of all identified cases of hepatitis C. As with HIV, the sharing of contaminated needles and other drug paraphernalia dramatically increases the chance of infection. Incidence of infection among IV drug users has surpassed 50% in many studies, and reached 100% in others. Cocaine users have also been shown to transmit the virus by sharing snorting straws.

Tattooing, as well as many body piercing practices, such as acupuncture and ear-piercing, have contributed to the spread of HCV, even in industrial nations. Needle-stick injuries, contaminated medical equipment, and blood spills in health care settings are also responsible for many cases of HCV.

Heterosexual or homosexual activity with multiple sexual partners has been clearly identified as a mode of transmission, but the exact risk is unknown. Because of the lack of sufficient information, persons in long-term, monogamous relationships are advised not to change sexual practices. Day-to-day contact with another household member who has hepatitis C has also been strongly implicated. Maternal-to-infant transmission has been documented as a mode of spread. Certain specialized risks have also been identified – such as manicures, shared toothbrushes, or straight razors in barber shops.

In more than 40% of all cases, the infected individuals cannot identify a source for their infection. It is believed that most of these are caused by known risk factors – however in more than 10% of all cases, no risk factor can be identified. There are clearly other, as yet unidentified, modes of transmission.

**Signs and symptoms**

Although hepatitis C damages the liver, 80% of people with the disease do not have symptoms. (See Chart I) In those who do, symptoms may not appear for 10-20 years, or even
longer. Even then, the symptoms usually come and go and are mild and vague. Unfortunately, by the time symptoms appear, the damage may be serious. A minority of people have symptoms during the early acute phase of the infection. These symptoms typically develop 5-12 weeks after exposure to HCV.

Over time, the liver in people with chronic infection may begin to experience the effects of the persistent inflammation caused by the immune reaction to the virus. Blood tests may show elevated levels of liver enzymes, a sign of liver damage, which is often the first suggestion that the infection may be present. However, up to 40% have a persistent normal alanine transaminase (ALT) but three-quarters of these have liver damage. Since the ALT levels may fluctuate in and out of the normal range, the ALT test should be repeated a number of times.

Patients may become easily fatigued or complain of nonspecific symptoms as the disease progresses. As cirrhosis develops, symptoms increase and may include weakness, weight loss, rash, and some mental disturbances such as confusion and lethargy.

**Acute Hepatitis C**

Acute hepatitis C refers to the first 6 months after infection with HCV although symptoms may appear within a day if infection was caused by any method of intravenous injection. Between 60% and 70% of people infected develop no symptoms during the acute phase. If infection was caused by direct access to the blood stream, the virus may cross the blood brain barrier 100 times more easily. In the minority of patients who experience acute phase symptoms, these are generally mild and nonspecific, and rarely lead to a specific diagnosis of hepatitis C. Symptoms of acute hepatitis C infection include decreased appetite, nausea, vomiting, fatigue, abdominal pain, itching, muscle aches, and fever, i.e., flu-like symptoms. Jaundice, a yellowing of the skin or eyes, is rare at this early stage of infection.

The hepatitis C virus is usually detectable in the blood by polymerase chain reaction (PCR) within one to three weeks after infection, and antibodies to the virus are generally detectable within three to 15 weeks. Spontaneous viral clearance rates are highly variable; between 10 and 60% of persons infected with HCV clear the virus from their bodies during the acute phase, as shown by normalization of the liver enzymes ALT and AST (aspartate transaminase), and plasma HCV-RNA clearance (this is known as spontaneous viral clearance). However, persistent infections are common and most patients develop chronic hepatitis C.

**Chronic Hepatitis C**

Chronic hepatitis C is defined as infection with the hepatitis C virus persisting for more than six months. Clinically, it is often asymptomatic, and it is mostly discovered accidentally (e.g. usual physical check-up).

The natural course of chronic hepatitis C varies considerably from one person to another. However, recent data suggest that among untreated patients, roughly one-third progress to liver cirrhosis in less than 20 years. Another one-third progress to cirrhosis within 30 years. The remainder of patients appears to progress so slowly that they are unlikely to develop cirrhosis within their lifetimes.

Once chronic hepatitis C has progressed to cirrhosis, signs and symptoms may appear that are generally caused by either decreased liver function or increased pressure in the liver circulation, a condition known as portal hypertension. Possible signs and symptoms of liver cirrhosis include ascites (accumulation of fluid in the abdomen), bruising and bleeding tendency, varices (enlarged veins, especially in the stomach and esophagus), jaundice, and a syndrome of
cognitive impairment known as hepatic encephalopathy. Hepatic encephalopathy is due to the accumulation of ammonia and other substances normally cleared by a healthy liver.

Mean ALT and bilirubin levels of patients with hepatitis C are significantly lower than those of patients with HBV, but the extensive overlap of the ranges of elevation precludes identification of the type of viral hepatitis by the use of these assays. Usually prothrombin and albumin results are normal, but may become abnormal once cirrhosis has developed.

The levels of elevation of liver tests do not correlate well with the amount of liver injury on biopsy. Viral genotype and viral load also do not correlate with the amount of liver injury. Liver biopsy is the best test to determine the amount of scarring and inflammation. Radiographic studies, such as ultrasound or CT scan, do not always show liver injury until it is fairly advanced. However, noninvasive tests (blood sample) are coming, with FibroTest and ActiTest, respectively, estimating liver fibrosis and necrotic inflammation. These tests are validated and recommended in Europe. FDA procedures have been initiated in U.S.

**Clinical Laboratory Testing for HCV**

There are three major types of assays available for HCV testing: Enzyme Immunoassay (EIA), Western Blot or Recombinant Immunoblot Assay (RIBA), and Polymerase Chain Reaction (PCR).

Hepatitis C tests are used to screen for and diagnose a hepatitis C virus infection, to guide therapy and/or to monitor the treatment of an HCV infection.

An HCV antibody test is used to screen for infection. It detects the presence of antibodies to the virus, indicating exposure to HCV. This test cannot distinguish between someone with an active or a previous HCV. The HCV RNA test that detects viral RNA in the blood is used to determine if there is an active infection.

The following tests may be used to diagnose a current infection and to guide and monitor treatment:

- **HCV RNA test.** Qualitative test is used to distinguish between a current or past infection.
- **HCV Viral Load (HCV RNA test, quantitative)** detects and measures the number of viral RNA particles in the blood. Viral load tests are often used before and during treatment to help determine response to therapy by comparing the amount of virus before and during treatment. Some newer viral load tests can detect very low amounts of viral RNA.
- **Viral genotyping** is used to determine the genotype of the HCV present. There are 6 major types of FCV; the most common (genotype 1) is less likely to respond to treatment than genotypes 2 or 3 and usually requires longer therapy. Genotyping is often ordered before treatment is started to give an idea of the likelihood of success and how long treatment may be needed.

Interpretation of the HCV screening and follow-up tests is in the following table:

<table>
<thead>
<tr>
<th>HCV Antibody</th>
<th>HCV RNA</th>
<th>HCV Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td></td>
<td>No infection (or too early after exposure)</td>
</tr>
<tr>
<td>Positive or indeterminate</td>
<td>Negative</td>
<td>Past infection or no infection (false pos. screen)</td>
</tr>
<tr>
<td>Positive or weak</td>
<td>Positive</td>
<td>Current Infection</td>
</tr>
</tbody>
</table>
Liver Biopsy

Liver biopsy is not necessary for diagnosis but is helpful for grading the severity of disease and staging the degree of fibrosis and permanent architectural damage. Hematoxylin and eosin stain and Masson’s trichrome stain are used to grade the amount of necrosis and inflammation and to stage the degree of fibrosis. Specific immunohistochemical stains for HCV have not been developed for routine use. Liver biopsy is also helpful in ruling out other causes of liver disease, such as alcoholic liver injury, nonalcoholic fatty liver disease, or iron overload. HCV causes the following changes in liver tissue:

- Necrosis and inflammation at the edge of the portal areas, so-called “piecemeal necrosis” or “interface hepatitis”
- Necrosis of hepatocytes and focal inflammation in the liver parenchyma
- Inflammatory cells in the portal areas (“portal inflammation”)
- Fibrosis may exist in:
  - an early stage, being confined to the portal tracts
  - an intermediate stage consisting of expansion of the portal tracts and bridging between portal areas or to the central area
  - a late stage of frank cirrhosis characterized by architectural disruption of the liver with fibrosis and regeneration

Several scales are used to stage fibrosis. One common classification is a scale from 0 to 4 where stage 0 indicates no fibrosis; stage 1 indicates enlargement of the portal areas by fibrosis; stage 2 indicates fibrosis extending out from the portal areas with rare bridges between portal areas; stage 3 indicates many bridges of fibrosis that link up portal and central areas of the liver; and stage 4 indicates cirrhosis.

Assigning scores for severity, grading, and staging of hepatitis is helpful in managing patients with chronic hepatitis. The degree of inflammation and necrosis can be assessed as none, minimal, mild, moderate, or severe. The degree of fibrosis can be similarly assessed. Scoring systems are particularly helpful in clinical studies on chronic hepatitis.

Treatment

Until new drugs were approved in May, 2011, in 2013, 2014, and 2015, the standard treatment for Hepatitis C was with peginterferon and ribavirin. This regimen required injections and had serious side effects.

In May 2011 the FDA approved Boceprevir (Victrelis, Merck & Co.) and Telaprevir (Incivek, Vertex Pharmaceuticals). Both drugs block an enzyme that helps the virus reproduce. If used alone the virus would become resistant. The drugs, given in addition to the standard treatments using the injected drug, peginterferon, and the pill, ribavirin, decreased resistance and increased effectiveness. The combination of the drug treatment increased the sustained virologic response rate to about 70%.

However, in late 2013 the FDA approved two new direct acting antiviral drugs, Sofosbuvir and Simeprevir. One or the other of these drugs, when used in combination with peginterferon and ribavirin, obtained sustained virologic response of 80-95% of patients.
Most recently, in late 2014 and in 2015, drug treatment regimes were approved, as follows: (see www.hepmag.com/articles/2512_18756.shtml)

- **Harvoni**, a combination of sofosbuvir and ledipasvir cures 90% of genotype 1 hepatitis C in 12 weeks.
- **Veikira Pac**, a combination of ombitasuir, paritaprevir, and ritonavir plus dasabuvir pills cures over 95% in 12 to 14 weeks.
- **Sovaldi** plus ribavirin is used for Hepatitis C genotypes 2 and 3.
- In July 2015 **declastasvir** in combination with sofosbuvir was approved for treating genotype 3.

These newer medications are super-expensive and may not be covered by some insurance. Other drugs are presently being reviewed.

**Prevention**

Hepatitis C virus can only be transmitted through blood transfer, but exposure to even small amounts of blood must be avoided:

- Never share needles
- Don’t share personal care items
- Choose tattoo and piercing parlors carefully
- Practice safe sex
- Healthcare workers must take precautionary measures to avoid needle-stick injuries and avoid coming into direct contact with blood. Equipment used in drawing blood should be discarded safely or sterilized appropriately.

Recent investigations have shown that excluding blood from donors who are positive for d anti-HBcAg from the blood supply and use of third-generation anti-HCV testing has reduced the incidence of post-transfusion hepatitis C.

Vaccines and immunoglobulin products do not exist for prevention or treatment of hepatitis C. Development of immunization appears unlikely in the near future because these products would require antibodies to all the genotypes and variants of hepatitis C; however, some type of vaccine may eventually be developed.

**HEPATITIS D**

Hepatitis D virus was discovered in 1977 by Mario Rizzetto as a nuclear antigen in patients infected with HBV who had severe liver disease. Hepatitis D is a unique sub-viral satellite infection. It is a small defective RNA containing virus that cannot replicate independently, but requires the Hepatitis B antigen for replication. Individuals cannot contract HDV unless they have had hepatitis B or are co-infected with HBV and HDV. Modes of transmission of HDV are very similar to HBV infections. Each year approximately 7,500 individuals contract HDV, and chronic HDV infections are responsible for approximately 1,000 deaths in the United States. It is found throughout the world and is more prevalent in the Mediterranean area.

HDV requires the help of viral particles from HBV to replicate and infect other hepatocytes. It is believed that HDV infections occur through the binding of the HDV virion to the same cellular receptor as the HBV.

In general during the acute phase of the infection HDV carriers develop severe hepatitis and 70-80% progress onward to chronicity. Infection with HDV causes more severe disease than infection with HBV alone, including faster progression to liver cirrhosis and greater possibility
of developing liver cancer. Hepatitis D combined with hepatitis B has the highest mortality rate of all hepatitis infections.

**Hepatitis D Diagnosis:**
Diagnosis of HDV relies on the detection of antibodies against hepatitis D antigen and serum HDV RNA, as well as HBV markers.

**Hepatitis D Prevention:**
There is no vaccine for hepatitis D but it can be prevented by giving hepatitis B vaccine to persons who are not infected by hepatitis B. Currently widespread use of the vaccine for hepatitis B has resulted in a decline in the incidence of hepatitis D.

**Hepatitis D Therapy:**
Interferon-a is currently the therapy in use for Chronic HDV infections, however hepatitis D requires a higher dose and the treatment period needs to be longer than for patients with hepatitis B.

**HEPATITIS E**
Hepatitis E virus (HEV) was first identified in 1955, in an outbreak in New Delhi, India. HEV is a non-enveloped single stranded RNA containing virus. It is the sole member of the genus Hepevirus.

HEV is transmitted primarily by the fecal-oral route via water borne and food contaminated sources. The most frequent source of infection is fecally contaminated drinking water. Therefore HEV outbreaks are most common in developing countries. Outbreaks most commonly occur after heavy rainfalls and monsoons because of their disruption of the water supplies. At particular risk are people living in refugee camps and overcrowded housing after natural disasters. There have been some reports that the virus was isolated from wild pigs and deer, as well as some species of rats and there is the possibility of zoonotic transfer.

Symptomatic Hepatitis E is uncommon in the United States. Infected individuals usually have a history of travel to a country where hepatitis E is endemic. Nonetheless rare cases have been found in people who have no history of travel to endemic countries. Surprisingly recent studies have found a high incidence of antibodies to HEV in the general population in the U.S.

**Symptoms of Hepatitis E**
The clinical presentation of HEV is very similar to hepatitis A; however the severity of the infection is greater than HAV infections.

**Hepatitis E Diagnosis:**
Diagnosis of HEV infections relies upon the presence of IgM anti-HEV markers. There are EIA and immunochromatography assays available and there is an increased use of PCR assays for limited confirmatory roles. The incubation period for HEV is 21-42 days. The virus can be detected in bile and feces by about seven days after infection.

**Hepatitis E Prognosis:**
In most cases the infection is somewhat mild, but in pregnant women HEV can be very severe. Literature indicates that mortality can be up to 20% in pregnant females.

**Hepatitis E Prevention:**
Currently, there are no commercially available HEV vaccines approved for use in the United States.
HEPATITIS G

Two viral agents related to hepatitis have been identified as G virus (HGV) and GB virus type C (GBV-C). Molecular characterization has shown them to be virtually identical. This virus has been detected worldwide and has been found in high prevalence in the U.S. It is common to find people with hepatitis C that are co-infected with GBV-C. The clinical significance is not well understood, but most evidence suggests that GBV-C does not cause hepatitis in humans.

CONCLUSION

Viral hepatitis continues today as a major public health concern, but because of improvements in food handling, water purification, improved testing of donor blood, the use of vaccines, and new medications, the numbers of infections and deaths have diminished for most of the hepatitis types. The exception is Hepatitis C, whose incidence has increased in the past few years. Although there is no vaccination for Hepatitis C, newer medications offer hope of cure to those infected.

Hepatitis causes damage to the liver, an organ that is essential for life; however the use of new medications allows the liver time to recover from the infection in many cases. The increased use of clinical laboratory testing in the areas of antibody detection and polymerase chain reaction technology has given the healthcare provider new tools. With many new cases reported each year of the several different types of hepatitis with varying degrees of severity, it is important for laboratorians to understand how to interpret test results and report them appropriately.

REFERENCES:
4. American Association for Clinical Chemistry:
   https://labtestsonline.org/understanding/analytes/hepatitis-c/tab/test/
5. www.hepmag.com/articles/2512_18756.shtml
www.uptodate.com/contents/gb-virus-c-hepatitis-g-infection
CHART #1.

**Hepatitis C History of Infection**

- Exposure
- Acute Disease
  - Resolved – 15%
  - Chronic – 85%
- Asymptomatic 10-20 years
  - Cirrhosis – 20%
  - End Stage Liver Disease (ESLD)
    - 6%/yr.
  - Hepatic carcinoma (HCC)
    - 4%/yr.

*Ref.: Ayoub, Walid, M.D. Hepatitis C Seminar, Eden Hospital Castro Valley, CA, 3/2/12
REVIEW QUESTIONS
Course #DL 004
Choose the one best answer

1. All of the following are functions of the liver except
   a. produces blood clotting factors
   b. converts harmless chemicals into harmful ones
   c. stores sugars, fats, and vitamins
   d. manufactures enzymes and hormones

2. Hepatitis A
   a. is called the long incubation hepatitis
   b. was isolated by Purcell in 1973
   c. occasionally develops into a chronic stage
   d. is most commonly diagnosed by enzyme immune-assay (EIA)

3. Hepatitis A infection is spread through:
   a. contact with blood
   b. droplets from sneezing
   c. fecal-oral pathway
   d. coughing from infected individuals

4. Hepatitis A is a:
   a. SS-DNA virus
   b. DS-DNA virus
   c. RNA virus
   d. TLC virus

5. Methods of prevention of Hepatitis A include all except
   a. good hand washing procedures
   b. avoiding using contaminated needles
   c. vaccination
   d. avoiding high risk sexual behavior

6. Hepatitis B viral infection is frequently referred to by the name:
   a. long incubation hepatitis
   b. short incubation hepatitis
   c. non A-non B hepatitis
   d. infectious hepatitis

7. Hepatitis B virus is a:
   a. picornavirus
   b. hepadnavirus
   c. flavivirus
   d. calicivirus
8. Hepatitis B virus is spread through contact with:
   a. body fluids
   b. contaminated water
   c. food
   d. feces

9. One way to test the progression of hepatitis B viral infection is:
   a. HPLC
   b. atomic absorption
   c. electron microscopy
   d. polymerase chain reaction

10. One of the antiviral medications currently used to treat hepatitis viral infection is:
    a. quinadine
    b. peginteferon
    c. laudinum
    d. heptoplasmin

11. A patient is HBsAg negative, anti-HBc negative, anti-HBs positive. The most likely interpretation is:
    a. hepatitis B susceptible
    b. immune due to hepatitis B infection
    c. immune due to hepatitis B vaccination
    d. chronically infected with HBV

12. In order for the vaccine for hepatitis B viral infection to be effective, a person needs to have how many injections of the vaccine?
    a. 1
    b. 2
    c. 3
    d. 4

13. Hepatitis C viral infection is spread through contact with contaminated:
    a. saliva
    b. food
    c. water
    d. blood

14. According to the course which hepatitis has shown an increase in incidence in the U.S. since 2009?
    a. hepatitis A
    b. hepatitis B
    c. hepatitis C
    d. hepatitis D
15. Hepatitis C viral infection has an incubation period of:
   a. 1-2 weeks
   b. 1-4 weeks
   c. 2-15 weeks
   d. 1-6 weeks

16. The newest treatment for patients with hepatitis C viral infection is:
   a. Harvoni
   b. alpha and beta interferon
   c. boceprevir
   d. ribaviron and pegylated interferon

17. Patients with hepatitis D viral infections have had previous or are co-infected with:
   a. hepatitis A virus
   b. hepatitis B virus
   c. hepatitis E virus
   d. non-A, non-B virus

18. Which of the following is not a mode of transmission of hepatitis E?
   a. contaminated blood
   b. fecal-oral route
   c. wild pigs that have been used for food
   d. wild deer that have been used for food

19. In the United States hepatitis E most often occurs
   a. along with hepatitis G
   b. in sporadic epidemics
   c. in people who have returned from a developing country
   d. in post-transfusion patients

20. A vaccine is not available for which type of viral hepatitis?
   a. hepatitis A virus
   b. hepatitis B virus
   c. hepatitis C virus
   d. hepatitis D virus