Marmara Medical Journal 2016; 29 (Special issue 1): 6-9
DOI: 10.5472/MMJsi.2901.03

REVIEW / DERLEME

Liver function tests in chronic viral hepatitis cases

Kronik viral hepatit olgularında karaciğer fonksiyon testleri

Goncagül HAKLAR

ABSTRACT

The liver has many vital functions and biochemical tests are used for the assessment. For the diagnosis of chronic hepatitis: (1) persistence of increased alanine aminotransferase (ALT) for 6 months after an episode of acute hepatitis; or (2) increased ALT (without another explanation) on more than one occasion over a period of 6 months are needed. The time period may be shorter for the patients with risk factors. Although the clinical picture of HBV and HCV in chronic cases is similar, aminotransferase activities are different. In HBV, ALT activity is generally constant, except increases are evident at seroconversion times. On the other hand, two-third of HCV cases experience fluctuation. In the evaluation of a patient with a new diagnosis of chronic viral hepatitis, a comprehensive medical history should be taken, followed by full physical examination. Laboratory workup should start with a comprehensive metabolic panel with liver function tests including assessment of synthetic function with prothrombin time and serum albumin level measurement. Screening for the chronic hepatic injury is not cost-effective and should be limited to high-risk individuals. ALT should be the preferred biochemistry parameter besides viral serology and increased activity should be confirmed before further evaluation. For treatment, patients with increased ALT are more likely to respond than those with initially normal ALT activity.

Keywords: Chronic viral hepatitis, Hepatitis B virus, Hepatitis C virus, Alanine aminotransferase, Aspartate aminotransferase

ÖZ

Karaciğerin pek çok hayati fonksiyonu vardır ve bu fonksiyonlar biyokimya testleri ile değerlendirilir. Kronik viral hepatit tanısı için: (1) akut hepatit atağından sonra 6 ay boyunca yüksek seyreden alanin aminotransferaz (ALT) aktivitesi; veya herhangi bir neden olmaksızın 6 ay içinde bir kereden fazla ALT yüksekliği gereklidir. Risk gruplarında süre kısaltılabilir. Kronik olgularda HBV ve HCV benzer klinik tablolar sergileseler de, aminotransferaz aktiviteleri farklıdır. HBV olgularında ALT aktivitesi genelde sabitken, serokonversiyon dönemlerinde artışlar izlenir. HCV olgularının üçte ikisinde ise dalgalanmalar olur. Yeni tanı almış kronik viral hepatit olgularında ayrıntılı tıbbi öykü alınmalı ve ayrıntılı fizik muayene yapılmalıdır. Laboratuvar incelemeleri karaciğer fonksiyon testleri ve protrombin zamanı ile serum albümin düzeyi ölçümleri gibi karaciğerin sentez fonksiyonlarını da değerlendiren testleri de içeren ayrıntılı bir metabolik panel ile başlamalıdır. Kronik viral hepatit taraması maliyet-etkin değildir ve sadece yüksek risk taşıyanlar ile sınırlı olmalıdır. Viral serolojinin yanı sıra biyokimya testi olarak ALT tercih edilmeli, artmış aktivite ileri incelemeden önce mutlaka teyid edilmelidir. Tedavi izleminde artmış ALT aktivitesi olan olgular başlangıçta normal olanlara göre daha iyi cevap verirler.

Anahtar kelimeler: Kronik viral hepatit, Hepatit B virüsü, Hepatit C virüsü, Alanin aminotransferaz, Aspartat aminotransferaz

Introduction

The liver has many vital biochemical functions including excretion, synthesis, metabolism, and storage. Endogenous and exogenous organic anions are extracted, biotransformed, and excreted into the bile, while the measurement of endogenously produced compounds like bilirubin and bile acids are used for the assessment of excretory function. Hepatic synthetic function not only involves glucose synthesis via gluconeogenesis, but also protein, triglyceride, fatty acid, cholesterol, and bile acid synthesis as well. Activation and detoxification of drugs, and the disposal of exogenous and endogenous substances, such as galactose and ammonia are included in hepatic metabolic functions.

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Liver is the major site for glycogen storage besides some minerals and vitamins. Liver function tests are useful in (1) detecting, (2) diagnosing, (3) evaluating severity, (4) monitoring therapy, and (5) assessing the prognosis of liver disease and dysfunction. Different tests are utilized to assess different functions of the liver (Table I) [1].

Table I. Main liver function tests and their usage

Test	Usage
Bilirubin, total	Diagnosing jaundice, modest correlation with severity
Alkaline phosphatase (ALP)	Diagnosing disorders of metabolism in children, cholestatic disorders in adults
Bilirubin, direct and indirect	Diagnosing cholestasis and space-occupying lesions
Aspartate aminotransferase (AST)	Sensitive test of hepatobiliary disease; AST>ALT in alcoholic disease
Alanine aminotransferase (ALT)	Sensitive and more specific test of hepatobiliary disease
Albumin	Indicator of chronicity and severity
Prothrombin time (PT)	Derangement of synthetic functions in acute cases, indicator of severity of cholestasis

Chronic hepatitis is defined as a chronic inflammation of the liver that persist for at least 6 months, or signs and symptoms of chronic liver disease in the presence of elevated cytosolic enzymes [2]. The clinical presentations of chronic hepatitis cases are highly variable. Most patients are diagnosed because of unexplained abnormalities of aminotransferases or positive results on screening tests for cause of chronic hepatitis. Moderate elevations of aminotransferase activities (twofold to fivefold) are characteristic, whereas results of most other tests are normal. Normal aminotransferase activities do not rule out pathological evidence of chronic hepatitis, especially in the presence of chronic viral hepatitis or nonalcoholic steatohepatitis [3,4].

'National Academy of Clinical Biochemistry Guidelines on Use of Laboratory Tests in the Diagnosis and Monitoring of Hepatic Injury' states that in the absence of liver biopsy showing evidence of chronic hepatitis, one of the following laboratory results should be used for the diagnosis of chronic hepatitis: (1) persistence of increased alanine aminotransferase (ALT; EC 2.6.1.2) for 6 months after an episode of acute hepatitis; or (2) increased ALT (without another explanation) on more than one occasion over a period of 6 months. In patients with risk factors for chronic viral hepatitis, genetic causes of hepatic injury, autoimmune liver injury, or in the presence of clinical signs or symptoms of liver disease the time period may be shorter (evidence IIB) [2].

Although the clinical picture of HBV and HCV in chronic cases is similar, aminotransferase activities are different. In HBV, ALT activity is generally constant, except increases are evident at seroconversion times. On the other hand, two-third of HCV cases experience fluctuation [5-7]. Serum ALT activities of chronic hepatitis C cases range between one to four times the upper reference limit, reaching maximum less than seven times. On the other hand, 15-50% of cases with chronic hepatitis C have normal ALT activities [6,8,9], but this rates decreases with increasing number of measurements [8]. From 43% of cases experiencing fluctuation between normal and abnormal, 16% of those with normal ALT on their first two visits and 11% of those with normal ALT on their first three visits subsequently develop increased ALT [8]. Chronic hepatitis C cases with persistantly normal ALT activities experience lower rates of progression to cirrhosis [7,10]. ALT activity is consistently higher than aspartate aminotransferase (AST; EC 2.6.1.1) with all causes of chronic hepatic injury except for alcoholic hepatitis [11-14]. Additionally, ALT may be normal in cirrhotic cases but AST activity increased [15,16]. Total bilirubin, direct bilirubin, and alkaline phosphatase (ALP; EC 3.1.3.1) are generally normal in chronic viral hepatitis cases [17,18].

In the evaluation of a patient with a new diagnosis of chronic viral hepatitis, a comprehensive medical history should be taken, followed by full physical examination. Laboratory workup should start with a comprehensive metabolic panel with liver function tests including assessment of synthetic function with prothrombin time and serum albumin level measurements. Although ALT reflects liver inflammation, its correlation with necroinflammation is not satisfactory and even poorer for fibrosis [19,20]. Thrombocytopenia (count<150000/μL) is often a sign of portal hypertension and liver cirrhosis, but its sensitivity and specificity is limited for the diagnosis of cirrhosis (68% and 76 %, respectively) [21]. AST/ALT ratio>1 is another laboratory marker suggestive of cirrhosis, which is also seen in alcoholism. Combined tests like AST/platelet ratio index (APRI) and Forn index show good correlation with liver fibrosis [22,23]. Measurement of 25-hydroxy-vitamin D3 level will be beneficial, as its deficiency has been found to be correlated with reduced antiviral activity of peg-interferon plus ribavirin treatment [24-26]. Chronic viral hepatitis cases

with cirrhosis should be monitored in every 3-6 months with clinical examinations and laboratory tests (comprehensive metabolic panel, coagulation profile, complete blood count) with the calculation of the Child-Turcotte-Pugh (CTP) and Model for End-Stage Liver Disease (MELD) score to determine the prognosis of patient [27,28].

According to 'European Association for the Study of the Liver Disease (EASL 2012)', ALT is considered as a classification criteria for the phases of chronic hepatitis B (CHB): (1) immune tolerant phase with HBeAg positive, HBeAb negative, Viral DNA>20000 IU/mL and normal ALT level, (2) HBeAg-positive CHB (immune reactive) phase with HBeAg positive, HBeAb negative, Viral DNA >2000 IU/mL and elevated ALT level, (3) low replicative phase with HBeAg negative, HBeAb positive, Viral DNA <2000 IU/mL and normal ALT level, and (4) HBeAg-negative CHB phase with HBeAg negative, HBeAb positive, viral DNA >2000 IU/ml and elevated ALT level [29-31].

Screening for the chronic hepatic injury is not cost-effective and should be limited to high-risk individuals like anyone who has ever injected illicit drugs, recipients of blood transfusions or solid organ transplants before July 1992, or clotting factor concentrates before 1987, patients who have ever received long-term hemodialysis treatment, persons with known exposure to hepatitis C, persons infected with HIV, children born to HCV-positive mothers, persons with persistently elevated levels of alanine aminotransferase, and anyone born from 1945 through 1965 according to "Centers for Disease Control and Prevention Hepatitis C Virus Testing Recommendations", August 2012 [32-34]. ALT should be the preferred biochemistry parameter besides viral serology and increased activity should be confirmed before further evaluation [17,18]. An increase of one to two times the upper reference limit should not be attributed to disease state [17,18,35]. Especially in young individuals creatine kinase (CK; EC 2.7.3.2) measurement should be considered to rule out skeletal muscle as the origin besides history of exercise [36]. Generally, chronic HBV carriers have normal ALT activity like many cases of chronic HCV cases but repeated testing increase the chance of revealing increased values [37]. Anti-HCV-positive chronic cases should be confirmed with HCV RNA tests and negative results in the presence of increased ALT activities should be repeated (evidence IIB).

For treatment, patients with increased ALT are more likely to respond than those with initially normal ALT activity [38,39]. For HCV treatment HCV RNA levels and ALT activity should be measured after 12 weeks of treatment to determine nonresponders (evidence IIB).

Key Points

- Liver function tests are useful in detecting, diagnosing, evaluating severity, monitoring therapy of chronic liver diseases.
- Persistence of increased alanine aminotransferase for 6 months after an episode of acute hepatitis indicates chronicity.
- In the treatment of Chronic hepatitis patients with increased ALT are more likely to respond than those with initially normal levels.

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Marmara Medical Journal 2016; 29 (Special issue 1): 10-17
DOI: 10.5472/MMJsi.2901.04

REVIEW / DERLEME

Microbiological diagnostics of viral hepatitis

Viral hepatitlerde mikrobiyolojik tanı

Ufuk HASDEMİR

Introduction

Viral hepatitis is an infection that primarily affects the liver but may also have systemic clinical manifestations. The vast majority of viral hepatitis are caused by one of five hepatotropic viruses: hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D (delta) virus (HDV), and hepatitis E virus (HEV) (Table I) [1]. HBV, HCV, and HDV also cause chronic hepatitis, whereas HAV does not. HEV causes acute hepatitis in normal hosts but can cause protracted and chronic hepatitis in immunosuppressed patients.

Keywords: Microbiologic diagnosis, Hepatitis A, hepatitis B, Hepatitis C

Table I. Characteristics of five hepatitis viruses and their infections

	HAV	HBV	HCV	HDV	HEV
Family	*Picornaviridae*	*Hepadnaviridae*	*Flaviviridae*	*Unassained*	*Hepeviridae*
Genus	*Hepatovirus*	*Orthohepadnavirus*	*Hepacivirus*	*Delta virus*	*Hepevirus*
Nucleic acid	RNA	DNA	RNA	RNA	RNA
Incubation period (days)	15-48 (mean 30)	30-180 (mean 60-90)	15-160 (mean 50)	30-180 (mean 60-90)	14-60 (mean 40)
Transmission					
Fecal-oral	Yes	No	No	No	Yes
Sexual	Possible	Yes	Rare	Yes	No
Blood	Rare	Yes	Yes	Yes	No[a]
Chronic infection	No	Yes	Yes	Yes	Yes[b]
Cirrhosis	No	Yes	Yes	with HBV	No[c]
HCC					

[a] Can be bloodborne in endemic areas
[b] Acute hepatitis in normal hosts, protracted and chronic infection only in immunosuppressed patients
[c] Cirrhosis can occur after chronic infection, which is confined to immunosuppressed patients

Liver may also be target some other viruses including Epstein-Barr virus (EBV), cytomegalovirus (CMV), adenovirus, Herpes simplex virus (HSV), enteroviruses, yellow fever virus, mumps, rubella, and rubeola. However liver is not the primarily infected organ with these viruses and its' involvement is seen on occasion as part of their systemic infections. Additionally a few viruses such as hepatitis G, hepatitis GB, transfusion-transmitted virus, SEN virus may account in cases of hepatitis presenting features similar to hepatitis caused by well known viruses.

In this section laboratory diagnostics of well known

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hepatitis viruses, HAV, HBV, HCV, HDV, and HEV will be addressed.

Hepatitis A

Serologic assays: Diagnosis of hepatitis A is most commonly confirmed by detection of specific IgM by an enzyme immune assay based techniques in a single acute-phase serum sample (Figure 1) (Table II, III) [1-3]. IgM Anti-HAV may be detected at the time of the first increase in alanine aminotransferase (ALT). However, specific antibody tests can be initially negative in 10% of patients who are tested soon after the onset of symptoms; testing should be repated for these patients if hepatitis A is clinically suspected. IgM Anti-HAV can be detected at least 2 days after ALT peak and remains positive in most for 3 to 6 months. It is rarely detected more than years after initial exposure. Assays for total HAV antibody are of little diagnostic value because IgG persists for many years and may be related to a past infection.

HAV antigen and nucleic acid detection: HAV or viral antigen can be detected in the stools of patients 1 to 2 weeks before symptoms develop, but such detection has little value in routine clinical diagnosis because the tests are not widely available and shedding often has stopped before the patient seeks medical attention. Nucleic-acid based techniques are seldom useful for general diagnostic purposes [1,2]. These techniques have been used in research laboratories when a sensitive test for the presence of HAV is required.

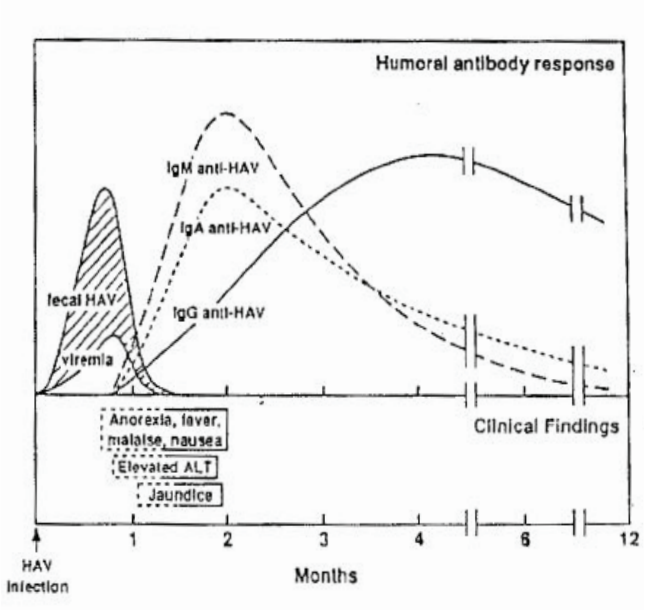


Figure 1. Serologic, virologic and clinical course of hepatitis A

Table II. Simplified diagnostic approach in patients with acute hepatitis

	Serologic tests of patient's serum			
Diagnostic interpretation	HbsAg	IgM Anti-HAV	IgM Anti-HBc	Anti-HCV
Acute hepatitis B	+	-	+	-
Chronic hepatitis B	+	-	-	-
Acute hepatitis A	-	+	-	-
Acute hepatitis A and B	+	+	+	-
Acute hepatitis A and B (HBsAg below detection threshold)	-	+	+	-
Acute hepatitis B (HBsAg below detection threshold)	-	-	+	-
Acute hepatitis C	-	-	-	+

HAV, hepatitis A virus; HBc, hepatitis B core; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus

Table III. Methods for detection and identification of hepatitis viruses

	Method				
Virus	Electron microscopy	Culture	Antigen detection	Nucleic acid detection	Antibody detection
HAV	C[c]	C	C	C	A[a]
HBV	C	C	A	A	A
HCV	C	C	C	A	A
HDV	C	C	B[b]	B	A
HEV	C	C	C	B	A

[a] A; test is generally useful for the indicated diagnosis
[b] B; test is useful under certain circumstances
[c] C; test is seldom useful for general diagnostic purposes

Hepatitis E

Hepatitis E virus may account for as much as 70% of the cases of acute viral hepatitis in endemic countries. It may cause fulminant hepatitis with case fatality rates of 0,9% to 2,8% in men and up to 20% in pregnant woman. Specific diagnosis of acute or chronic HEV infection remains a challenge. Figure 2. has shown typical serologic and virologic course during HEV infection. Commercially available diagnostic assays vary widely in sensitivity and specificity (Table III) [1,4]. False-positive results may be seen because of high sensitivity of these assays. There is no

FDA-approved serologic or nucleic acid based techniques for diagnosis of hepatitis E. In HEV nonendemic countries, the infections of more common agents (i.e. HAV, HBV, and HCV) should first be excluded. Detection of HEV-specific IgM can be used for serologic diagnosis of acute infection in areas of low prevalence, but comperative studies have shown a wide range of assay performance. Detection of HEV-specific IgG has little impact for diagnosis of acute infection in HEV endemic countries where a majority of population has antibody from prior infection. Specificity of assays is less critical than assay sensitivity in HEV endemic countries. So rapid point-of-care IgM test may have promise for HEV diagnosis in such areas.

Detection of HEV-RNA in serum sample by RT-PCR is used as a confirmatory test for positive IgM anti-HEV results in nonendemic countries and can be used to monitor infection (Table III). Virus isolation by tissue culture and detection of viral antigen are not appropriate methods for diagnostic purposes (Table III).

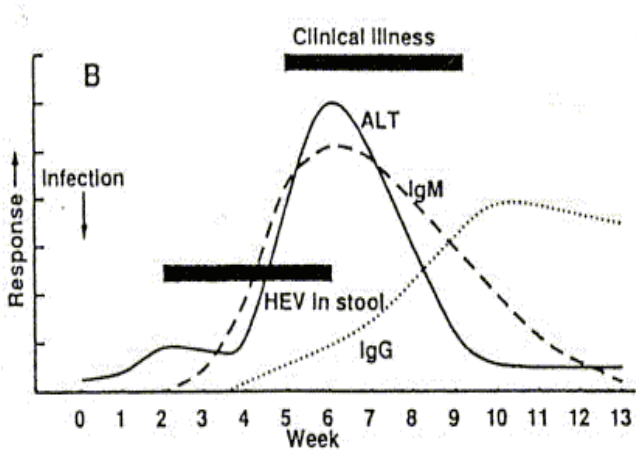


Figure 2. Serologic, and virologic course of hepatitis E

Hepatitis B

Acute hepatitis B: The diagnosis of acute Hepatitis B is based on the detection of HBsAg and IgM anti-HBc (Figure 3) (Table II, IV) [1,5]. Surface antigen (HBsAg) is the serologic hallmark of HBV infection and is the first marker to appear after acute infection. HBsAg appears in serum within 1 to 12 weeks, usually in 8 to 12 weeks after an acute exposure to HBV, before the onset of symptoms or elevation of serum ALT. In patients who subsequently recover, HBsAg usually becomes undetectable after 4 to 6 months, followed by development of specific IgM against core antigen (IgM anti-HBc) and then development of IgG anti-HBc. Anti-HBc can be present weeks to months before detection of anti-HBs (Figure 3). After HBsAg clears, anti-HBs becomes detectable and persists indefinitely. Typically anti-HBs appears in serum as early as 8 months after infection ((Figure 3) (Table II, IV). Markers of viral replication, such as HBsAg and HBV-DNA in the serum, will be present at the same time as anti-HBc. Molecular methods used for HBV-DNA detection has been shown in Table IV. Detection of HBeAg may be useful as a marker for high levels of viral replication and for relative infectivity late in the course of illness. Loss of HBV-DNA, HBeAg, HBsAg and IgM Anti-HBc and the development of anti-HBe and anti-HBs all characterize immunity. The 'a' determinant is the predominant B cell epitope common to all HBV subtypes. Antibodies against to this epitope confer immunity to all HBV subtypes. Anti HBs is sufficient for protection against HBV infection.

Patients who recover from acute hepatitis B are probably not truly cured of infection because a significant number of patients will have HBV-DNA detectable by PCR many years after clinical recovery. They generally have lifelong protection from disease unless there is significant immunosuppression with loss of protective immune responses, such as in the setting of HIV, chemotherapy, or bone marrow transplantation. Table IV, has shown various serologic profiles during acute hepatitis B infection resulting with recovery.

Recovery from acute infection and Vaccination: Recovery from acute infection and vaccination can be distinguished by testing for total anti-HBc [1,5]. Recovery is characterized by the presence of both anti-HBs and total anti-HBc. The presence of anti-HBs is the only marker of HBV vaccination (Table IV).

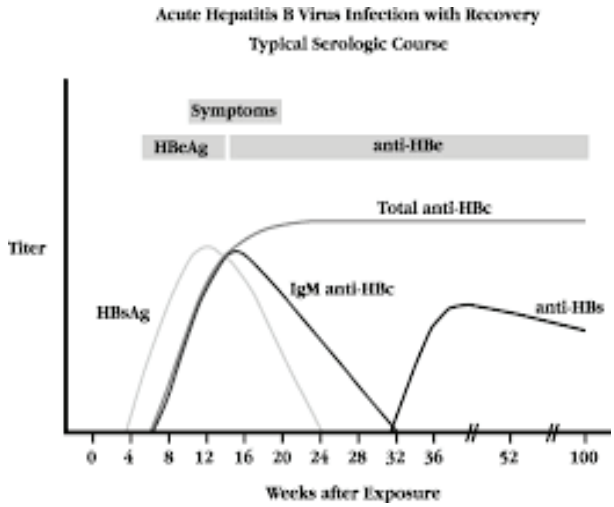


Figure 3. Serologic course of acute hepatitis B infection

Table IV. Serologic and replication markers in various phases of hepatitis B infection

	HBsAg	HBeAg	Anti-HBe	Anti-HBc IgM	Anti-HBc total	Anti-HBs	HBV-DNA
Acute hepatitis B							
Early infection	+	-	-	-	-	-	+
Acute infection	+	+	-	+	+/-	-	+
Windows period	-	-	-	+	+/-	-	+
Acute infection (Recovery period)	-	-	+	+	+	-	+
Recovery	-	-	+/-	-	+	+	-
Chronic hepatitis B							
Chronic infection (high infectivity)	+	+	-	-	+	-	+
Chronic infection	+	-	+	-	+	-	+
Chronic infection	-	-	+	-	+	-	+
Chronic infection (low level infection)	-	-	-	-	+	-	+/-
Inactive chronic infection	+	-	-	-	+	-	-
Vaccination	-	-	-	-	-	+	-

Table V. Nucleic acid based tests for detection of hepatitis viruses

Technology	Manufacturer	Product	Targets
Polymerase Chain reaction	Abbott Molecular Diagnostics (Abbott Park, IL)	Real-time PCR	HBV, HCV
	Roche Molecular Diagnostics Pleasanton, CA)	Amplicor	HCV
		AmpliPrep/COBAS	HBV, HCV
		AmpliScreen	HBV, HCV
		COBAS TaqMan	HBV, HCV
		COBAS TaqScreen	HBV, HCV
Transcription mediated amplification (TMA)	Siemens Healthcare Diagnostics (Tarrytown, NY)	VERSANT	HCV
	Hologic Gen-Probe	Aptima	HCV
Branched DNA amplification (bDNA)	Siemens Healthcare Diagnostics	VERSANT	HCV

Chronic hepatitis B: Chronic hepatitis B develops in less than 5% of adults, 10% to 25% of young children, and 80% to 90% of infants after acute infection. The persistence of HBsAg for more than 6 months is a marker for development of chronic disease (Figure 4) [1,5]. Patients may have additional markers of viral replication, HBeAg or HBV DNA, as well. HBeAg and HBV-DNA in serum, should be investigated for further classification of the disease and to determine the need for treatment. Among cases with chronic hepatitis B, a distinction can be drawn between those with highly replicative HBV infection (>10^5 to 10^6 IU/mL) and those with levels of replication below this threshold. In the relatively replicative phase of chronic hepatitis B, levels of HBV-DNA are high, HBeAg is detectable, HBcAg in hepatocytes is demonstrable, and clinical markers of liver injury are present. Such patients tend to have moderate to severe chronic hepatitis B. HBV-DNA level in patients having HBeAg tends to be higher than in those with HBeAg negative patients. Flares of disease activity may be due to changes in the level of baseline HBV replication. Approximately 10% to 15% of persons with highly replicative chronic hepatitis B lose markers of high replication and undergo spontaneous conversion to a relatively low replicative state characterized by limited infectivity and liver injury and loss of HBeAg and of detectable HBcAg in hepatocytes. Such patients tend to have clinically mild chronic hepatitis or are even inactive carriers of the virus (Table IV).

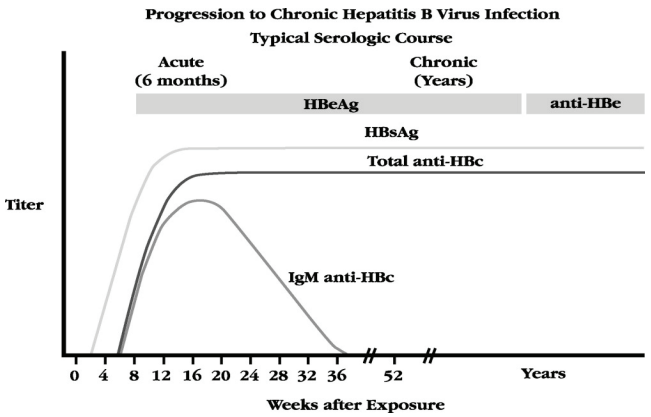


Figure 4. Serologic course in chronic hepatitis B infection

Development of anti-HBe in HBeAg positive patients may occur at any time during chronic infection. Patients with flares of disease activity should be followed for the development of anti-HBe, which indicates control of viral replication. Rising in anti-HBe titers is often accompanied by an increase in serum aminotransferases, followed by a disappearance of HBeAg and HBV-DNA from the serum and improvement in liver inflammation. However, some patients with active liver disease continue to have HBV-DNA in serum after HBeAg seroconversion. Unlike hepatitis C, in which any level of virus replication can be associated with liver injury, in chronic HBV infection, a threshold exists, on the order of approximately 10^3 to 10^6 IU per mL, below which liver injury is negligible or absent. Babies born to mothers with high level of HBV replication (HBeAg positive, HBV-DNA ≥10^6 IU/mL) have a 90% chance for development of chronic HBV infection. Whereas only 10% or less of babies born to mothers with low level of HBV replication develops chronic hepatitis B. Inactive hepatitis B carriers who have low level viremia can undergo HBsAg-to-anti-HBs seroconversion. In the setting of immunologic compromise (e.g., cytotoxic chemotherapy, immunusuppresion for organ transplantation, anti-TNF-α therapy, rheumatologic disorders, HIV infection), inactive carriers and even persons who have recovered from heptatitis B can have reactivation. Among the complications of chronic hepatitis B is HCC, postulated to be associated with incorporation of HBV-DNA into the host genome. Recent observations show that, among persons with HBsAg, the risk of HCC resides primarily among those with markers of high level HBV replication. Thus, the level of HBV replication has a profound influence over the clinical expression of chronic hepatitis B.

Additional information regarding HBV-DNA and its' detection have been shown in Box 1, Table IV and Table V [1,6].

Box 1. The role of HBV-DNA quantification in patients with hepatitis B infection

1. HBV-DNA assay plays major role in the evaluation of patients with either HBeAg positive or HBeAg negative chronic hepatitis B to assess candidacy for treatment.

2. Following HBV-DNA is essential to monitor the treatment response in patients with chronic hepatitis B.

3. Distinguishing the window period of acute infection from chronic infection in patients with anti-HBc IgG only as marker of HBV infection.

4. HBV-DNA measurement is also useful in cases of fulminant hepatitis, where there may be undetectable levels of HBsAg.

5. HBV-DNA testing is also indicated in patients in whom there may be false-negative HBsAg, which occurs due to mutants in the surface protein.

6. Several real-time PCR assays have lower limits of sensitivity of approximately 10 IU/mL and upper limits of 4 x 10^9 IU/mL.

Some people infected with HBV may represent unusual serologic profiles (isolated ant-HBc positiveness, HBeAg negative mutants, HBsAg escape mutants) as stated in Box 2, Box 3 and Box 4 [1,7,8].

Box 2. Isolated anti-HBc positiveness

1. The isolated presence of anti-HBc without other HBV serologic markers are not uncommon. Isolated anti-HBc is more common in patients with HIV and in those with HCV.

2. Isolated anti-HBc can be only marker of HBV infection in patient during window period.

3. Isolated anti-HBc can be only serologic marker of infection due to a decline of anti-HBs many years after resolution in patients recovered from acute HBV infection.

4. Rarely, after years of chronic infection with decline of HBsAg to titers below detection limit.

5. Isolated anti-HBc positiveness should be confirmed to exclude false-positive results.

6. Anti-HBc IgM testing should be performed to exclude acute hepatitis if that is suspected.

7. If there are unexplained aminotransferase elevations in a patient with isolated anti-HBc, HBV-DNA testing should be done.

8. Patients having isolated anti-HBc with or without low level HBV-DNA should be considered potentially infectious.

Box 3. Pre-core or HBeAg negative mutants

1. Absence of HBeAg in a patient having a high level of HBV-DNA is typically associated severe liver disease. This is due to the development of HBV mutants that cannot produce HBeAg due to mutations in the pre-C or core promoter.

2. The most frequent pre-C mutation is a G to A change at nucleotide 1896, which creates a premature stop codon in the pre-C region and abolishes production of HBeAg.

3. Pre-C HBV mutants often become dominant viral quasi-species in the viral populations within an individual.

4. HBeAg negative disease is more frequent with genotypes B, C, and D than with genotype A infection.

Box 4. HBsAg escape mutants

1. The mutations (G145R, sD144A) in the 'a' determinant sequence of HBsAg decrease binding of HBsAg to anti-HBs.

2. These mutations result in development of 'escape' infection in infants infected with HBV following passive-active vaccination.

3. G145R mutation has also been observed in liver transplant recipients who developed recurrent HBV infection despite HBIG prophylaxis.

Hepatitis D

Hepatitis D virus (HDV) requires the presence of HBV for replication. It may either infect the host at the same time as HBV (coinfection), or it can superinfect a host that is already infected with HBV. Simultaneous infection (coinfection) of HBV and HDV generally presents as an acute self-limited hepatitis with full recovery, and chronic infection is seen in 2% of patients. Whereas chronic hepatitis D generally occurs if a patient with chronic hepatitis B acquires HDV. Chronic hepatitis B is converted to more severe chronic hepatitis after HDV superinfection [1]. Serologically, the diagnosis of hepatitis D is established by the presence of circulating antibody to HDV (anti-HDV) [1]. IgM anti-HDAg antibody develops with acute HDV infection, becoming positive 7-15 days after onset of clinical illness and waning with recovery and resolution of HDV infection (Figure 5). During acute infection, HDV RNA can also be detected by polymerase chain reaction (1). After clearance of HDV, IgG anti-HDAg becomes positive. With chronic HBV/HDV infection, both serum IgM and IgG anti-HDAg can be present in conjunction with detectable circulating HDV RNA. In patients with chronic HDV, HDv is the dominant virus because it suppresses HBV replication. Thus, most HBV-HDV coinfected patients have low serum HBV-DNA levels. HDV RNA testing can be variable, and false-negative results occur in actively infected patients. HDV RNA levels do not correlate with the severity of disease or with clinical outcomes. HDV RNA may be most useful in following treatment outcomes rather than in supporting the clinical diagnosis of HDV infection.

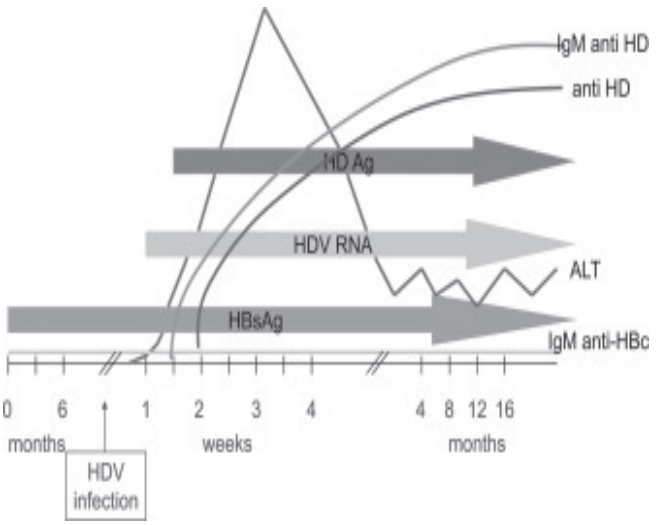


Figure 5. HDV superinfection: serological profile of HDV markers over time in a chronic HBV carrier

Hepatitis C

Table I. has shown typical virologic characteristics of HCV infection. There are at least six major genotypes or clades of hepatitis C virus (HCV). The high level of virion turnover coupled with the absence of proofreading by the NS5B polymerase results in generation of quasi-species in an infected individual. The incubation period of hepatitis C is from 15 to 160 days (mean of 60 days). Viremia peaks 8 to 12 weeks after infection and then plateaus or drops to lower levels and persists in 85% to 90% of individuals (Figure 5) [1,9]. Simplified diagnostic approach in patients with acute hepatitis C has been shown in Table II. After infection, HCV-RNA can be detected within days of exposure. HCV-RNA is found in blood, saliva, tears, seminal fluid, ascitic fluid, and cerebrospinal fluid. The diagnosis of HCV infection is based principally on detection of antibodies to recombinant HCV polypeptides and by assays for HCV-RNA [1,9,10].

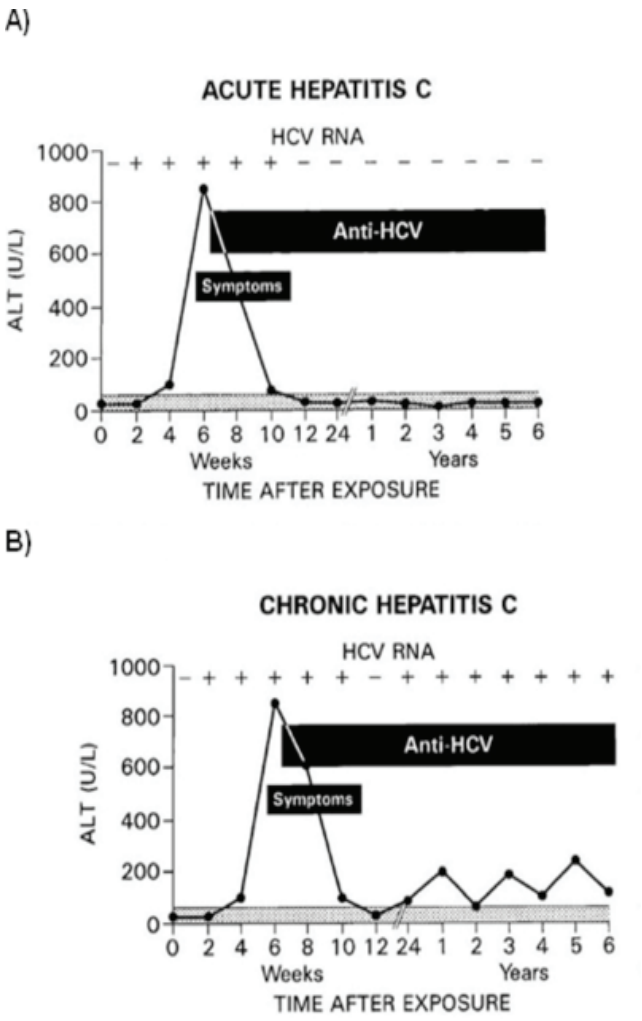


Figure 6. Serologic, biochemical, clinical course of acute (A) and chronic (B) HCV infection

There have been several generations of EIAs that measure antibodies against NS4, core, NS3, and NS5 sequences. The sensitivity of the third-generation EIA is 97%, and it can detect HCV antibody within 6 to 8 weeks of exposure. These assays determine either past or recent HCV infection, not immunity. Assays for IgM HCV antibodies are not clinically useful. In the past, supplemental serologic testing such as with the recombinant immunoblot assay has been used to improve the specificity of the screening tests. However, supplemental antibody assays are no longer commercially available in the United States. Today, HCV testing recommendations generally call for screening for HCV antibodies followed by RNA testing [1,9,10]. In acute HCV infection or immunosuppressed states, a negative anti-HCV EIA result does not exclude HCV infection.

HCV-RNA testing is helpful if seronegative acute HCV infection is suspected because HCV-RNA can be detected within 2 to 3 days after an exposure, while antibodies to HCV are detectable an average of 7 to 8 weeks later. HCV-RNA testing can also be used to screen for HCV infection in persons with negative anti-HCV EIA results who are known to have conditions associated with diminished antibody production, such as HIV infection and hemodialysis. Various molecular techniques, including reverse-transcription PCR (RT-PCR), real-time PCR, transcription-mediated amplification (TMA), and branched DNA (bDNA) are used for detection of HCV-RNA (Table V). Real-time PCR and TMA assays quantify virus across a wide range and have comparable sensitivity (10 to 50 IU/mL) to qualitative assays. In persistently infected persons not receiving treatment, HCV-RNA levels tend to remain stable (within one-half $\log_{10}$) over years. In the absence of treatment, there is little need to repeat HCV-RNA tests frequently.

The HCV core antigen titer corresponds closely with the HCV-RNA level, but the test is not widely used in practice [1,9].

Determination of HCV genotype may be useful in predicting the response to therapy (Box 5).

Box 5. Commercially available tests for genotyping of HCV
1. Direct evaluation of 5' noncoding sequence; Trugene 5'NC HCV Genotyping kit (Bayer Healthcare Diagnostics Division, Tarrytown, NY).
2. Hybridization to genotype-specific 5' noncoding oligonucleotide probes; INNO-LiPa HCV II (Innogenetics, Ghent, Belgium).
3. Versant HCV Genotyping Assay 2.0 (Bayer Healthcare Diagnostics Division, Tarrytown, NY).

Chronic HCV infection is usually diagnosed by testing for HCV antibodies and then by testing reactive sera for HCV-RNA to assess if the infection is ongoing. HCV-RNA level is useful for predicting and monitoring treatment response. Ideally, the quantitative HCV-RNA test should have a sensitivity limit of less than 50 IU/mL. A negative HCV-RNA test result (<50 IU/mL) in an individual found to have antibodies directed HCV (anti-HCV) most likely indicates that HCV infection has resolved.

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