

Indexed, Abstracted and Cited: **ISRA Journal Impact Factor, International Impact Factor Services (IIFS), Directory of Research Journals Indexing (DRJI), International Institute of Organized Research and Scientific Indexing Services**

## World Journal of Biology and Medical Sciences

Published by Society for Advancement of Science®

ISSN 2349-0063 (Online/Electronic)

Volume 1, Issue- 4, 62-71, October-December, 2014



WJBMS 1/3/15/2014

All rights reserved

A Double Blind Peer Reviewed Journal

[www.sasjournals.com](http://www.sasjournals.com)

[wjbmedsc@gmail.com](mailto:wjbmedsc@gmail.com) / [wjbms.lko@gmail.com](mailto:wjbms.lko@gmail.com)

REVIEW ARTICLE

Received: 01/06/2014

Revised: 27/10/2014

Accepted: 28/10/2014

## Recent Advances in Diagnosis of Viral Hepatitis: An updated Overview

S. Malhotra, Shweta Sharma, P. Kumar, N.J.K. Bhatia and C. Hans

PGIMER and Associated Dr. RMLH, CCRUM, New Delhi, India

### ABSTRACT

*Viral hepatitis is a common form of liver disease that affects roughly 1% to 2% of the population. Infections with hepatitis viruses account for majority of acute hepatitis. Moreover, the chronic stages of hepatitis B and C infection are associated with an increased risk of hepatic decompensation, cirrhosis, and hepatocellular carcinoma. Timely diagnosis of all forms of viral hepatitis is useful for preventing transmission and understanding outbreak patterns. This is a brief review presenting the development in the diagnostic techniques of various hepatitis viruses especially Hepatitis A, B, C, D and E. The gold standard for the diagnosis of acute HAV is the detection of IgM anti-HAV against capsid proteins in the serum. For definitive diagnosis of HBV infection, serologic testing for specific particles and their corresponding antibodies or HBV DNA levels by RT-PCR is done. Monitoring HBV DNA levels or HBsAg titre is also useful in predicting response to treatment with pegylated interferon. HCV RNA testing is essential in the diagnosis of chronic HCV infection because serologic testing cannot distinguish chronic infections from resolved acute infections. Quantitation for HCV RNA by Real-time PCR or TMA (transcription-mediated amplification) is important before making the decision for treatment. Detection of HDAG in the liver by immunostaining is the gold standard of diagnosis of HDV infection, however serological testing is preferred. Serologic testing for hepatitis E IgG and IgM antibodies is done by ELISA. To conclude, diagnosis of hepatitis virus is necessary for further management of such cases as these viruses cannot be differentiated on the basis of clinical presentation.*

**Keywords:** Viral Hepatitis, Diagnosis, Clinical Spectrum, RT- PCR and Serologic testing.

## INTRODUCTION

Viral hepatitis is a disease with multiple causes that was first described in the fifth century BC. Viral hepatitis is a common form of liver disease that affects roughly 1% to 2% of the population [CDC, 2013]. There are a large number of viruses, including cytomegalovirus, Epstein-Barr, herpes simplex, mumps, and varicella, known to cause liver inflammation and cell death, but the term *viral hepatitis* commonly refers to the 5 well described hepatotropic viruses: hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), and hepatitis E virus (HEV). HAV, HBV, and HCV are the most frequent causes of viral hepatitis however HDV infection is rare and is seen among HBV-infected individuals. HEV is considered an uncommon form of viral hepatitis in the developed world but it is common in developing countries. Whether the recently described hepatitis G virus is pathogenic in humans remains unclear. Infections with hepatitis viruses account for majority of acute hepatitis. Moreover, the chronic stages of hepatitis B and C infection are associated with an increased risk of hepatic decompensation, cirrhosis, and hepatocellular carcinoma (HCC) [Harris and Dighe, 2002].

Clinical manifestations vary widely between different forms of viral hepatitis. HAV manifests as acute or asymptomatic disease, has no chronic carrier state, and rarely causes acute fulminant liver failure. HBV, HCV, and HDV can also cause asymptomatic or acute infection, but often establish chronic infection which may lead to liver cirrhosis and hepatocellular carcinoma resulting in significant morbidity and mortality. HDV is a "defective" virus and it replicates only in the presence of HBV. HBV/HDV co infection (simultaneous acquisition of HBV and HDV) and super infection (acquisition

of HDV by a person with chronic HBV infection) significantly increase the severity of disease relative to HBV infection alone [Gish, et al 2013]. Acute HBV/HDV co infection tends to resolve spontaneously, whereas HBV/HDV super infection has a high likelihood of progressing to chronic infection.

Timely diagnosis of all forms of viral hepatitis is useful for preventing transmission and understanding outbreak patterns. Treatment for HAV is supportive, whereas specific antiviral therapies are available for HBV and HCV infection. Prompt and accurate diagnosis of HBV and HCV is needed to avoid complications of chronic disease. Vaccines are available for only HAV and HBV.

## HEPATITIS A

Hepatitis A virus (HAV), an RNA-containing virus is considered to be the most common cause of acute viral hepatitis. Worldwide, it is estimated to infect 1.4 million people annually, most prominently in South America, Africa, and Asia. Accurate diagnosis is not only important for the care of the infected individual but also for close contacts who may be candidates for immunoglobulin prophylaxis [CDC, 2004].

### Laboratory Testing for HAV

The gold standard for the diagnosis of acute HAV is the detection of IgM anti-HAV against capsid proteins in the serum by radioimmunoassay (RIA) or enzyme-linked immuno sorbent assay (ELISA) or immunoblotting [Cuthbert et al, 2001]. Positive IgM anti- HAV along with elevated transaminases and clinical symptoms of acute hepatitis is suggestive of acute HAV infection. IgM anti-HAV is detectable in the serum for 4 to 6 months after infection, although an infection that is relapsing can have a positive IgM anti-HAV for upto 12 months before resolution [Gliksun et al, 1992]. The presence of IgG

anti- HAV is indicative of immunity (after vaccination or natural infection). HAV vaccination provides immunity for an estimated 10 to 20 years, whereas natural infection provides lifelong immunity [Van Damme et al, 1994]. HAV can be replicated in African green monkey kidney cells and fetal rhesus monkey kidney cells, without any cytopathic effect, hence growth detected and quantitated with fluorescent and immunological assays like radioimmunofocus assay, fluorescent focus assay, in situ radioimmunoassay, and in situ hybridization. Other methods of documenting HAV infection are Nucleic acid detection techniques such as nucleic acid hybridization, Southern blotting, reverse transcription-PCR (RT-PCR), nucleic acid sequencing-based amplification, restriction fragment length

polymorphism etc [Omana et al, 2006]. However, most sensitive and widely used method for detection of HAV RNA from clinical samples and also from environmental isolates is RT-PCR. Multiplex RT-PCR is better than RT-PCR, because genome sequences of multiple organisms are amplified simultaneously like HAV and HEV and of HAV, rotavirus, and poliovirus [Jothi Kumar et al, 2000]. Real-time PCR, a rapid, sensitive, reproducible with minimum risk of contamination, can be used for the detection and quantification of HAV. Nucleic acid sequencing can be done on PCR products to identify and characterize the organism and to determine the genetic relatedness of isolates [Hussain et al, 2005].

**Table 1. Clinical Spectrum of Viral Hepatitis.**

<b>Virus</b>	<b>Route of transmission</b>	<b>Incubation Period</b>	<b>Mortality</b>	<b>Carrier rate</b>	<b>Chronic Disease</b>	<b>Associated with HCC</b>
HAV	Faeco-oral	2-6 wk	1%	None	None	No
HBV	Parenteral, perinatal, sexual	4-26 wk	1-2%	10%(adults) 90%(infants)	1%-10%	Yes
HCV	Parenteral, perinatal, sexual	2-23 wk	1-5%	50%-80%	80%-90%	Yes
HDV	Parenteral, sexual, perinatal	6-26 wk	2-20%	Variable*	80% in Super-infection*	Yes*
HEV	Fecal-oral	2-9 wk	-1% -10-30% in pregnant women	Rare	Rare	No

**\*Requires co-infection with HBV. Simultaneous infection with HBV is associated with severe acute disease and low likelihood of chronic infection (<5%); super-infection with HBV carries high likelihood of fulminant disease (2%-20%), chronic HDV infection (up to 80%), and cirrhosis (60%-70%), and may progress to HCC.**

## HEPATITIS B

Hepatitis B virus (HBV) is the ninth leading cause of death worldwide, with estimates of more than 350 million carriers worldwide [Kim and Saab 2004]. HBV is a complex double-shelled DNA virus. The outer membrane contains hepatitis B surface antigen (HBsAg), while the inner core of the virus contains hepatitis B core antigen (HBcAg) and hepatitis B e antigen (HBeAg). Because the clinical symptoms of HBV infection are indistinguishable from other forms of viral hepatitis, definitive diagnosis is dependent on serologic testing for HBV infection. Tests for these specific particles and their corresponding antibodies aid in the diagnostic evaluation of an HBV-infected patient.

### Laboratory Testing for HBV

When HBV infection is suspected, it is the appearance of serum markers that establishes the diagnosis of the disease. Acute HBV infection is characterized by the presence of HBsAg in serum and the development of IgM anti-HBc (Table 2) [Francis, 1999]. Detection of HBsAg can be done by ELISA and radioimmunoassays, which can detect HBsAg at concentrations of  $\geq 0.1$  ng/ml. HBeAg is also detectable during acute infection. During convalescence, HBsAg and HBeAg are cleared, and anti-HBs, anti-HBc, and anti-HBe develop. Anti-HBs is a protective antibody that neutralizes the virus. The presence of anti-HBs following acute infection indicates recovery and immunity from reinfection. Anti-HBs titre of  $>10$  IU/ml is considered protective [Greenberg et al, 1996]. Anti-HBs are also detected among persons who have received hepatitis B vaccine. Immunoassays for the detection of total anti-HBc involve both IgM and IgG and indicate current or past exposure to virus and viral replication. The detection of IgM anti-HBc is diagnostic of acute HBV infection. In persons with chronic HBV infection, HBsAg remains

persistently detectable, generally for life. HBeAg (Hepatitis B<sub>e</sub> antigen) indicates high viral replication and person is considered highly infectious. IgM anti-HBc generally becomes undetectable 6 months after acute infection.

HBV DNA is detectable in the serum of persons with acute and chronic HBV infection. A commercial liquid hybridization assay (Abbott) detects 1.5 pg of HBV DNA per ml and the branched-DNA hybridization assay detects 2.5 pg of HBV DNA per ml. PCR is much more sensitive than direct hybridization and detects HBV DNA levels of  $10^{-3}$  pg/ml (approximately 100 to 1,000 genomes); however, PCR assays are prone to false-positive results [Francis, 1999]. Real-time PCR is based on continuous quantitative monitoring during the exponential phase of the PCR reaction, reduce contamination, and increase the accuracy of quantification thereby increasing the utility of these assays [Harris and Dighe, 2002]. Generally, detection by PCR has the same significance as detection of HBsAg and indicates current HBV infection. In contrast, detection by hybridization indicates significant viral replication and a high probability of active liver disease (similar to HBeAg). Fewer than 10% of all HBV-infected patients develop chronic HBV. Patients who tend to be at greater risk for chronic disease are the very young (up to 90% in newborns) and the immunocompromised, including hemodialysis patients [Greenberg et al, 1996]. About 15-20% of chronic carriers develop end-stage liver disease. Chronic HBV is suggested by the presence of HBsAg in the serum for at least 6 months. The two types of chronic HBV infection are the active and inactive forms. Active chronic HBV is characterized by HBeAg positivity, HBV DNA exceeding  $10^5$  copies/mL, elevated transaminases, and liver biopsy findings showing chronic

hepatitis. Inactive chronic HBV is characterized by seroconversion from HBeAg to antibody for HBeAg (anti-HBe), HBV DNA below  $10^5$  copies/mL, normal transaminases, and the absence of significant hepatitis on liver biopsy [Hyams, 1995]. Monitoring HBV DNA levels or HBsAg titre is useful in predicting response to treatment with pegylated interferon. Eight HBV genotypes (A-H) identified based on > 8% of sequence divergence over the entire genome of HBV DNA and each genotype has characteristic geographic or ethnic distribution, however has limited application in natural history of disease and treatment of patients [Ferruccio et al, 2010]. Nucleic acid sequence analysis of the PCR products has been used to identify genetic variants of the virus and to investigate common-source outbreaks

#### **Precore or HBeAg negative mutants**

- Due to mutation in precore (abolishes HBeAg production) or core promoter region (down-regulates HBeAg production)
- No effect on viral replication (may be enhanced)
- More difficult to treat; greater risk of cirrhosis [CDC, 2004].

## **HEPATITIS C**

HCV is a major public health problem: there are 170 million HCV carriers worldwide [Harris et al, 2002]. Acute infection with HCV progresses to a chronic infection in 50% to 90% of infected individuals. Chronic infection with HCV is an important cause of chronic liver disease and is strongly associated with the development of liver cirrhosis (10-20%) and hepatocellular carcinoma (1-2%). Six major HCV genotypes and numerous subtypes have been identified based on molecular relatedness. Molecular differences between genotypes are significant, and they differ at least 30% at the nucleotide level. Genotypes 1, 2, and 3 have a worldwide distribution, while genotypes 4, 5, and 6 are localized to

of HBV infection. Initially nucleic acid sequencing was based on Sanger's technique, later newer techniques like Ultradeep sequencing developed. Ultradeep sequencing, allows direct sequencing of the mixed population sample and relative quantification of individual mutations with extremely high coverage (up to  $10^5$  reads per base pair) over a relatively short time frame [Radford et al, 2012 and Julianne et al, 2013].

HBsAg and anti-HBs may co-exist in up to 24% of chronically infected individuals; likely due to mutations in the "a" determinant of the S gene [Lok et al, 2001]. Surface antigen escape mutants are well described in infants infected with HBV after HBIG + vaccination and in Liver transplants after prolonged HBIG.

specific geographic locations. In India, genotype 1 is the most common genotype. Historically, HCV genotype 1, particularly 1b, has not responded to therapy as well as genotypes 2 and 3. Currently, the only clinically relevant distinction is between genotype 1 and genotypes 2 and 3 [Krajden et al, 2005].

Isolates from individual patients also may contain many closely related genomes termed quasi species. The presence of quasi species in HCV is thought to be the mechanism whereby the virus escapes host immune surveillance and probably contributes to the high rates of chronic infection, the difficulty in vaccine development, and the variable response to therapy [Dusheiko et al, 1994].

**Table 2. Serological markers in hepatitis B virus infection.**

	HBV DNA/ DNA polymerase	HBsAg	HBeAg	Anti-HBe	Anti-HBc	Anti-HBs
Incubation period	+	+	+	-	-	-
Acute infection	+	+	+	-	+	-
Convalescence	-	+/-	-	+	+	+
History of past infection	-	-	-	+	+	+/-
History of vaccination	-	-	-	-	-	+
Carrier state (high infectivity)	+	+	+	-	+	-
Carrier state (low infectivity)	+/-	+	-	+	+	-

**Anti-HBc = antibody to hepatitis B core antigen; anti-HBs = antibody to HBsAg; HBsAg = hepatitis B surface antigen; HBV = hepatitis B virus.**

### Laboratory Testing for HCV

The screening test for HCV is an ELISA based assay to detect specific HCV antibodies. In first generation, yeast-expressed recombinant protein containing the epitope C100-3 from the NS4 region was used, with very high false positivity rate. Further improvement was done in second generation assay which used a multiantigen format, including antigens from the core, NS3 and NS4 regions, which markedly improved sensitivity and specificity of the assay. However, differences in serologic reactivity to HCV antigens among different HCV genotypes were reported. Hence the most recent third-generation EIA developed which involves the HCV core protein and NS3, NS4, and NS5 proteins, which is better than second generation and less strongly influenced by the HCV genotypes. This EIA can be used to detect antibodies within 4 to 10 weeks following infection, with diagnostic specificity of >99% [Ayaz et al, 2002 and Farci et al, 2000]. However, still false positive results can occur in patients with autoimmune diseases, mononucleosis, pregnancy etc. False-negative results can occur in persons with compromised immune systems, such as

people with HIV-1 infection, patients with renal failure, and patients with HCV associated essential mixed cryoglobulinemia [Uyttendaele et al, 1994]. A saliva-based test for HCV antibody detection may soon be available [González et al, 2008]. The recombinant immunoblot assay (RIBA) traditionally has been used to confirm HCV infection [Lok and Gunaratnam, 1997]. A positive immunoblot assay result is defined as the detection of antibodies against 2 or more HCV antigens, and an indeterminate assay result is defined as the detection of antibodies against a single antigen. However, the RIBA, as well as the EIA, is unable to distinguish acute infection from chronic infection. Direct detection of the HCV genome has been made possible with RT-PCR and the branched DNA signal amplification assays [Gretch et al, 1995]. The advantages of direct HCV RNA detection include the early diagnosis of acute infection, the diagnosis of infection in patients with compromised humoral immunity (immune compromised patients, and chronically ill patients such as those with chronic renal failure), and the confirmation of active infection in patients with indeterminate antibody

results. Furthermore, HCV RNA testing is essential in the diagnosis of chronic HCV infection because serologic testing cannot distinguish chronic infections from resolved acute infections. HCV RT-PCR testing also has proven useful in assessing viremia in patients with positive antibody tests, evaluating treatment response, and examining patients with suspected false-negative serologic test results. Serum samples should be frozen within 3 hours because the HCV viral RNA genome is unstable. HCV-RNA RT-PCR assays are more than 90% sensitive and specific. Branched-chain testing for HCV, while perhaps less variable and technically easier, lacks the sensitivity of RT-PCR based testing [Gretch et al, 1995]. In recent years, automated quantitative HCV core antigen tests were developed, which can also be detected during the window period of acute infection and may constitute a useful alternative to HCV-RNA measurement for predicting and monitoring response to treatment [Kesli et al, 2011 and Vermehren et al, 2012]. However, it has lower sensitivity than RT-PCR and hence not recommended for blood screening, when PCR is available. Quantitative assays for HCV RNA are Real-time PCR or TMA (transcription-mediated amplification), which are somewhat less sensitive but quantitation is important before making the decision for treatment. All patients with compensated liver disease and detectable HCV RNA are candidates for therapy. Treatment regimen and duration is based on HCV genotype, hence genotype should be determined before initiating treatment. Genotype is determined with direct sequence analysis (gold standard) or reverse hybridization (line probe assay). New technologies like next generation sequencing (NGS), are now available for analyzing viral genome sequences, particularly amino acid substitutions

associated with drug resistance [Stephane, 2013 and Marco Ciottia et al, 2013]. The serum ALT level may be elevated in patients with acute hepatitis C, and useful for monitoring the effectiveness of therapy for HCV infection.

## HEPATITIS D

Hepatitis D Virus (HDV) is a satellite virus, which is dependent on hepatitis B virus (HBV) for the production of envelope proteins. HBV/ HDV co infection most commonly occurs in the Mediterranean area and parts of South America. The availability of HBV vaccines and public health education on the prevention of transmission of HBV infection has led to a significant decline in the prevalence of HDV infection in the past decade [Gaeta et al, 2000]. HDV infection occurs into two forms. The first form is caused by the co infection of HBV and HDV; this usually results in a more severe acute hepatitis with a higher mortality rate than is seen with acute hepatitis B alone but rarely results in chronic infection. A second form is a result of a super infection of HDV in a HBV carrier and can manifest as a severe "acute" hepatitis in previously asymptomatic HBV carriers or as an exacerbation of underlying chronic hepatitis B. Unlike co infection, HDV super infection in HBV carriers almost always results in chronic infection with both viruses. A higher proportion of persons with chronic HBV/HDV co infection develop cirrhosis, hepatic decompensation, and liver cancer compared to those with chronic HBV infection alone. Approximately 5% of HBsAg carriers are infected with HDV infection worldwide [Farci, 2003]. Recognition of this infection is very important because knowing the presence of the HDV infection in a HBV infected patient allows a more accurate prognosis. Moreover, patients with acute HDV

infection are at a higher risk of development of fulminant /severe hepatitis, and chronic HDV infected patients are highly at risk of progression to cirrhosis and liver failure. HDV infection effects on the response of patients to antiviral therapy and a higher dosage of antiviral is needed than that of cases with chronic hepatitis B alone.

#### **Laboratory Testing for HDV**

Detection of HDAg in the liver by immunostaining is the gold standard of diagnosis of HDV infection, this test is not used for routine practice. Serum HDAg is usually detected by micro plate-based, enzyme-linked (EIA) or radioimmunoassay (RIA). Since HDAg is often present transiently and there are no commercial assays to detect HDAg, it is only a research test. IgM anti-HDV correlates to the levels of HDV replication but in the chronic HDV infected cases, an IgG anti-HDV titre higher than 1:1000 is a strong clue of persistent viral replication. HDV infection happens only in the HBsAg positive patients and is diagnosed by IgM-anti-HDV for acute or IgG anti-HDV for chronic infection. In 10% patients, the markers of HBV infection may not be detected because of rapid HBsAg clearance in fulminant or acute hepatitis cases or due to inhibitory effect of HDV on HBV replication. In such cases, the presence of IgM anti- HBcAg in serum helps to diagnose acute hepatitis B. There is suppression effect of HDV on HBV replication; hence HBV markers may resemble a carrier state of HBsAg, HBeAg negative, anti-HBe positive, and HBV DNA negative. HDV RNA is a useful marker of HDV replication in patients with chronic infection. It is also an early marker of acute infection. HDV RNA detection in the serum is made by either molecular hybridization or reverse transcriptase-polymerase chain reaction (RT-PCR). RT-PCR is more sensitive and has a lower limit

of detection of 10 genomic copies. HDV RNA may be useful for antiviral therapy monitoring. 90% of chronic HDV have HDV RNA detectable in the serum [Hajiani and Alavi , 2011 and Hajiani and Hashemi, 2005].

#### **HEPATITIS E**

HEV is a single-stranded RNA virus of the Caliciviridae family. The highest rates of HEV infection occur in Asia, Africa, the Middle East, and Central America [Mast and Krawczynski, 1996]. The incubation period following exposure to HEV ranges from 15 to 60 days. For unknown reasons, in pregnant women hepatitis E infection carries a high mortality (20%) from fulminant hepatitis. Causes of death among pregnant women with hepatitis E include hepatic encephalopathy and disseminated intravascular coagulation. Serologic testing for hepatitis E IgG and IgM antibodies by ELISA is available [Tsega et al, 1992].

#### **ACKNOWLEDGEMENTS**

I would like to acknowledge the technical staff for their immense support in providing practical issues related to various laboratory tests for hepatitis.

#### **REFERENCES**

- Centers for Disease Control and Prevention. (2013).** Viral Hepatitis Surveillance – United States.
- Harris, K. and Dighe, A.S. (2002).** Laboratory Testing for Viral Hepatitis. *Am J Clin Pathol*, 118 (Suppl 1): S18-S25.
- Gish, R.G., Yi, D.H., Kane, S., Clark, M., Mangahas, M., Baqai, S., Winters, M.A., Proudfoot, J and Glenn, J.S., (2013).** Coinfection with hepatitis B and D: epidemiology, prevalence and disease in patients in Northern California. *J Gastroenterol Hepatol.* 28(9):1521-5.

- Centers for Disease Control and Prevention. (2004).** Disease burden from hepatitis A, B, and C in the United States. National Center for Infectious Diseases Viral Hepatitis Surveillance.
- Cuthbert, J. A. (2001).** Hepatitis A: old and new. *Clin. Microbiol. Rev.* 14:38–58.
- Glikson, M., Galun, E., Oren, R., Turkaspa, R., Shouval, D. (1992).** Relapsing hepatitis A. Review of 14 cases and literature survey. *Medicine (Baltimore)*, 71:14–23.
- Van Damme, P., Thoelen, S., Cramm, M., De Groote, K., Safary, A., Meheus, A. (1994).** Inactivated hepatitis A vaccine: reactogenicity, immunogenicity, and long-term antibody persistence. *J Med Virol*, 44: 446–51.
- Omana V. Nainan, Guoliang Xia, Gilberto Vaughan, and Harold S. Margolis. (2006).** Diagnosis of Hepatitis A Virus Infection: a Molecular Approach. *Clinical Microbiology Reviews*, pp. 63–79.
- Jothi Kumar, N., Paulmurugan, R., Padmanabhan, P., Sundari, R.B., Kamatchiammal, S., and Rao, K.S. (2000).** Duplex RT-PCR for simultaneous detection of hepatitis A and hepatitis E virus isolated from drinking water samples. *J. Environ. Monit.* 2:587–590.
- Hussain, Z., Das, B.C., Husain, S.A., Asima, M., Chattopadhyay, S., Malik, A. (2005).** Hepatitis A viral genotypes and clinical relevance: clinical and molecular characterization of hepatitis A virus isolates from northern India. *Hepatol. Res.* 32:16–24.
- Kim, A.I., and Saab, S. (2004).** Interpretation of Laboratory Tests for Diagnosing Viral Hepatitis. *Hospital Physician*, pp. 15-19.
- Francis, J. Mahoney. (1999).** Update on Diagnosis, Management, and Prevention of Hepatitis B Virus Infection. *Clinical Microbiology Reviews*, pp. 351–366.
- Greenberg, D. P., C. M. Vadheim, V. K. Wong, S. M. Marcy, S. Partridge, T. Greene, C. Y. Chiu, H. S. Margolis, and J. I. Ward. (1996).** Comparative safety and immunogenicity of two recombinant hepatitis B vaccines given to infants at two, four and six months of age. *Pediatr. Infect. Dis. J.* 15:590–596.
- Hyams, K.C. (1995).** Risks of chronicity following acute hepatitis B virus infection: A review. *Clinical Infectious Diseases*, 20: 992-1000.
- Ferruccio Bonino, Teerha Piratvisuth, Maurizia, R. Brunetto, Yun-Fan Liaw. (2010).** Diagnostic markers of chronic hepatitis B infection and disease- Review. *Antiviral Therapy. Suppl* 3:35–44.
- Radford, A.D., Chapman, D., Dixon, L., Chantrey, J., Darby, A.C. and Hall, N. (2012).** Application of next-generation sequencing technologies in virology. *J Gen Virol*, 93 (Pt 9):1853–1868.
- Julianne Bayliss, Tin Nguyen, C. Rinaldi, A. Lesmana, Peter Revill. (2013).** Advances in the Molecular Diagnosis of Hepatitis B Infection: Providing Insight into the Next Generation of Disease. *Semin Liver Dis*, 33: 113–121.
- Lok, A.S. and McMahon, B.J. (2001).** Chronic hepatitis B. Guidelines Committee, American Association for the Study of Liver Diseases. *Hepatology*, 34: 1225–41.
- Krajden, M., McNabb, G., and Petric, M. (2005).** The laboratory diagnosis of hepatitis B virus. *Can J Infect Dis Med Microbiol*; 16(2):65-72.
- Dusheiko, G., Schmilovitz-Weiss, H., Brown, D., McOmish, F., Yap, P.L., Sherlock, S., McIntyre, N., Simmonds, P. (1994).** Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. *Hepatology*, 19: 13-18.
- Ayaz, M., Majid, A., David and R. Gretch. (2002).** Current and future hepatitis C virus diagnostic testing: problems and advancements. *Microbes and Infection* 4, 1227–1236

- Farci, P., Shimoda, A., Coiana, A., Diaz, G., Peddis, G., Melpolder, J.C., Strazzer, A., Chien, D.Y., Munoz, S.J., Balestrieri, A., Purcell, R.H., Alter, H.J. (2000). The outcome of acute hepatitis C predicted by the evolution of the viral quasispecies. *Science*; 288: 339-344.
- Uyttendaele, S., Claeys, H., Mertens, W., Verhaert, H., Vermynen, C. (1994). Evaluation of third-generation screening and confirmatory assays for HCV antibodies. *Vox Sang*; 66: 122-129.
- González, V., Martro, E., Folch, C., Esteve, A., Matas, L., Montoliu, A., Grifols, J.R., Bolao, F., Tural, C., Muga, R., Parry, J.V., Ausina, V., Casabona, J. (2008). Detection of hepatitis C virus antibodies in oral fluid specimens for prevalence studies. *Eur J Clin Microbiol Infect Dis*; 27(2):121-126.
- Lok, A.S. and Gunaratnam, N.T. (1997). Diagnosis of hepatitis C. *Hepatology*. 26 (3 suppl 1): 48S-56S.
- Gretch, D.R., Dela Rosa, C., Carithers, R.L. Jr, Willson, R.A., Williams, B., Corey, L. (1995). Assessment of hepatitis C viremia using molecular amplification technologies: correlations and clinical implications. *Ann Intern Med*; 123: 321-329.
- Kesli, R., Polat, H., Terzi, Y., Kurtoglu, M.G. and Uyar, Y. (2011). Comparison of a newly developed automated and quantitative hepatitis C virus (HCV) core antigen test with the HCV RNA assay for clinical usefulness in confirming anti-HCV results. *J Clin Microbiol*. 49(12): 4089-4093.
- Vermehren, J., Susser, S., Berger, A., Perner, D., Peiffer, K.H., Allwinn, R., Zeuzem, S., Sarrazin, C. (2012). Clinical utility of the ARCHITECT HCV Ag assay for early treatment monitoring in patients with chronic hepatitis C genotype 1 infection. *J Clin Virol*, 55(1):17-22.
- Stephane Chevaliez. (2013). New markers for diagnosis and management of chronic hepatitis C virus infection. *Annals of Gastroenterology*, 26, 98-99.
- Marco Ciottia, E., Cartesio D'Agostinib, C. and Aldo Marrone. (2013). Advances in the Diagnosis and Monitoring of Hepatitis C Virus Infection. *Gastroenterology Research*; 6(5): 161-170.
- Gaeta, G.B, Stroffolini, T., Chiamonte, M., Ascione, T., Stornaiuolo, G. and Lobello, S. (2000). Chronic hepatitis D: a vanishing Disease ? An Italian multicenter study. *Hepatology*, 32: 824-7.
- Farci, P. (2003). Delta hepatitis: an update. *J Hepatol*; 39 (Suppl 1): S212-9.
- Hajiani, E. and Alavi, S.M. (2011). A review on epidemiology, diagnosis and treatment of hepatitis D virus infection. *Jundishapur J Microbiol*; 4(Supplement 1): S1-S8.
- Hajiani, E. and Hashemi, S.J. (2005). Evaluation of AFP in patients with hepatitis B virus infection in diagnosis of hepatocellular carcinoma. *Yafteh*; 1: 45-51.
- Mast, E.E. and Krawczynski, K. (1996). Hepatitis E: an overview. *Annu Rev Med*; 47:257-266.
- Tsega, E., Hansson, B.G., Krawczynski, K., Nordenfelt, E. (1992). acute sporadic viral hepatitis in Ethiopia: causes, risk factors, and effects on pregnancy. *Clin Infect Dis*; 14: 961-965.

---

Corresponding author: Dr. Shalini Malhotra, PGIMER and Associated Dr. RMLH, CCRUM, New Delhi, India

Email: [drshalinimalhotra@yahoo.com](mailto:drshalinimalhotra@yahoo.com)