Viral Hepatitis – B & C

Hepatitis - B

Five percent of the earth’s populations have hepatitis –B, making it one of the most common infective diseases in the world today. There are estimated 400 million causes of hepatitis - B virus (HBV) worldwide 80% of whom reside in Asia and western pacific. Effective vaccines have been available for more than 20 years now, but perinatal exposure continues in high prevalence areas.

Clinical outcome of HBV infection

Patients in whom Hepatitis B surface antigen (HbsAg) persists in serum for more than 6 months are referred to as “chronic HBs Ag carriers.” In common usage the term “carrier” often has been used to refer to persistently infected individuals with normal serum aminotransferase levels (sometimes also referred to as healthy HBV carriers). (1) The term “healthy carrier” seems to be a poor one in that these patients may have evidence for hepatic inflammation on biopsy, particularly when serologic markers of viral replication are evident. (2) Because of potentially confusing nomenclature, it has been proposed that the carrier state be categorized as inactive or active, with the former referring to patients who have evidence for HBV replication by PCR – based assay only and normal or only mildly abnormal serum aminotransferase levels. Long-term follow-up of inactive carriers suggests that the majority of patients do not have progressive liver disease and do not develop complications. (2,3). However, there are major exceptions to this rule. Some of these patients have periodic reactivations of their liver disease in association with an increase in viremia and immunologic responses: while some patients with the inactive carrier state may develop hepatocellular carcinoma. Active carriers, in contrast, have evidence for HBV replication by standard hybridization assays, abnormal serum aminotransferase levels, and evidence for chronic hepatitis, with or without concomitant cirrhosis.

The age at which the individual becomes infected with HBV correlate with clinical outcome and the possible route of exposure. (4, 5) Liver injury and extrahepatic disorders are caused by cell-mediated and humoral patterns of response of HBV infection. The severity of the liver injury reflects the activity of the immune response: the strongest response causes the greatest hepatocellular injury and higher likelihood of viral clearance. Thus HBV infection in adults with intact immune systems is likely to cause clinically apparent acute hepatitis – B, with only 1 percent to 5 percent of cases becoming chronically infected. (6) By contrast, as much as 95 percent of infected neonates become chronic HBV carriers and generally develop subclinical infection because of their immature immune systems. (6, 7). Spontaneous survival in acute liver failure resulting from hepatitis B is between 20 and 30 percent. (8). Liver transplantation has resulted in 50 percent to 60 percent survival rate, and recurrent disease in the allograft is infrequent in this situation. (9, 10) Anti-viral therapy has little rationale for fulminant hepatitis B because most of the immunologically mediated liver damage and elimination of HBV has already occurred by the time of presentation. Patients in whom the diagnosis of acute fulminant hepatitis B is suspected are often HBe-Ag-negative and may be HBs-Ag-negative when first seen. Thus the accurate diagnosis of fulminant liver
failure resulting from hepatitis B may require testing with immunoglobulin (Ig) M antibody to HBc antigen (HBcAg).

Between one third and one quarter of people chronically infected with HBV are expected to develop progressive liver disease (including cirrhosis and primary liver cancer). An estimated 15 percent to 25 percent of all age groups with chronic HBV infection will die prematurely from these condition.

Patients who lack evidence for HBeAg and have normal serum aminotransferase levels rarely transmit the infection to others and usually do not have progressive liver disease even after prolonged follow-up. (3, 12)

Several large cohort studies have demonstrated that the presence of active viral replication and long-standing necroinflammatory liver disease, resulting from HBV infection, strongly influence the rate of progression to cirrhosis. Cirrhosis is associated with decreased survival and higher frequency of complications, including hepatocellular carcinoma.

Serologic Markers of HBV infection

HBsAg is the serologic hallmark of HBV infection. HBsAg appears in serum 2 to 10 weeks after exposure to the virus and before the onset of symptoms or the elevation of serum aminotransferase levels. In patients who subsequently recover, HBsAg usually becomes undetectable after 4-6 months. Persistence of HBsAg for more than 6 months implies progression to chronic HBV infection.

The disappearance of HBsAg is followed by the appearance of anti-HBs. In most patients, anti-HBs persist for life, conferring long-term immunity. In some patients anti – HBs may not become detectable, but these patients do not appear to be susceptible to recurrent infection. (13). Anti – HBs may not be detectable during a window period of several weeks to months after the disappearance of HBsAg. (14, 15). During this period diagnosis of HBV infection is made by detection of IgM antibodies against HBcAg (16) Anti – HBc is detectable in acute and chronic HBV infection. During acute infection anti – HBc is predominantly of the IgM class. IgG anti HBc persists in those who recover from acute hepatitis – B.

Isolated presence of anti HBc in the absence of HBs Ag and anti HBs may occur in the following four settings. (a) During the window period of acute hepatitis B when it is predominantly of the IgM class, (b) many years after recovery from acute hepatitis B when anti-HBs has fallen to undetectable levels; as a (c) false positive serologic test and after (d) many years of chronic infection when the HBs Ag titer has decreased below the level of detection. (17)

HbeAg is a soluble viral protein that is found in serum early during acute infection. HbeAg positive 3 or more months after the onset of illness indicates a high likelihood of transition to chronic infection (14, 18,
19). HbeAg is an indication of viral replication and infectivity. In general seroconversion from HbeAg to anti Hbe is associated with disappear of HBVDNA in serum and remission of liver disease. (20, 21). Some patients however, continue to have active liver disease and detectable HBV DNA in serum resulting from either low levels of wild -type virus or the presence of precore mutation that impairs e antigen secretion. (22, 23)

**HBV DNA Tests**

HBV DNA can be measured in serum using **qualitative or quantitative assays**. PCR assay are more popular than non-PCR based assay. Quantitation of serum HBV DNA is useful in several situations such as candidacy for antiviral treatment, response to antiviral treatment, determining etiology of biochemical flares, detection of phenotypic and genotypic resistance to lamivudine and other nucleosides etc.

Qualitative HBV DNA testing is useful in cryptic HBV infection (ie. HBs Ag negative patients) and determining the infectivity of anti HBc positive donor and in patients with fulminant hepatitis B who frequently have cleared HBsAg by the time they obtain medical attention.

**HBV Genotypes**

HBV has 8 genotypes. Each genotypes has its distinct geographical and ethnic distribution (24, 25) Genotypes A and D occur frequently in Africa, Europe and India. Genotypes B and C are prevalent in Asia.

Several studies have shown that compared with genotypes C, genotype B is associated with spontaneous HBeAg seroconversion at a younger age (26, 27) less active liver disease (28, 29) slower progression to cirrhosis (30) and less frequent development of HCC (24, 28). A study from India indicated that genotype D is more often associated with HBeAg negative chronic hepatitis – B, more severe disease and may predict the occurrence of HCC in your patients (31)
Table 1. Diagnosis of hepatitis B virus infection

<table>
<thead>
<tr>
<th></th>
<th>HBsAg</th>
<th>HBeAg</th>
<th>IgM anti-HBc</th>
<th>IgG anti-HBc</th>
<th>Anti HBs</th>
<th>Anti HBe</th>
<th>HBV DNA</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute HCV infection</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+/-</td>
<td>Early phase</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+/-</td>
<td>+/-</td>
<td>Window phase</td>
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<td>-</td>
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<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Recovery phase</td>
</tr>
<tr>
<td><strong>Chronic HBV infection</strong></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Replicative phase (HBeAg positive chronic hepatitis)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Low / non-replicative phase</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Precore mutant variants (HBeAg negative chronic hepatitis)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+/-</td>
<td>+/-</td>
<td>+</td>
<td>-</td>
<td>+/-</td>
<td>+/-</td>
<td>Exacerbations of chronic hepatitis</td>
</tr>
</tbody>
</table>

HBV DNA detected by unamplified assay

HbsAg, hepatitis B surface antigen; HbeAg, hepatitis B e antigen; HBc, hepatitis B core IgM immunoglobulin

The diagnosis of acute hepatitis B is based on the detection of HBsAg and IgM anti-HBc. During the initial phase of infection, the markers of HBV replication (HBeAg and HBV DNA) are present. Recovery is accompanied by the disappearance of HBV DNA, seroconversion from HBeAg to anti-HBe, and subsequently seroconversion from HBsAg to anti-HBs. Rarely, patients may have entered the window period at the time of presentation; IgM anti-HBc is the sole marker of acute HBV infection in these cases. This situation is more common in patients with fulminant hepatitis B, in whom virus clearance tends to be relatively rapid.

Past HBV infection is diagnosed by the detection of anti-HBs and IgG anti-HBc. Immunity to HBV infection after vaccination is indicated by the presence of anti-HBs only.

The diagnosis of chronic HBV infection is based on the detection of HbsAg. Additional tests for HBV replication (HBeAg and serum HBV DNA) should be performed to determine if the patient should be considered for antiviral therapy. PCR assays are more sensitive; however, the pathogenic significance of minute amounts of HBV DNA that can be detected only by PCR but not by hybridization or signal amplification assays is uncertain. HBeAg-negative patients who have normal ALT levels do not have to be evaluated further, but those with elevated ALT levels should be tested for serum HBV DNA to determine if the liver disease is related to persistent HBV (wild type or variant) replication. Additional tests for hepatitis C and hepatitis D should be performed to rule out super infection with other hepatitis virus(es).
Management

The main aim of the treatment of chronic hepatitis B is to suppress HBV replication before irreversible liver damage occurs. Currently, IFN-α and lamivudine are the only approved treatments. Many new antiviral and immunomodulatory therapies are being evaluated; some of these have shown promise and may play a key role in the treatment of chronic HBV infection.

Treatment of Chronic Hepatitis B

- Interferon α.
- Antiviral agents.
  - Lamivudine.
  - Adefovir.
  - Emtricitabine.
  - Entecavir.
  - Clevudine.
  - β-L-Thymidine (L-dT).
- Immunomodulatory therapy.
  - Interleukin 12.
  - Thymosin.
- HBV –specific.
  - DNA vaccination.
- Combination treatment.
  - Interferon + Lamivudine.
  - Lamivudine + adenofovir dipivoxil.

Aims of Treatment

The initial goals are to suppress HBV replication and induce a remission of liver disease. The ultimate goals are to eliminate HBV, prevent progression to cirrhosis and HCC, and improve survival. Response is usually defined as sustained clearance of HBeAg with or without detectable anti-HBe, failure to detect HBV DNA in serum (with unamplified assays), and regression of liver disease (normalization of ALT levels and decrease in necrotic inflammation on liver biopsy). At a recent National Institutes of Health workshop on the management of hepatitis B, it was proposed that the definitions and criteria of the response to antiviral therapy of chronic hepatitis B be standardized. The proposal categorized responses as biochemical (BR), virologic (VR), or histologic (HR), and as on-therapy or sustained off-therapy (32).
Table 2- Definition Of Response To Antiviral Therapy Of Chronic Hepatitis B

<table>
<thead>
<tr>
<th>Category of response</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Biochemical (BR)</td>
<td>Decrease in serum level to within the normal range.</td>
</tr>
<tr>
<td>Virological (VR)</td>
<td>Decrease in serum HBV DNA to undetectable levels in unamplified assays (&lt; $10^5$ copies/mL) and loss of HbeAg in patients who were initially HbeAg-positive.</td>
</tr>
<tr>
<td>Histologic (HR)</td>
<td>Decrease in histology activity index by at least 2 points in comparison with value on pretreatment liver biopsy.</td>
</tr>
<tr>
<td>Complete (CR)</td>
<td>Fulfillment of criteria of biochemical and Virological response and loss of HbsAg.</td>
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<table>
<thead>
<tr>
<th>Time of assessment</th>
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<tbody>
<tr>
<td>End-of-treatment</td>
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<tr>
<td>Sustained response (SR-6)</td>
</tr>
<tr>
<td>Sustained response (SR-12)</td>
</tr>
</tbody>
</table>

HBV- Hepatitis B virus, HbeAg-Hepatitis B e antigen, HbsAg-Hepatitis B s antigen.

Antiviral therapy

Interferon

At present the standard therapy of chronic HBV infection among children is interferon-alpha (IFN). As per the consensus advice of 1999, the indications for treatment of children with IFN are HBsAg positivity, HbeAg positivity more than 6 months, active viral replication (HBV DNA positivity) and elevated serum alanine aminotransferase (ALT) more than twice the normal value in children older than two years (33). These criteria, however, excluded a majority of Asian children with normal serum ALT levels, who have perinatally acquired disease.

Side effects

<table>
<thead>
<tr>
<th>Side effects</th>
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<tbody>
<tr>
<td>Flulike illness</td>
</tr>
<tr>
<td>Fatigue</td>
</tr>
<tr>
<td>Anorexia, nausea</td>
</tr>
<tr>
<td>Emotional lability, anxiety, irritability, depression</td>
</tr>
<tr>
<td>Bone marrow suppression: neutropenia, thrombocytopenia</td>
</tr>
<tr>
<td>Sleep disturbance</td>
</tr>
<tr>
<td>Unmasking or exacerbation of autoimmune disorders, thyroid dysfunction</td>
</tr>
</tbody>
</table>

It is contraindicated in case of decompensated liver disease, cytopenia, autoimmune disease and severe renal or cardiac disease. Results of IFN in trials vary from 20%-60% success. It is found to be higher in European children as compared to Asian population, probably because of perinatal transmission and young age at infection acquisition in the latter. Israeli experience in children with chronic hepatitis B is 31.5% response rate. They concluded that IFN treatment is to be given only for children with HBV and significantly elevated liver enzymes.
Interferon is available as 3MU and 5 MU and is given as a subcutaneous injection, is available as single injection or multidose injection as in pen-interferon. It is debatable weather IFN treatment, compared to results of untreated patient, and might just accelerate the natural clearance of HBV infection (33, 34). Bortolotti et al. Have examined the long-term effects of IFN treatment in children with HBV and have seen HBeAg clearance rates of 60% versus 65% in the control group and HBsAg loss in 25% of the cases after a 5-year follow-up (35).

**PEG Interferon**

Shortcomings of interferon treatment are its fast absorption, large volume of distribution, short serum half life (2-5 hours), rapid renal clearance, limited efficacy, acute and chronic side effects. To achieve optimum results, constant viral suppression is needed. Polyethylene glycol or PEG is an inert, non-toxic and water-soluble polymer which when linked to interferon results in increase in its in vivo biological activity. PEG- interferon is interferon linked to polyethylene glycol moiety, which is given as weekly injections and provides constant viral suppression during that time. (Figure 2)

Characteristics of PEG interferon are rapid and sustained absorption, increased half life, improved antiviral activity, reduced frequency of dosing, reduce clearance, less side effects and improved patient compliance. Studies of PEG interferon in chronic hepatitis B infection is adults have shown definite advantages over interferon therapy (SVR ~ 30 - 40%), though results are not as good as compared to chronic hepatitis C infection. However studies with PEG interferon are children are still undergoing and therefore data from children is not available.

**Lamivudine**

Lamivudine (LAM) is a nucleoside analogue that prevents HBV DNA synthesis by competitively inhibiting the viral reverse transcriptase and DNA-polymerase stages of virus replication and by termination of proviral DNA chain extension. Clinical trials have been performed among children with a recommended dose of 3mg/kg with a maximum dose of 100mg, which have shown a satisfactory tolerability profile. (36, 37). Kocak et al, has shown HBV DNA clearance of 64.8% but HbeAg clearance of 7.4% with less than 6% seroconversion of anti HbeAg (38), when used in children with low viral replication (low HBV DNA levels). Patients with severe histologic activity and high baseline ALT seem to show better results in terms of seroconversion after treatment with Lamivudine However, in majority of the cases there is reappearance of HBV DNA replication on stopping lamivudine. Another major concern with the use of lamivudine is development of resistance to LAM.
treatment with the emergence of mutant HBV species. Certain HBV species show mutations in the tyrosine-methionine-aspartate-aspartate (YMDD) locus of the HBV-RNA -dependent DNA polymerase and therefore develop resistance to therapy with LAM after 6-9 months. Studies suggest that the incidence of YMDD mutations increases with the duration of treatment, from 22% after 24 weeks to 38-40% and 67% after 2 and 4 years (39, 40), respectively. The initial positive response to LAM treatment may then be followed by virological rebound and exacerbation of hepatitis. Fig 3 Seroconversion to low-level viral conversion.

There is no consensus on how to manage patients who develop a mutation. This phenomenon poses a great difficulty in deciding the ideal treatment time and in balancing positive response to adverse effects. This uncertainty of treatment is of paramount importance in pediatrics, as children may require LAM therapy for an indefinite length of time with an inevitable risk of mutation and relapse. Concerns have, therefore, been expressed about the use of LAM outside research trials and LAM has not been licensed for use in hepatitis B in children universally. Experience in adults has shown that adefovir may be effective in patients who develop Lamivudine resistant mutant.

**Adefovir Dipivoxil**

Adefovir Dipivoxil is a synthetic acyclic adenine nucleotide analogue. It is a potent inhibitor of HBV reverse transcriptase of the wild – type HBV, famciclovir-resistant and lamivudine – resistant mutants.

Two large international multicentric double-blinded, placebo-controlled studies in adults have shown that 10 mg oral Adefovir dipivoxil daily given for 40 weeks is effective in terms of histologic improvement, HBV DNA suppression, ALT normalization and HBsAg loss (1.6 -2% vs. 0%) in both HBeAg positive and HBeAg negative CHB (41, 42). In HBeAg positive patients, HBeAg loss and HBeAg seroconversion also increased as compared with controls (12% vs. 6%) (41). The development of resistance was 2% after 2 years of therapy. There is a lot of interest generated presently with the use of the drug in chronic hepatitis and trials are underway in children.
Famciclovir

Famciclovir is known to induce seroconversion, but seems to show weaker suppression than Lam (43) and therefore might be useful only in combination with LAM or other antiviral agents (44).

Entecavir

Entecavir is a guanosine nucleoside analogue and has been tested in clinical trials among adults (45, 46). With a safety profile similar to LAM, it has shown to be more effective in viral suppression compared to LAM, in wild type and in LAM resistant infections with HBV. Both Entecavir and Adefovir may play an important role in the further development of antiviral treatment of HBV.

Other antiviral therapy agents include thymosin, clevudine, etc.

Combination Therapy

There has been interest in using a combination of IFN or PEG - IFN with LAM hoping that the combination might have additional antiviral effects (47, 48). What remains to be identified is the most appropriate regimen; that is, whether LAM is to be used before or along with IFN or after initial dose IFN etc. Studies are still undergoing in Pediatrics on PEG interferon in children and on newer antiviral agents. The only pediatric studies on combination therapy have used IFN and LAM. While Selimoglu et al. compared a combination treatment to IFN monotherapy (49), Dikici et al. compared different regimens of IFN and LAM combination (47).

Hepatitis B Vaccination

The use of hepatitis B vaccination and need for universal hepatitis B vaccine cannot be more emphasized.

Hepatitis C

Hepatitis - C is the result of infection with hepatitis C virus (HCV). Though common cause of both acute and chronic liver disease in adults (50, 51) it is an uncommon infection in children. It is estimated to affect 170 million people worldwide and approximately 1% of the population based on studies carried out in blood donors. The incidence is probably lower in children although there is no sufficient data on HCV in children.

Hepatitis C Virus

It is a double RNA shelled virus, approximately 50 to 50 nm. The virus genome has structural and non-structural protein genes. A distinctive feature of HCV is its sequence diversity or heterogeneity. This variability is called species diversity. HCV can be classified into genotypes, subtypes and isolates based on sequence diversity of the genome.
Genomic Organization and Viral Proteins

HCV consists of a 9.6-kb, positive (sense), single-strand RNA genome that comprises a highly conserved 341-base 5′ noncoding region, a single long open reading frame (ORF) of 9,033 to 9,099 bases, and a 3′ noncoding region. The ORF encodes a polyprotein precursor of approximately 3,000 amino acids (52). This polyprotein is cleaved cotranslationally and posttranslationally by both cellular and viral proteases to produce at least 10 polypeptides with various functions in replication and virus assembly (53,54).

Table-3: Heterogeneity of HCV

<table>
<thead>
<tr>
<th>Classification</th>
<th>Average degree of diversity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quasispecies</td>
<td>&lt;1% - 5%</td>
</tr>
<tr>
<td>Isolate</td>
<td>2% - 15%</td>
</tr>
<tr>
<td>Subtype a, b, c</td>
<td>10% - 30%</td>
</tr>
<tr>
<td>Genotype 1,2,3,4,5,6</td>
<td>&gt;30%</td>
</tr>
</tbody>
</table>

*Variability in nucleotide sequence in representative regions of the viral ribonucleic acid.

Genotypes 1 & 2 are found worldwide; genotype 3 is common in the Indian subcontinent and Southeast Asia. The clinical importance of genotypes is related to the response to antiviral therapy. Patients with genotypes 1 and 4 are more likely to be resistant to interferon-based therapy than are patients with genotypes 2 or 3 (55, 56, 57, 58).

Table- 4: The Modes of Transmission of Hepatitis C

- Blood transfusion
- Administration of blood products (anti - hemophilic factor, factor IX, intravenous immunoglobulin)
- Injection drug use
- Cocaine snorting
- Accidental occupational exposure (i.e. needlestick)
- Exposure to contaminated medical equipment (reusable syringes, inadequately sterilized medical instruments, contamination of intravenous fluids or injectable medications)
- Tattooing or body piercing
- Sexual spread
- Maternal - infant spread
Hepatitis C can be spread from HCV -positive mother to newborn, but such spread is uncommon. In large prospective studies, 5% to 10% of infants born to anti-HCV - positive mothers acquire hepatitis C during the first 1 to 2 years of life (59, 60, 61). The transmission appears to occur in the postnatal period, although instances of HCV infection detected at the time of birth have been reported. All infants born to anti-HCV -positive mother have anti-HCV in serum as a result of passive transfer of immunoglobulin in utero. The passively transferred anti-HCV can persist for 12 months and possibly longer, which makes anti HCV testing unreliable for documenting transmission. Most studies have relied upon HCV RNA testing to detect transmission, but HCV RNA intermittent positivity may be transient. Also complicating the issue is the occurrence of false-positive HCV RNA results from PCR, particularly when testing cord blood at the time of delivery. Thus proof of transmission of hepatitis C usually requires the presence of detectable HCV RNA on two separate occasions or persistence of anti-HCV after 12 months of age. Using these criteria the rate of transmission ranges from 5% to 10%. Upto 50% of infected newborns clear HCV RNA spontaneously, so that chronic infection eventuates in only 2% to 5% of children. Rarely is neonatal infection associated with symptoms or jaundice. The importance of maternal-infant transmission is shown by studies of hepatitis C in children that demonstrate that vertical transmission now accounts for the majority of cases of chronic hepatitis C in children. (63)

Maternal-infant transmission of hepatitis C occurs only if the mother has HCV RNA in addition to anti-HCV in serum. Other factors that are reported to correlate with a higher likelihood of transmission of hepatitis C are high maternal titers of HCV RNA, co-infection with HIV, a prolonged or difficult delivery, and the use of internal fetal monitoring during delivery. (59, 60, 63)

Transmission of HCV has not been associated with breast – feeding. If breast-feeding is elected, careful breast hygiene and avoidance of feeding if there are cracks in the nipples are appropriate.

Clinical Course of Hepatitis C

Acute Hepatitis C

The clinical, biochemical, and serologic course of a “typical” case of acute hepatitis C is shown in Figure 5. The incubation period of acute hepatitis C lasts for 15 to 75 days, averaging 50 days. During this period and within a week or two of exposure, HCV RNA becomes detectable in serum and levels of virus gradually rise.
Diagnosis of Acute Hepatitis – C

The diagnosis of acute hepatitis C is suggested by the presence of clinical or biochemical evidence of acute hepatitis accompanied by anti-HCV or HC RNA in serum. The presence of anti-HCV or HCV RNA in a patient with biochemical evidence of acute hepatitis is suggestive but not completely diagnostic of acute hepatitis C. Neither anti-HCV nor HCV RNA testing can reliably distinguish between acute and chronic hepatitis C with a superimposed form of acute liver injury or acute exacerbation.

A more common issue in managing patients with acute hepatitis is the need to rule out hepatitis C as a cause of acute liver injury in a patient who lacks other serologic markers for hepatitis A (IgM anti-HAV) and B (hepatitis B surface antigen [HbsAg] and IgM anti-HBc). In this situation testing for HCV RNA during the acute illness or testing for anti-HCV both during the acute illness and during convalescence effectively rules out acute hepatitis C.

Table 5 - Hepatitis C virus peptides in immunodiagnostic assays

<table>
<thead>
<tr>
<th>Peptide</th>
<th>EIA-1</th>
<th>EIA-2</th>
<th>EIA-3</th>
<th>RIBA-1</th>
<th>RIBA-2</th>
<th>RIBA-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-11 (NS4)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C 100-3 (NS3-4)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C33c (NS 3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C200 (fusionC100/C 3)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C22-3 (core)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NS5</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

EIA- Enzyme immunoassay; RIBA- recombinant immunoblot assay.

Table 6 - Sensitivity and predictive value of enzyme immunoassay (EIA) for HCV infections

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sensitivity(%)</th>
<th>Time for positivity after infection (wks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIA –1</td>
<td>70 - 80</td>
<td>16</td>
</tr>
<tr>
<td>EIA –2</td>
<td>92- 95</td>
<td>10</td>
</tr>
<tr>
<td>EIA –3</td>
<td>97</td>
<td>7 – 8</td>
</tr>
</tbody>
</table>

Clinical Course of Chronic Hepatitis C

The course of a patient developing chronic hepatitis C is shown in Figure 6.

Figure - 6: Typical course of chronic hepatitis C HCV RNA is detectably by polymerease chain reaction within 2 weeks of exposure. ALT levels rise thereafter and symptoms appear 6 to 8 weeks after viremia. Anti – HCV generally arises late, after onset of ALT elevations and symptoms. With development of chronic disease, serum ALT and HCV RNA levels fluctuate, being intermittently normal or undetectable. Chronic infection is shown by persistence of HCV RNA positivity with or without ALT elevations. (61) HCV, hepatitis C virus; RNA, ribonucleic acid; ALT, serum alanine aminotransferase.
Diagnosis of chronic hepatitis C

The diagnosis of chronic hepatitis C is generally made on the basis of persistence of ALT elevation or HCV RNA in serum for 6 months or longer.

Serologic and Virologic markers of hepatitis C

Anti – HVC Assays

The most widely used tests for anti-HCV are EIAs (Elisa immune assays). Several third generation EIAs are commercially available and approved for use in screening blood. Current assays have a high degree of sensitivity and specificity.

Immunoblot Assays

Immunoblot assays for anti-HCV are available for confirmation of EIA test results. (64, 65). In patients with biochemical evidence of liver disease, testing for HCV RNA is a more appropriate approach for confirming anti-HCV reactivity. (66,67)

HCV RNA Tests (Qualitative Assay)

Testing for HCV RNA is the most direct method of demonstrating active HCV infection. (66,67) The levels of HCV RNA found in serum are generally too low to be detected using direct hybridization techniques, but can be readily detected after amplification by polymerase chain reaction PCR (68) or transcription - mediated amplification. The food and drug administration (FDA) has approved to PCR based tests for qualitative detection of HCV - RNA; (1) Amplicor Hepatitis C Virus Test, version2.0, and (2) Cobas Amplicor Hepatitis C virus Test, version 2.0 (Roche Molecular Systems, Branchburg, NJ), which have limits of detection of approximately 50 IU/mL. A third approved test is the VERSANT® HCV RNA Qualitative Assay.

HCV RNA Levels (Quantitative Assays)

Testing for the level of HCV RNA can be helpful in assessing the likelihood of a response before or during therapy. Two major commercial assays are widely available, one being based upon competitive qualitative PCR (qPCR) and one using signal amplification of branched DNA (bDNA). The level of HCV RNA in blood helps in predicting the likelihood of response to treatment, and the change in the level of HCV RNA during treatment can be used to monitor response.

HCV Genotyping

There are 6 major HCV genotypes (69) although genotype does not predict the outcome of infection, it does predict the likelihood of treatment response, and in many cases, determines the duration of treatment. (70,71). Genotyping can be done by sequence analysis of various regions of the virus, by restriction fragment length polymorphism analysis, by probe-specific hybridization, or by line probe assay (LiPA) (72,73). These tests are expensive. Common Genotypes in Indian patients are Genotype 2 & 3.
AASLD recommendations for testing with HCV RNA assays are:

1. Patients suspected of having chronic HCV infection should be tested for HCV antibodies.
2. HCV RNA testing should be performed in
   a. Patients with a positive anti-HCV TEST
   b. Patients for whom antiviral treatment is being considered, using a quantitative assay
   c. Patients with unexplained liver disease whose anti-HCV test is negative and who are immune - compromised or suspected of having acute HCV infection
3. HCV genotype should be determined in all HCV - infected persons prior to treatment in order to determine the duration of therapy and likelihood of response.

Liver Biopsy

Liver biopsy furnishes information about the staging of fibrosis and the degree of hepatic inflammation. It also provides information on prognosis. However it's not mandatory in order to initiate therapy.

Natural History of Chronic Hepatitis - C

After chronic HCV infection is established, spontaneous resolution is uncommon. (63,74). Disease activity in chronic hepatitis C may improve or worsen spontaneously, but loss of viremia is rare.

The relatively benign natural history of chronic hepatitis C in young individuals are supportive of studies of children infected with hepatitis C as a result of maternal - infant exposure or blood transfusion (75). In a large study from Germany 10 - to 20 years follow - up was available on a cohort of children who underwent open-heart surgery in the 1980s. (76) It usually progresses to cirrhosis after 15-20 years. The progression is more severe or rapid in the presence of excess iron in liver or associated HIV. Unlike hepatitis –B infection hepatocellular carcinoma does not develop in the absence of cirrhosis, with chronic hepatitis –C infection.

Treatment Objectives and Outcomes

The goal of treatment is to prevent complications of HCV infection; this is principally achieved by eradication of infection. Infection is considered eradicated when there is sustained virologic response (SVR), defined as the absence of HCV RNA in serum by a sensitive test at the end of treatment and 6 months later. Persons in whom HCV RNA levels remain stable on treatment are considered non responders, while those whose HCV RNA levels decline (e.g. by >2 logs), but never become undetectable, are referred to as partial responders.
Therapy of Children

There have been substantial improvements in the success of HCV treatment, and there are currently several treatments approved by the FDA. In randomized clinical trials in adults, the highest overall SVR rates have been achieved with the combination of weekly subcutaneous injections of long-acting peg interferon alfa and oral ribavirin, which represents the current standard of care. The response is better for non-genotype 1 (65-70% as compared to < 40%). Response to therapy is measured by estimating the number of patients who are HCV RNA negative by PCR 6 months after stopping therapy. Present recommendation is to use the combination therapy for a period of 1 year in genotype 1 as compared to 6 months in other genotypes.

Interferon and ribavirin are approved by FDA for use in treatment of hepatitis -C in children. The drug has been used in the dose of 3MU/M$^2$ three times a week for 6-12 months, along with a dose of 15-mg/kg bid of ribavirin. Interferon is contraindicated in children less than 2 years of age due to its negative effect on growth and possible role in the development of spastic diplegia. Preliminary uncontrolled studies suggest that children have a better rate of response to interferon therapy than adults and that they tend to have fewer side effects. (77, 79). A metaanalysis of 10 studies of IFN use in children has shown a response rate as high as 70% in non-type 1 & 27% in type 1 patients. One of largest group of children in India with HCV are those who have been multi-transfused. These children often have high ferritin levels and excess iron load results not only in more liver damage but also poorer response to therapy. Also, the most common adverse effect of ribavarin is anemia and this translates to increased blood transfusion requirement. Our study of interferon therapy for treatment of chronic hepatitis C in thalassemia children in India (n=11) has shown that the response to interferon is good (79). Chronic hepatitis C rarely resolves spontaneously: and children with this disease are likely to have the infection for life. For these reasons, early treatment may he appropriate and is often requested. Recently FDA has also approved PEG interferon therapy for use in chronic hepatitis C infection in children.

Children who should be treated include -

1. Acute HCV infection that has not resolved (HCV viremia present) after 12 weeks

2. Progressive HCV infection with or without elevated transaminases and biopsy showing necro-inflammatory changes.

Liver Transplantation

Liver transplantation has been performed in children with end stage HCV disease. Like in adults, the re-infection of the graft with HCV is almost universal.

Hepatitis C Vaccine

There has been some progress made in developing a vaccine against hepatitis – C, which is still in experimental stages.
The rapid advances in knowledge of HCV and availability of new tools to evaluate virus replication and inhibition promise to provide new, more effective, and better tolerated approaches to the treatment and prevention of this important liver disease.

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