58 Hepatitis Viruses

INTRODUCTION

The term 'viral hepatitis' refers to a primary infection of the liver by any one of a heterogeneous group of 'hepatitis viruses', which currently consists of types A, B, C, D, E and G. (The designation 'type F' had been proposed for a putative virus believed to cause transfusion-associated hepatitis, distinct from types A to E. But it proved to be a mutant [HBx] of the type B virus and not a separate entity. Type F was therefore deleted from the list of hepatitis viruses.)

Hepatitis viruses are taxonomically unrelated. Except for type B, which is a DNA virus, all the others are RNA viruses. The features common to them are their hepatotropism and ability to cause a similar icteric illness, ranging in severity from the unapparent to the fulminant fatal forms.

As all types of hepatitis viruses cause a clinically indistinguishable acute illness, their differentiation is based on their serological and molecular markers. Hepatitis may occur incidentally during many other viral infections, such as yellow fever, Lassa fever, Marburg, EB, cytomegalovirus, herpes simplex, varicella zoster, measles, rubella or coxsackie viruses. These are not included in the category of viral hepatitis.

Types of viral hepatitis

By epidemiological and clinical criteria, two types of viral hepatitis had been recognised for long:

- One type occurred sporadically or as epidemics, affecting mainly children and young adults, and transmitted by the fecal–oral route. This was called **infective** or **infectious hepatitis**, later termed **type A hepatitis.**
- A second type of viral hepatitis, transmitted mainly by inoculation, was originally observed in persons receiving serum inoculation or blood transfusion. This had been given various names such as **homologous serum jaundice, serum hepatitis** (because of its association with human or homologous antisera so commonly used for prophylaxis or therapy early in the twentieth century) and **transfusion hepatitis.** It was later called **type B hepatitis.**

Non-A non-B hepatitis (NANB): For a time it was believed that all viral hepatitis was caused by either of the two hepatitis viruses, type A accounting for all infectious hepatitis and type B for all post-transfusion or serum hepatitis. However, with the development of techniques for identifying type A and type B viruses, it became apparent that in many cases of infectious and post-transfusion hepatitis no evidence could be found of infection with either type A or B viruses. It therefore became evident that the clinical syndrome of type A or B hepatitis could also be caused by one or more other uncharacterised viruses. The term **non-A non-B hepatitis** was applied to this group but is no longer used now as it is now possible to diagnose specific infections. These non-A non-B viruses include the following types:

- **Type C virus** was later identified as causing many non-A non-B transfusion-associated hepatitis cases.
- A defective virus which depends on the helper functions of type B virus was called **delta** or **type D viruses**.
- Type E virus is yet another type of non-A non-B hepatitis transmitted by the fecal–oral route, prevalent mostly in the developing nations was found to be caused by the **type E virus**.

- **Type G hepatitis** can also cause hepatitis virus, can also cause hepatitis, but its role has not yet been adequately understood.

TYPE A HEPATITIS

Type A hepatitis (infectious hepatitis) is a subacute disease of global distribution, affecting mainly children and young adults.

Clinical features: The large majority of infections are asymptomatic. Overt illness is seen in only about 5 per cent of those infected. The incubation period is 2–6 weeks. The clinical disease consists of two stages: the prodromal or preicteric and the icteric stages. Onset may be acute or insidious, with fever, malaise, anorexia, nausea, vomiting and liver tenderness. These usually subside with onset of jaundice. Recovery is slow, over a period of 4–6 weeks. Very rarely a rapidly fatal fulminant hepatitis may occur. The disease is milder in children, in whom many infections may be anicteric. Mortality is low (0.1–1 per cent), with most of the deaths occurring in adults.

Hepatitis A virus (HAV)

In 1973, Feinstone and co-workers, using immuno-electron microscopy (IEM), demonstrated this virus in the feces of experimentally infected human volunteers. Chimpanzees and marmosets can be infected experimentally. HAV can be grown in some human and simian cell cultures and is the only human hepatitis virus which can be cultivated in vitro. It has also been cloned.

Morphology: HAV is a 27-nm, non-enveloped RNA virus belonging to the picornavirus family. It was originally designated as 'enterovirus 72' (Fig. 58.1). Because of its unique features, HAV is now recognised as the prototype of a new genus *Hepatovirus*. Only one serotype of the virus is known.

Resistance: HAV is resistant to inactivation by heat at 60°C for one hour, ether and acid at pH 3, but is inactivated by boiling for one minute, 1:4000 formaldehyde at 37°C for 72 hours, and chlorine 1 ppm for 30 minutes. It is not affected by anionic detergents. It survives prolonged storage at a temperature of 4°C or below.

Epidemiology: Natural infection with HAV is seen only in humans. Though primates such as chimpanzees have been shown to acquire the infection from humans and transmit it to human contacts, there is no evidence of any extrahuman source of the virus in nature.

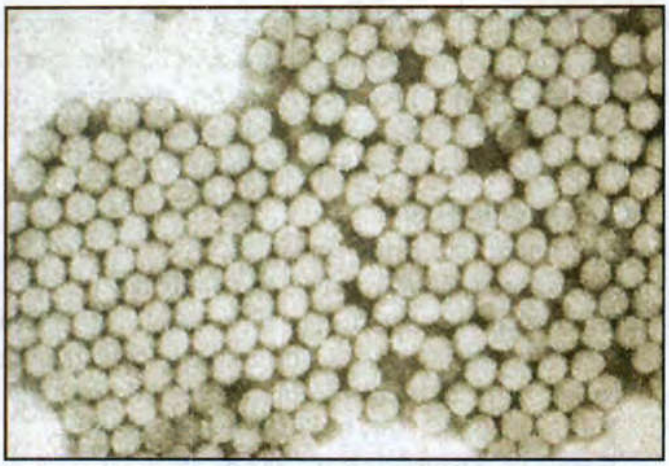

Fig. 58.1 Electron micrograph of 27-nm hepatitis A virus aggregated with antibody

HAV transmission is by the fecal–oral route. Infection is by ingestion. The virus multiplies in the intestinal epithelium and reaches the liver by hematogenous spread. It is shed in feces during the late incubation period and prodromal phase of the illness. Once jaundice develops, it is rarely detectable in feces. Chronic carriers are not seen. Virus persistence in nature depends on continuing inapparent infections.

A brief viremia occurs during the preicteric phase, but ceases with the onset of jaundice. Chronic viremia does not occur. Parenteral transmission is therefore very rare. Infection has been reported in recipients of some clotting factor concentrates. Transplacental infection has not been documented. HAV may be present occasionally in the saliva and urine of patients, but this is not considered relevant in its spread.

Type A hepatitis occurs sporadically or as outbreaks, which may be caused by contaminated food, water or milk. Shellfish have been known to be responsible for outbreaks. Domestic or institutional spread of infection among children is common. Overcrowding and poor sanitation favour its spread.

The epidemiology of type A hepatitis resembles that of poliomyelitis. In the developing countries, infection is acquired in childhood and by the age of 10, 90 per cent of the population possess the antibody to the virus and are immune. In India, type A hepatitis is the most common cause of acute hepatitis in children, but is much less frequent in adults. In affluent countries, and even in those developing countries with improved personal hygiene and sanitation, its incidence has

been declining, with an upward shift in the age group affected. In the temperate regions, the disease shows an autumn–winter predilection, but in the tropics no seasonal distribution is evident. In India, the disease tends to be associated with heavy rainfall.

Laboratory diagnosis:

1. **Specimen:** Feces or serum may be collected for demonstration of the virus or its antibody.
2. **Direct demonstration:** The virus can be visualised by IEM in fecal extracts during the late incubation period and the preicteric phase, but seldom later. This is not commonly used for diagnosis.
3. **Serology:** Diagnosis is usually by detection of antibody. IgM anti-HAV antibody appears during the late incubation period, reaches peak levels in 2–3 weeks and disappears after 3–4 months. The IgG antibody appears at about the same time, peaks in 3–4 months and persists much longer, perhaps for life. Demonstration of IgM antibody in serum indicates current or recent infection, while the IgG antibody denotes recent or remote infection and immunity. ELISA kits for detection of IgM and IgG antibodies are available (Fig. 58.2).

Prophylaxis:

General prophylaxis consists of improved sanitary practices and prevention of fecal contamination of food and water. A safe and effective formalin inactivated, alum conjugaged vaccine containing HAV grown in human diploid cell culture is available. A full course consists of two intramuscular injections of the vaccine.

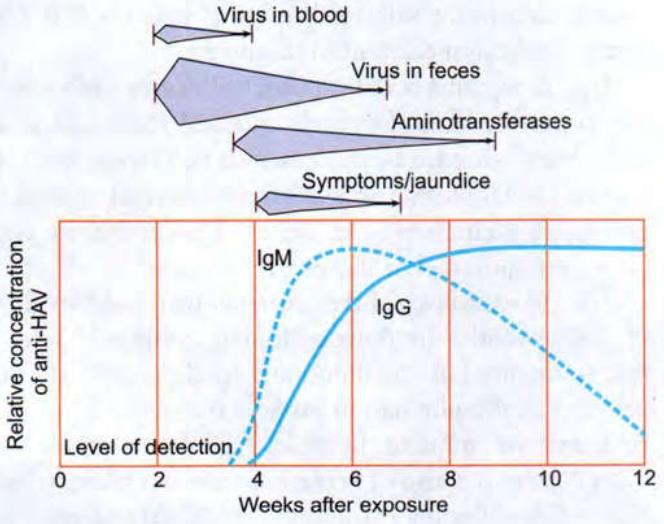

Fig. 58.2 Immunologic and biologic events associated with human infection with hepatitis A virus

Protection begins 4 weeks after injection and lasts for 10–20 years.

Specific passive prophylaxis by pooled normal human immunoglobulin (16% solution in a dose of 0.2–0.12 ml/kg body weight) IM, before exposure or in the early incubation period, can prevent or attenuate clinical illness, while not necessarily preventing infection and virus excretion.

Natural infection with HAV, clinical or subclinical, leads to lifelong immunity. There is no cross-immunity between HAV and any of the other hepatitis viruses.

Treatment: This is symptomatic. No specific antiviral drug is available.

TYPE B HEPATITIS

Type B hepatitis is the most widespread and the most important type of viral hepatitis. More than a third of the world's population is estimated to be have been infected by HBV. About a quarter of them become HBV carriers. A quarter of these develop serious liver disease, including chronic hepatitis, cirrhosis and primary hepatic cancer. As there is an effective vaccine against HBV, hepatocellular carcinoma has become the only human cancer which is vaccine-preventable. The WHO estimates that HBV infection causes more than a million deaths a year worldwide.

Clinical features: The incubation period is long, about 1–6 months. The clinical picture of hepatitis B is similar to that of type A, but it tends to be more severe and protracted. Onset is insidious and fever is not prominent. Extrahepatic complications like arthralgia, urticaria and rarely polyarteritis or glomerulonephritis may occur. These are ascribed to circulating immune complexes containing the viral surface antigen.

About 90–95 per cent of adults with acute hepatitis B infection recover within 1–2 months of onset and eliminate the virus from the body within about six months, remaining immune thereafter. Mortality is about 0.5–2.0 per cent, but may be more in post-transfusion cases. About 1 per cent of patients, particularly those having simultaneous delta virus infection develop fatal fluminant hepatitis.

A proportion of cases (1–10 per cent) remain chronically infected. They may be asymptomatic carriers or may progress to recurrent or chronic liver disease or cirrhosis. A few of them may develop hepatocellular carcinoma after many decades.

The pathogenesis of hepatitis appears to be immune mediated. Hepatocytes carry viral antigens and are subject to antibody-dependent NK cell and cytotoxic T cell attack. In the absence of adequate immune response, HBV infection may not cause hepatitis, but may lead to carrier state. Therefore infants and immunodeficient persons are more likely to become asymptomatic carriers following infection.

Hepatitis B virus (HBV)

Hepatitis

Clinical Case A 40-year-old woman who had received multiple blood transfusions over the previous six months presented with persistent fatigue, loss of appetite, nausea, vomiting and abdominal pain for a duration of 10 days. She had a history of passing high-coloured urine. Her liver function tests showed elevated serum bilirubin and liver enzymes. A viral hepatitis panel was advised and showed HBsAg positive, anti-HBc IgM positive, anti-HAV IgM negative and anti-HCV IgM negative. She was diagnosed with acute HBV infection, placed on supportive therapy and the liver enzymes and viral markers monitored every month to check for seroconversion from HBsAg to anti-HBsAg positivity (indicative of resolution of the disease).

Morphology: HBV is a 42-nm DNA virus with an outer envelope and an inner core, 27 nm in diameter, enclosing the viral genome and a DNA polymerase. Because of its unique features, HBV is assigned to a separate family Hepadnaviridae (hepatotropic DNA viruses), which consists of two genera: *Orthohepadnavirus* containing HBV as well as the woodchuck and ground squirrel hepatitis viruses, and *Avihepadnavirus*, containing the Pekin duck and grey heron hepatitis viruses. HBV is Hepadnavirus type 1.

The discovery of HBV was serendipitous. In 1965, Blumberg, studying human serum lipoprotein allotypes, observed in the serum of an Australian aborigine, a new antigen which gave a clearly defined line of precipitation with sera from two hemophiliacs who had received multiple blood transfusions. This was named the **Australia antigen**. By 1968 it was found to be associated with serum hepatitis. It was subsequently shown to be the surface component of HBV. Therefore the name Australia antigen was changed to **hepatitis B surface antigen (HBsAg)**.

Under the electron microscope, sera from type B hepatitis patients show three types of particles (Fig. 58.3):

- The most abundant form is a spherical particle, 22 nm in diameter.
- The second type of particle is filamentous or tubular with a diameter of 22 nm and of varying length. These two particles are antigenically identical and are surface components of HBV (HBsAg) which are produced in great excess.
- The third type of particle, far fewer in number, is a double-walled spherical structure, 42 nm in diameter. This particle is the complete hepatitis B virus. It was first described by Dane in 1970 and so is known as the Dane particle.

The envelope proteins expressed on the surface of the virion and the surplus 22-nm-diameter spherical and filamentous particles constitute the hepatitis B surface antigen. HBsAg consists of two major polypeptides, one of which is glycosylated.

Antigenic diversity:

HBsAg exhibits antigenic diversity. It contains two different antigenic components: the common group reactive antigen a, and two pairs of type specific antigens d-y and w-r, only one member of each pair being present at a time. HBsAg can thus be divided into four major antigenic subtypes: **adw, adr, ayw and ayr**. The subtypes do not seem to be important in immunity because of the dominant antigen a shared by all. The subtypes

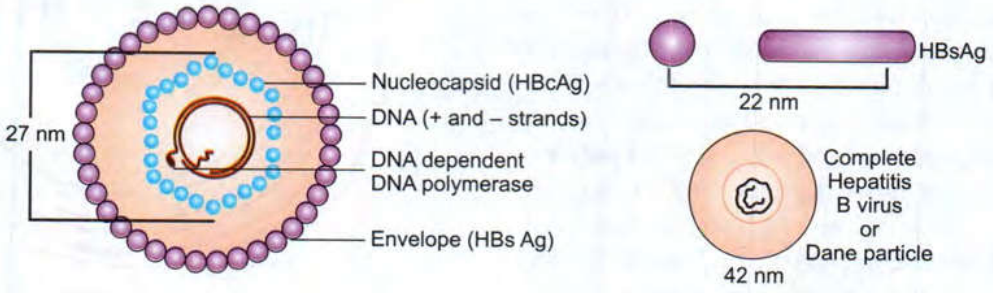

Fig. 58.3 Structure of hepatitis B virus

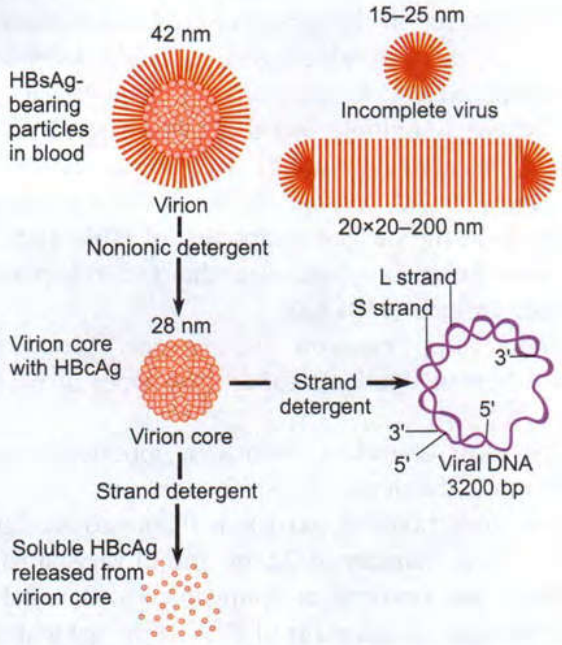

Fig. 58.4 Hepatitis B virus

breed true, and the index case and contacts in an outbreak have the same subtype. They show a distinct geographical distribution. Subtype ayw is common from West Asia through the Middle East, to Western and Northern India; adw is common in Europe, Australia and the Americas; adr is prevalent in South and East India and the Far East; ayr is very rare. A number of other surface antigenic reactivities (a, x, f, t, j, n, g) have been reported, but not been adequately studied.

HBcAg: Mild detergent treatment disrupts the viral envelope and exposes the core or nucleocapsid. The antigen expressed on the core is called the hepatitis B core antigen (HBcAg) (Fig. 58.4).

HBeAg: A third antigen called the hepatitis B antigen (HBeAg) is a soluble non-particulate nucleocapsid protein. HBcAg and HBeAg, though immunologically distinct, are coded for by the same gene.

Viral genome: The nucleocapsid encloses the viral genome consisting of two linear strands of DNA held in a circular configuration. One of the strands (the plus strand) is incomplete, so that the DNA appears partially double-stranded and partially single-stranded. Associated with the **plus strand** is a viral **DNA polymerase**, which has both DNA dependent DNA polymerase and RNA dependent reverse transcriptase functions. This polymerase can repair the gap in the plus strand and render the genome fully double-stranded (Fig. 58.5).

The genome has a compact structure with four overlapping genes:

- The **S gene** codes for the surface antigen. It consists of the S region and two Pre-S regions: Pre-S2 and Pre-S1. The protein coded for by the S region is called the S or major protein. When translation begins from the Pre-S2 region, the M or middle protein is formed. When the entire gene from Pre-S1 is translated, the L or large protein results. The L protein is present only in the virion, while the M and S proteins are found in the circulating HBsAg particles also.
- The **C gene** has two regions, C and Pre-C. When the C region alone is translated, the core antigen (HBcAg) is formed. HBcAg is assembled as the nucleocapsid core particles. It is not secreted and does not circulate in blood, but can be demonstrated in hepatocytes by immunofluorescence. Antibodies to HBc, both IgM and IgG, appear in blood. The IgG antibody to HBcAg persists in blood long after all other serological markers have disappeared and so provides a useful marker of prior infection with HBV. If translation begins from the Pre-C region, the resulting protein is HBeAg, a non-particulate soluble antigen possessing a signal protein which enables it to be secreted. It is therefore present in circulation. The presence of HBeAg in blood provides a convenient and readily detectable marker of HBV replication and high infectivity.
- The **P gene** is the largest and codes for the DNA polymerase enzyme.

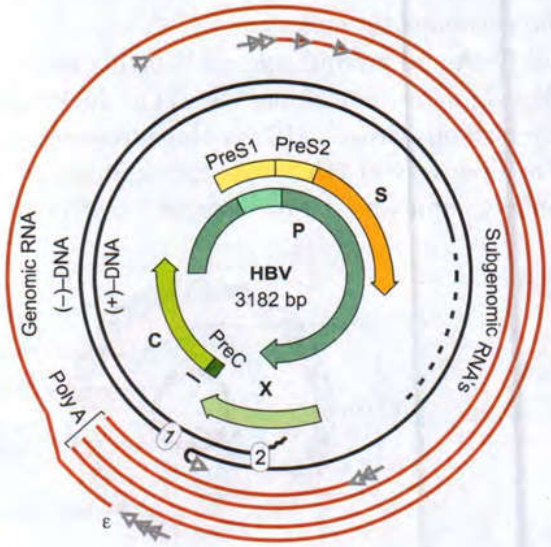

Fig. 58.5 Genetic organisation of the HBV genome

- The **X gene** codes for a small non-particulate protein (HBxAg), which has transactivating effects on both viral and some cellular genes. This leads to enhanced replication of HBV, as well as of some other viruses, such as the human immunodeficiency virus. HBxAg and its antibody are present in patients with severe chronic hepatitis and hepatocellular carcinoma.

Mutations: A few cases of infection by mutant viruses have been identified. Two types of mutations have been studied:

- One type, initially identified in Mediterranean countries, presents as severe chronic hepatitis, caused by pre-core mutants unable to synthesise HBeAg. Those infected with **precore mutants** may be positive for anti-HBe and anti-HBc.
- The second group of so-called '**escape mutants**' have been seen in some infants born to HBeAg-positive mothers, and in liver transplant recipients who had received combined immunisation with anti-HBV immunoglobulin and vaccine. They show mutation in the common a determinant of HBsAg, preventing them from being neutralised by the anti-HBsAg antibody. If such mutants become more common, they may pose problems in hepatitis B prophylaxis.

HBV replicates within hepatocytes. Viral DNA exists in the hepatocyte nucleus in the free extrachromosomal state or integrated with the cell chromosome. Replication resembles that seen in retroviruses, in that DNA is synthesised from an RNA template by reverse transcription.

HBV DNA and protein have also been identified in extrahepatic sites such as the bone marrow, spleen, lymph nodes and circulating lymphocytes, but apparently no damage is produced in these locations. The significance of this extrahepatic presence is not understood.

HBV does not grow in any conventional culture system. However, limited production of the virus and its proteins can be obtained from several cell lines transfected with HBV DNA. HBV proteins have been cloned in bacteria and yeast. The chimpanzee is susceptible to experimental infection and can be used as a laboratory model.

Resistance: HBV is a relatively heat stable virus. It remains viable at room temperature for long periods. Heat at 60°C for 10 hours reduces infectivity by 100- to 1000-fold. It is susceptible to chemical agents. Exposure to hypochlorite (10,000 ppm available chlorine) or 2% glutaraldehyde inactivates infectivity, though HBsAg may not be destroyed by such treatment.

Epidemiology: Hepatitis B occurs throughout the world. Natural infection occurs only in humans. There is no animal reservoir. The virus is maintained in the large pool of carriers whose blood contains circulating viruses for long periods, in some even lifelong. There is no seasonal distribution. The infection is usually sporadic, though occasional outbreaks have occurred in hospitals, orphanages and institutions for the mentally handicapped.

The prevalence of hepatitis carriers varies widely in different countries, in relation to their living standards. India falls in the intermediate group: carrier rate 2–7 per cent, with higher carrier rates in the southern part of the country and lower rates in the northern part.

A carrier is a person with detectable HBsAg in blood for more than six months. Following infection, about 5–10 per cent of adults, 30 per cent of children and 90 per cent of neonates become carriers. The carrier state is more common among males. There are over 350 million carriers now worldwide. Of them, about 45 million are in India, which has the second largest carrier pool, next only to China.

Carriers: Carriers are of two categories:

- **Super carriers:** These are highly infectious, having high titre HBsAg, along with HBeAg, DNA polymerase and HBV in circulation, and generally elevated transaminases. Some of them have enormous antigenemia and viremia, up to 10^{13} HBsAg particles equal to 500 µg of protein, and 10^{8} HBV per ml of blood. About a quarter of the carriers in India are HBeAg positive.
- **Simple carriers** have low infectivity and low titre HBsAg in blood, with negative HbeAg, HBV and DNA polymerase. Many super carriers in time become simple carriers.

Transmission: HBV is a bloodborne virus and the infection is transmitted by parenteral, sexual and perinatal modes.

- **Parenteral transfusion:** Blood of carriers, and less often of patients, is the most important source of infection. The virus may also be present in other body fluids and excretions, such as saliva, breast milk, semen, vaginal secretions, urine, bile and feces. Of these, semen and saliva are known to transmit

the infection; others may also do so, though much less efficiently than blood. Feces is not known to be infectious.

Transfusion of carrier blood, once the most widely known mode of infection has largely been eliminated wherever donor screening is strictly enforced. Therapeutic and prophylactic preparations from pooled human blood and serum have led to hepatitis, but this risk is now minimal, with screening of donors and production techniques ensuring virus inactivation. However, HBsAg screening is not a totally fail safe method as infection has occurred even with HBsAg-negative, anti-HBc–positive blood, which may have had undetectable amounts of virus.

Many other therapeutic, diagnostic, prophylactic and even non-medical procedures are now the main modes of infection. HBV is very highly infectious, far more that HIV. Any object or procedure than can convey minute traces of infected blood or other material, as little as 0.00001 ml, can be infectious. These include shared syringes, needles and other sharp items or endoscopes, personal articles such as razors, nail clippers or combs, and practices such as acupuncture, tattooing, ritual circumcision, ear or nose piercing, and field camps for surgery or disease detection by blood testing where separate sterile articles may not be available. Professionals using sharp articles like barbers, dentists and doctors may unwittingly transmit the virus if great care is not taken.

Infection by direct contact with open skin lesions such as pyoderma, eczema, cuts and scratches is very common among young children in developing countries, as also through household transmission where opportunities exist for contact with blood or saliva among members.

HBV has been said to survive in mosquitoes and bed bugs for about two weeks after blood meal, but no virus multiplication occurs. They do not appear to transmit the infection.

- **Perinatal transmission**: Congenital or vertical transmission is quite common from carrier mothers. The risk to babies is high if the mother is HBeAg positive (60–90 per cent) and low if negative (5–15 per cent). True congenital infection (in utero, transplacental) is rare. Infection is usually acquired during birth by contact of maternal blood with the skin and mucosa of the fetus, or in the immediate postnatal period. Infection by ingestion has been reported, but its efficiency is very low. However it is safer if carrier mothers do not breastfeed when proper nutrition of their babies can be otherwise ensured. HBV-infected neonates generally do not suffer from any clinical illness, but remain carriers for life and some of them may develop hepatocellular carcinoma after many decades.
- **Sexual transmission** of HBV occurs everywhere, but is more important in the developed countries, particularly in the promiscuous homosexual. The risk of transmission by heterosexual and homosexual contact increases with the number of partners and the duration of such relationships. HBV infection has occurred after artificial insemination. Semen donor screening is therefore obligatory.

Occupational risk: Certain groups and occupations carry a high risk of infection. These include medical and paramedical personnel, staff of blood banks, dialysis units, medical laboratories and mental health institutions, barbers and sex workers. Dentists and doctors have been responsible for small outbreaks. In non-endemic countries like Britain, HBV carriers are barred from invasive medical practice. Carriers are also not permitted to be medical students.

Laboratory diagnosis: Detection of viral markers: Specific diagnosis of hepatitis B rests on serological demonstration of the viral markers. It is therefore necessary to understand the sequence of their appearance in blood (Fig. 58.6).

1. **HBsAg** is the first marker to appear in blood after infection, being detectable even before elevation of transaminases and onset of clinical illness. It remains in circulation throughout the icteric or symptomatic course of the disease. In the typical case, it disappears within about two months of the start of clinical disease, but may sometimes last for six months and even beyond. When it is no longer detectable, its antibody, anti-HBs, appears and remains for very long periods. Anti-HBs is the protective antibody.
2. **HBcAg** is not demonstrable in circulation because it is enclosed within the HBsAg coat, but its antibody, anti-HBc, appears in serum a week or two after the appearance of HBsAg. It is therefore the earliest antibody marker to be seen in blood, long before anti-HBe or anti-HBs. As anti-HBc remains lifelong, it serves as a useful indicator of prior infection with

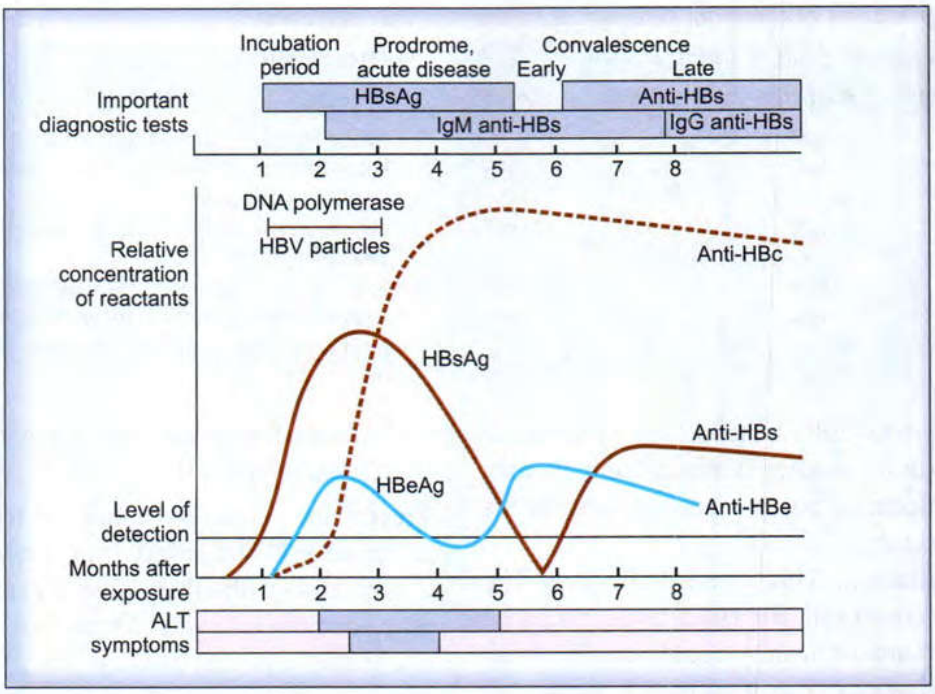

Fig. 58.6 Clinical and serologic events occurring in a patient with acute hepatitis B virus infection

HBV, even after all the other viral markers become undetectable. Initially, anti-HBc is predominantly IgM, but after about six months, it is mainly IgG. Selective tests for IgM or IgG anti-HBc therefore enable distinction between recent or remote infection respectively.

3. **HBeAg** appears in blood concurrently with HBsAg, or soon afterwards. Circulating HBeAg is an indicator of active intrahepatic viral replication, and the presence in blood of DNA polymerase, HBV DNA and virions, reflecting high infectivity. The disappearance of HBeAg coincides with the fall of transminase levels in blood. It is followed by the appearance of anti-HBe.

For the diagnosis of HBV infection, detection of HBsAg in blood is all that ordinarily necessary. The simultaneous presence of IgM anti-HBc indicates recent infection and the presence of IgG anti-HBc remote infection. Occasionally, when the level of HBsAg is too low to be detectable, diagnosis has to be made by testing for IgM anti-HBc (*Case*).

HBeAg provides information about relative infectivity. Its presence denotes high infectivity and its absence, along with the presence of anti-HBe, indicates low infectivity. As it is invariably present during acute hepatitis, its testing is indicated only in chronic infection and carriers.

The presence of anti-HBs without any other serological virus marker indicates immunity following vaccination. Table 58.1 shows the interpretation of various serological patterns in hepatitis B.

Like HBeAg, HBV DNA is also an indicator of viral replication and infectivity. Molecular methods such as DNA:DNA hybridisation and PCR, at present used for HBV DNA testing are highly sensitive and quantitative. HBV DNA level in serum reflects the degree of viral replication in the liver and so helps to assess the progress of patients with chronic hepatitis under antiviral chemotherapy.

Prophylaxis: General prophylaxis consists in avoiding risky practices like promiscuous sex, injectable drug abuse and direct or indirect contact with blood, semen or other body fluids of patients and carriers. Health education, use of the disposable syringes and needles, screening of blood, semen and organ donors, have all helped to an extent, but these alone cannot eliminate the risk altogether, particularly in the developing countries.

Immunisation: The only certain method appears to be universal immunisation. Both passive and active methods of immunisation are available.

- **Passive immunisation:** Hyperimmune hepatitis B immune globulin (HBIG) prepared from human volunteers with high titre anti-HBs, administered

Table 58.1 *Interpretation of commom serological patterns in HBV infection*

Virus/Antibody markers					Interpretation
HBsAg	HBeAg	Anti-HBc	Anti-HBs	Anti-HBe	
+	+	IgM	–	–	Acute HBV infection; highly infectious
+	+	IgG	–	–	Late/chronic HBV infection or carrier state; highly infectious
+	–	IgG	–	+/–	Late/chronic HBV infection or carrier state; low infectivity
–	+/–	IgM	–	+/–	Seen rarely in early acute HBV infection; infectious
–	–	IgG	+/–	+/–	Remote HBV infection; infectivity nil or very low
–	–	–	+	–	Immunity following HBV vaccine

IM in a dose of 300–500 i.u. soon after exposure to infection constitutes passive immunisation. It may not prevent infection, but protects against illness and the carrier state.

- **Active immunisation:** This is more effective. The first vaccine introduced in 1982, was prepared from pooled plasma of healthy human carriers with high level antigenemia. This was immunogenic, but became unacceptable because its source was human plasma, limited in availability and not totally free from possible risk of unknown pathogens.

  The vaccine currently preferred is genetically engineered by cloning the S gene of HBV in baker's yeast. It consists of non-glycosylated HBsAg particles alone. It is given with alum adjuvant, IM into the deltoid or, in infants into the anterolateral aspect of the thigh. Gluteal injection is not recommended as it may result in poor immune response. Three doses given at 0, 1 and 6 months constitute the full course. Seroconversion occurs in about 90 per cent of the vaccinees. A special vaccine containing all antigenic components of HBsAg (Pre-S1, Pre-S2 and S) has been developed, which gives greater seroconversion. Seroconversion can be checked by testing for anti-HBs which is usually detectable for about five years. Clinical protection is believed to last much longer. Booster doses are needed only for those at high risk.

  Now that the vaccine is manufactured in India, and is available at lower cost, it should be possible to include this in the national immunisation schedule.
- **Combined immunisation:** For non-immune persons exposed to HBV, combined immunisation is recommended. For babies born to carrier mothers, a single injection of 0.5 ml of HBIG given IM immediately after birth, is followed by the full course of vaccine at a different anatomical site, the first dose being given within 12 hours of birth. When HBIG is not available, the vaccine given alone has been reported to provide protection.

Treatment: No specific antiviral treatment is available for acute HBV infection. Interferon alpha, alone or in combination with other antiviral agents such as lamivudine and famcyclovir, has been beneficial in some cases of chronic hepatitis. There is no effective treatment for the carrier state, though spontaneous resolution takes place in some of them.

TYPE C HEPATITIS

Attempts to identify the group of 'non-A non-B' viruses by experimental infection in chimpanzees led to the discovery of hepatitis C virus (HCV). It is now the most common cause of post-transfusion hepatitis in the developed countries.

Clinical features: The incubation period is long, 15–160 days, with a mean of 50 days. The acute illness is usually mild or anicteric. Overt jaundice is seen in about 5 per cent of patients only. The important part in type C hepatitis is the chronic illness. About 50–80 per cent of patients progress to chronic hepatitis, with some developing cirrhosis and hepatocellular carcinoma.

Epidemiology: HCV infection is seen only in humans. The source of infection is the large number of carriers, estimated to be about 200 million worldwide. In general the epidemiology resembles that of hepatitis B.

Infection is mainly by blood transfusion and other modes of contact with infected blood or blood products. Injectable drug abusers, transplant recipients and immunocompromised persons are at high risk. Sexual transmission is probably less important. Vertical transmission from mother to baby may take place.

The infection occurs throughout the world, with carrier rates of 1–20 per cent. HCV infection is prevalent in India too, with an estimated 12.5 million cases.

A quarter of all chronic hepatitis cases in India are believed to be due to HCV infection.

Hepatitis C virus (HCV)

The virus has not been grown in culture, but has been cloned in *Escherichia coli*. HCV is a 50–60 nm virus with a linear, single-stranded RNA genome, enclosed within a core and surrounded by an envelope, carrying glycoprotein spikes (Fig. 58.7). HCV resembles flaviviruses in structure and organisation, and has been classified as a new genus *Hepacivirus* in the family Flaviviridae.

The virus shows considerable genetic and antigenic diversity. At least six different genotypes and many subtypes have been identified, indicating high mutability. Some genotypes are seen worldwide, while others are localised. Because of this diversity there is little heterologous or even homologous postinfection immunity in hepatitis C.

Laboratory diagnosis: The standard method of diagnosis is antibody detection by ELISA. The antigens used are various structural and non-structural proteins cloned in *E.coli*. Three successive generations of such antigens have been introduced to improve sensitivity and specificity of serological diagnosis. Even the third-generation ELISA currently in use, employing NS-5 region protein and synthetic peptides, becomes positive only months after the infection and shows non-specific reactions. Confirmation by immunoblot assay is therefore recommended. In HCV infection, antibodies appear irregularly and late, limiting their diagnostic utility. Culture is not yet established.

Identification of HCV RNA in blood provides more sensitive and specific results within a few days of exposure to HCV. Molecular methods like PCR and branched DNA assay are employed for the purpose.

Fig. 58.7 HCV virus

Prophylaxis: Only general prophylaxis, such as blood screening, is possible. No specific active or passive immunising agent is available.

Treatment: Prolonged treatment with interferon alpha, either alone or in combination with antiviral agents like ribavirin has been reported to be useful in some cases.

TYPE D (DELTA) HEPATITIS

In 1977, Rizzetto and colleagues in Italy identified a new viral antigen in the liver cell nuclei of patients infected with hepatitis B virus. This has been shown to be due to the hepatotropic virus delta or Hepatitis D Virus (HDV). Delta is a defective RNA virus dependent on the helper function of HBV for its replication and expression. Therefore, it has no independent existence and can survive and replicate only as long as HBV infection persists in the host.

Morphology: HDV is a spherical, 36-nm particle with an outer coat composed of the hepatitis B surface antigen surrounding the circular single-stranded RNA genome. Though it resembles some plant viruses, such as viroids or satellite viruses, it has been proposed to be classified in a new genus *Deltavirus*, because of its special features.

Clinical features: Its mode of transmission is the same as for HBV. Two types of infection are recognised:

- **Co-infection:** Here delta and HBV are transmitted together at the same time. Co-infection clinically presents as acute hepatitis B, ranging from mild to fulminant disease.
- **Superinfection:** Here delta infection occurs in a person already harbouring HBV. It usually leads to more serious and chronic illness, with deterioration of the underlying HBV infection. No association has been noted between HDV and hepatocellular carcinoma.

Laboratory diagnosis: The delta antigen is primarily expressed in liver cell nuclei, where it can be demonstrated by immunofluorescence. It is only occasionally present in serum. Anti-delta antibodies appear in serum and can be identified by ELISA. The IgM antibody appears 2–3 weeks after infection and is soon replaced by the IgG antibody in acute delta infection. However, in chronic infection, the IgM antibody per-

sists for years. Delta RNA sequences have been cloned and DNA probes have been developed for the rapid identification of delta particles in circulation. The woodchuck has been found to be a suitable experimental model for the study of HDV infection.

Epidemiology: HDV is distributed worldwide but is more common in certain endemic areas. In the Mediterranean countries, where it is endemic, infection is spread commonly by non-percutaneous routes, especially by close personal contact. In the non-endemic areas, such as northern Europe and North America, infection is more often through blood and blood products and is commonly seen in drug addicts and hemophiliacs. Introduction of HDV into non-endemic areas where HBV infection is common may lead to outbreaks of severe hepatitis with high mortality.

Prophylaxis: No specific prophylaxis exists, but immunisation with the HBV vaccine is effective as HDV cannot infect persons immune to HBV. Screening of blood donors for HBsAg automatically limits blood-borne HDV infection.

TYPE E HEPATITIS
(ENTERICALLY TRANSMITTED NANB OR EPIDEMIC NANB HEPATITIS)

Hepatitis viruses A and B account for less than half the cases of acute hepatitis in many developing countries. The bulk of NANB hepatitis in these areas is transmitted enterically through fecal pollution of drinking water (hence the name **enterically transmitted NANB or E-NANB**). It often appears as epidemics (hence also called **epidemic NANB**). The largest such epidemic occurred in Delhi during the winter of 1955–56, affecting over 30,000 persons within six weeks. Several outbreaks and sporadic infections have been reported from many parts of the Indian subcontinent, Central and South Asia, North Africa and Central America. This hepatitis was not seen in Western countries except when imported from endemic areas, but recently occasional cases have been reported from Europe. The disease is now called type E hepatitis and its causative agent hepatitis E virus (HEV). In India, HEV is responsible for the majority of epidemic and sporadic hepatitis in adults.

Type E hepatitis was previously mistaken for hepatitis A because of clinical and epidemiological similarities. It was recognised as a separate entity because of the absence of serological and virological evidence of HAV infection in these cases. The source of infection is fecal contamination of drinking water and the environment. Secondary attack rate among household contacts is very low in type E hepatitis, 2–3 per cent as against 10–20 per cent in HAV infection.

Clinical features: The incubation period ranges 2–9 weeks with an average of six weeks. Most cases occur in the young to middle aged adults (15–40 years old). The disease is generally mild and self-limited, with a low case fatality of about 1 per cent. A unique feature is the clinical severity and high case fatality rate of 20–40 per cent in pregnant women, especially in the last trimester of pregnancy.

Morphology: HEV is a spherical non-enveloped virus, 32–34 nm in diameter, with a single-stranded RNA genome. The surface of the virion shows indentation and spikes. The virus is very labile. In morphology and physical characteristics, it resembles Caliciviruses such as the Norwalk virus. It has been provisionally classified in *Hepeviridae*.

Laboratory diagnosis: HEV can be demonstrated by immuno-electron microscopy (IEM) in the bile and feces of patients in the incubation period or acute phase of illness. The carrier state has not been observed. Experimental infection can be transmitted to many species of primates. It has been reported to be prevalent in animal reservoirs such as pigs. In vitro cultivation has not been successful so far. The viral genome has been cloned. Comparison of virus strains from different areas indicates that only one serotype of the virus exists. ELISA kits are available for IgG and IgM antibodies, using recombinant and synthetic peptide antigens.

Table 58.2 lists out the comparative features of the various viral hepatitis types.

HEPATITIS G VIRUS

Two flavivirus-like isolates were obtained in 1995 from Tamarin monkeys inoculated with blood from a young surgeon (GB) with acute hepatitis. A similar virus was isolated from another human specimen the same year. These isolates were called GB viruses A, B and C, respectively.

In 1996, an isolate closely resembling GBV-C was obtained from a patient with chronic hepatitis. This has been called the hepatitis G virus (HGV). It has not been grown, but its RNA genome has been cloned. HGV RNA has been found in patients with acute, chronic