VIROLOGY

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DNA Enveloped Virus

- Hepatitis B Virus

Defenition

- HBV is classified as a hepadnavirus. HBV establishes chronic infections, especially in those infected as infants; it is a major factor in the eventual development of liver disease and hepatocellular carcinoma in those individuals.

General Properties of HBV

Virion: About 42 nm in diameter overall (nucleocapsids, 18 nm)

Genome: One molecule of double-stranded DNA, circular, 3.2 kbp. In virion, negative DNA strand is full-length and positive DNA strand is partially complete. The gap must be completed at beginning of replication cycle

Proteins: Two major polypeptides (one glycosylated) are present in HBsAg; one polypeptide is present in HBcAg

Envelope: Contains HBsAg and lipid

Replication: By means of an intermediate RNA copy of the DNA genome (HBcAg in nucleus; HBsAg in cytoplasm). Both mature virus and 22-nm spherical particles consist of HBsAg secreted from the cell surface

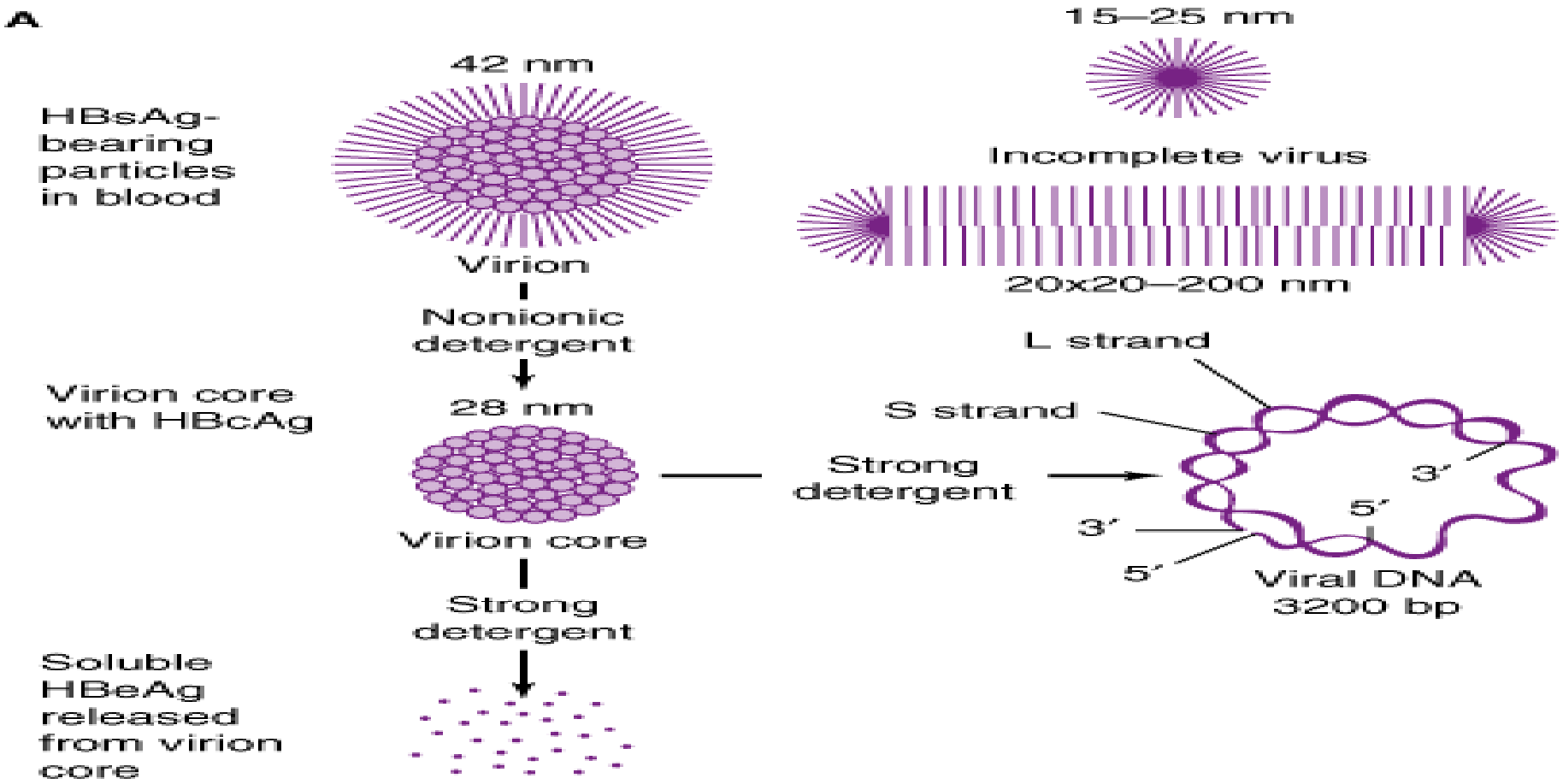
Outstanding characteristics:

Family is made up of many types that infect humans and lower animals (eg, woodchucks, squirrels, ducks)

Cause acute and chronic hepatitis, often progressing to permanent carrier states and hepatocellular carcinoma

Structure & Composition

- Electron microscopy of HBsAg-positive serum reveals three morphologic forms . The most numerous are spherical particles measuring 22 nm in diameter. These small particles are made up exclusively of HBsAg—as are tubular or filamentous forms, which have the same diameter but may be over 200 nm long—and result from overproduction of HBsAg. Larger, 42-nm spherical virions (originally referred to as Dane particles) are less frequently observed. The outer surface, or envelope, contains HBsAg and surrounds a 27-nm inner nucleocapsid core that contains HBcAg. The variable length of a single-stranded region of the circular DNA genome results in genetically heterogeneous particles with a wide range of buoyant densities.



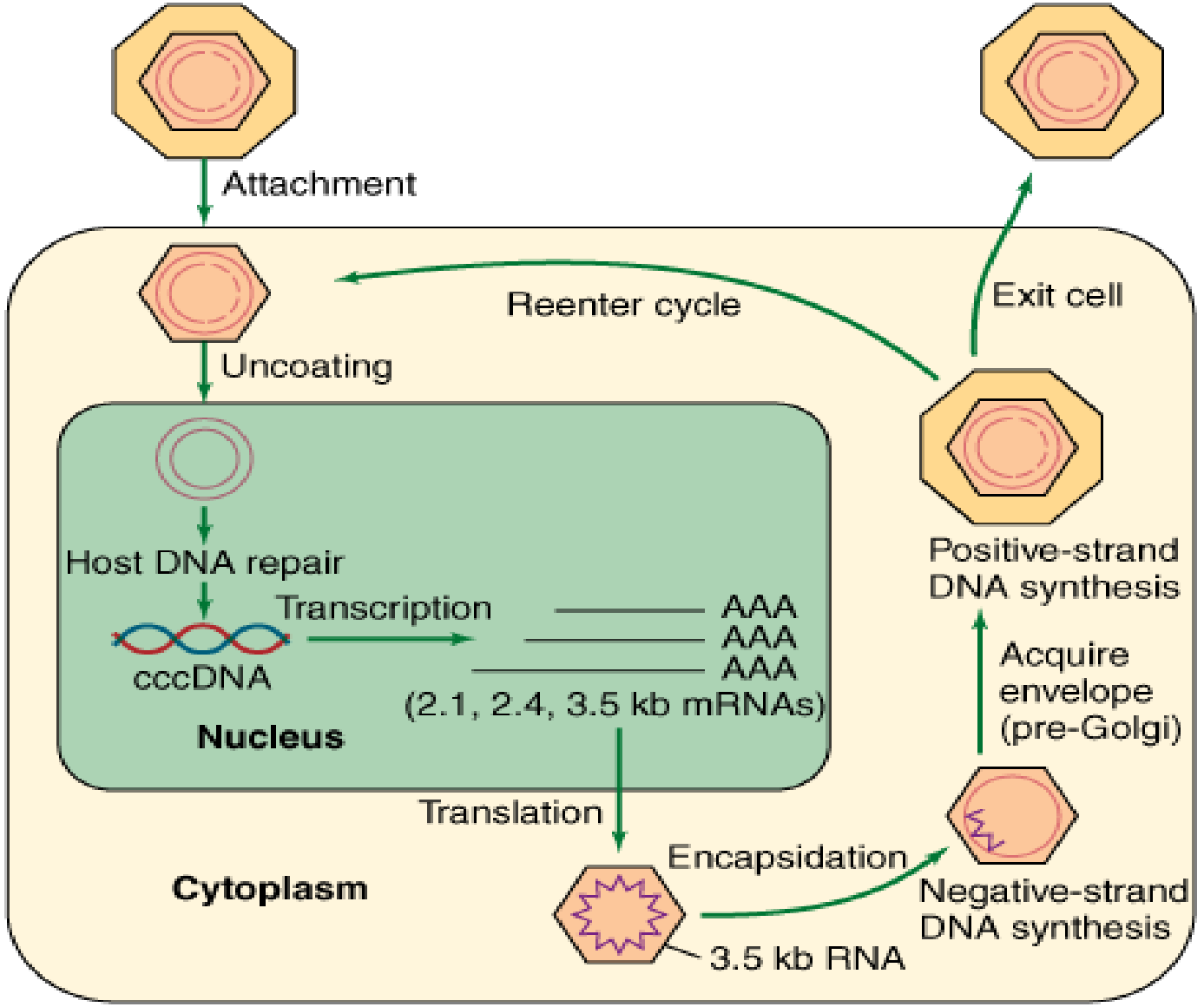
Hepatitis B viral and subviral forms. **A:** Schematic representation of three HBsAg-containing forms that can be identified in serum from HBV carriers. The 42-nm spherical Dane particle can be disrupted by nonionic detergents to release the 28-nm core that contains the partially double-stranded viral DNA genome. A soluble antigen, termed HBeAg, may be released from core particles by treatment with strong detergent.

HBV Genome

- The viral genome consists of partially double-stranded circular DNA, 3200 bp in length. Different HBV isolates share 90–98% nucleotide sequence homology. The full-length DNA negative strand (L or long strand) is complementary to all HBV mRNAs; the positive strand (S or short strand) is variable and between 50% and 80% of unit length.

Replication of Hepatitis B Virus

- The infectious virion attaches to cells and becomes uncoated. In the nucleus, the partially double-stranded viral genome is converted to covalently closed circular double-stranded DNA **(cccDNA)**. The cccDNA serves as template for all viral transcripts, including a 3.5-kb pregenome RNA. The **pregenome RNA** becomes encapsidated with newly synthesized HBcAg. Within the cores, the viral polymerase synthesizes by **reverse transcription** a negative-strand DNA copy. The polymerase starts to synthesize the positive DNA strand, but the process is not completed. Cores bud from the pre-Golgi membranes, acquiring HBsAg-containing envelopes, and may exit the cell. Alternatively, cores may be reimported into the nucleus and initiate another round of replication in the same cell.



Four stages in the viral life cycle

- The first stage is immune tolerance. The duration of this stage for healthy adults is approximately 2-4 weeks and represents the incubation period. For newborns, the duration of this period often is decades.
- Active viral replication is known to continue despite little or no elevation in the aminotransferase levels and no symptoms of illness.

- In the second stage, an inflammatory reaction with a cytopathic effect occurs. HBeAg can be identified in the sera, and a decline of the levels of HBV DNA is seen. The duration of this stage for patients with acute infection is approximately 3-4 weeks (symptomatic period). For patients with chronic infection, 10 years or more may elapse before cirrhosis develops.

- In the third stage, the host can target the infected hepatocytes and the HBV Viral replication no longer occurs. HBeAb can be detected. The HBV DNA levels are lower or undetectable, and aminotransferase levels are within the reference range. In this stage, an integration of the viral genome into the host's hepatocyte genome takes place. HBsAg still is present.

- In the fourth stage, the virus cannot be detected and antibodies to various viral antigens have been produced. Different factors have been postulated to influence the evolution of these stages, including age, sex, immunosuppression, and co-infection with other viruses.

Pathology

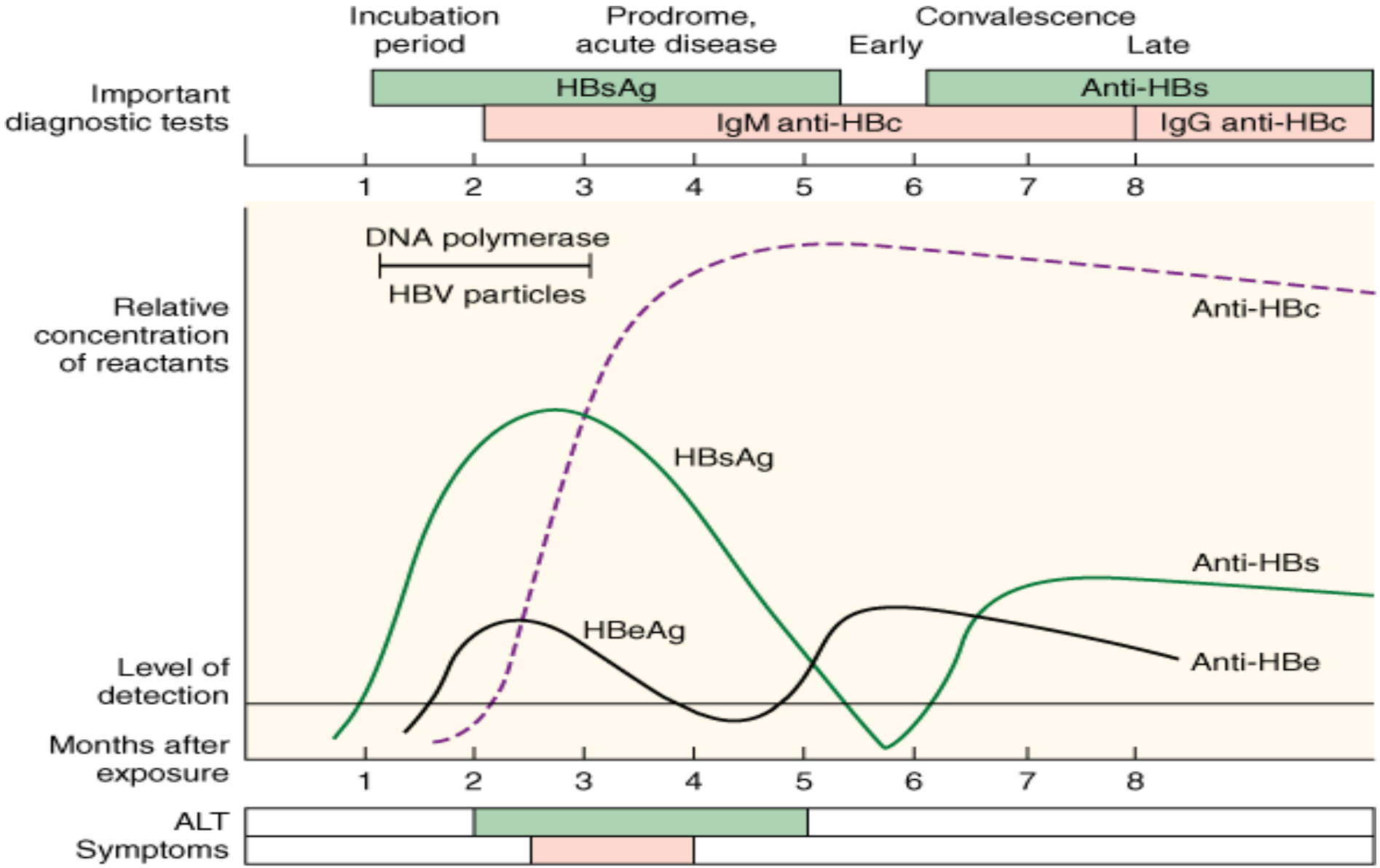
- Hepatitis is a general term meaning inflammation of the liver.
- Microscopically, there is spotty parenchymal cell degeneration, with necrosis of hepatocytes, a diffuse lobular inflammatory reaction, and disruption of liver cell cords. These parenchymal changes are accompanied by reticuloendothelial (Kupffer) cell hyperplasia, periportal infiltration by mononuclear cells, and cell degeneration.
- Localized areas of necrosis are frequently observed. Later in the course of the disease, there is an accumulation of macrophages near degenerating hepatocytes.
- Preservation of the reticulum framework allows hepatocyte regeneration so that the highly ordered architecture of the liver lobule can be ultimately regained.
- The damaged hepatic tissue is usually restored in 8–12 weeks.

Spectrum of Chronic Hepatitis B Diseases

1 Chronic Persistent Hepatitis - asymptomatic
2. Chronic Active Hepatitis - symptomatic exacerbations of hepatitis
3. Cirrhosis of Liver
4. Hepatocellular Carcinoma

Immunity to HBV

- DNA polymerase activity, HBV DNA, and HBeAg, which are representative of the viremic stage of hepatitis B, occur early in the incubation period, concurrently or shortly after the first appearance of HBsAg. High concentrations of HBV particles may be present in the blood (up to 10^{10} particles/mL) during the initial phase of infection; communicability is highest at this time.
- HBsAg is usually detectable 2–6 weeks in advance of clinical and biochemical evidence of hepatitis and persists throughout the clinical course of the disease but typically disappears by the sixth month after exposure.



Source: Brooks GF, Carroll KC, Butel JS, Morse SA, Mietzner TA: *Jawetz, Melnick, & Adelberg's Medical Microbiology, 25th Edition*: http://www.accessmedicine.com

Interpretation of HBV serological marker in Hepatitis patients

- A battery of serological tests are used for the diagnosis of acute and chronic hepatitis B infection.
- HBsAg - used as a general marker of infection.
- HBsAb - used to document recovery and/or immunity to HBV infection.
- anti-HBc IgM - marker of acute infection.
- anti-HBcIgG - past or chronic infection.
- HBeAg - indicates active replication of virus and therefore infectiveness.
- Anti-Hbe - virus no longer replicating. However, the patient can still be positive for HBsAg which is made by integrated HBV.
- HBV-DNA - indicates active replication of virus, more accurate than HBeAg especially in cases of escape mutants. Used mainly for monitoring response to therapy.

CHRONIC HEPATITIS

- A history of acute hepatitis is elicited in only a small percentage of patients with chronic HBV infection.
- Many patients with chronic hepatitis B are asymptomatic, while others have nonspecific symptoms such as fatigue. Some patients experience exacerbations of the infection which may be asymptomatic, mimic acute hepatitis, or manifest as hepatic failure.
- Physical examination may be normal or there may be stigmata of chronic liver disease. Jaundice, splenomegaly, ascites, peripheral edema, and encephalopathy may be present in patients with decompensated cirrhosis. Laboratory tests may be normal, but most patients have mild to moderate elevation in serum AST and ALT.
- During exacerbations, the serum ALT concentration may be as high as 50 times the upper limit of normal and alfa-fetoprotein (AFP) concentrations as high as 1000 ng/mL may be seen .

Treatment

- Interferon - for HBeAg +ve carriers with chronic active hepatitis. Response rate is 30 to 40%.
- Lamivudine - a nucleoside analogue reverse transcriptase inhibitor. Well tolerated, most patients will respond favorably. However, tendency to relapse on cessation of treatment. Another problem is the rapid emergence of drug resistance.
- Adefovir – less likely to develop resistance than Lamivudine and may be used to treat Lamivudine resistance HBV. However more expensive and toxic
- Successful response to treatment will result in the disappearance of HBsAg, HBV-DNA, and seroconversion to HBeAg.

Prevention

- Vaccination - highly effective recombinant vaccines are now available. Vaccine can be given to those who are at increased risk of HBV infection such as health care workers. It is also given routinely to neonates as universal vaccination in many countries.
- Hepatitis B Immunoglobulin - HBIG may be used to protect persons who are exposed to hepatitis B. It is particular efficacious within 48 hours of the incident. It may also be given to neonates who are at increased risk of contracting hepatitis B i.e. whose mothers are HBsAg and HBeAg positive.
- Other measures - screening of blood donors, blood and body fluid precautions.

Primary Hepatocellular Carcinoma

- The WHO estimates that 80% of all cases of PHC can be attributed to chronic HBV infections.
- HBV may induce PHC by promoting continued liver repair and cell growth in response to tissue damage or by integrating into the host chromosome and stimulating cell growth directly.