AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

LESSON 18.

Microbiology diagnosis of viral hepatitis

FACULTY: ***General Medicine***
SUBJECT: ***Medical microbiology - 2***

Discussed questions:

1. The problem of viral hepatitis

2. Classification of hepatitis viruses.

- Hepatitis A virus. Taxonomy. Virion structural features, cultivation, resistance, ways of infection. Pathogenesis of the disease, clinic. Microbiological diagnosis, specific treatment and prevention.

- Hepatitis B virus. Taxonomy. Structural characteristics of the virion, antigens - HBs, HBc, HBe, HBx, reproduction characteristics, cultivation, persistence, ways of infection. Pathogenesis of the disease, mechanism of persistence, clinic. Immunity. Microbiological diagnosis, specific treatment and prevention

- Hepatitis D virus. Virion structural features, antigens, cultivation. Features of delta-infection (coinfection, superinfection), pathogenesis of the disease. Microbiological diagnosis, treatment and prevention

- Hepatitis C virus. Virion structural features, genotypes, antigenic variability, cultivation, persistence, ways of infection. Pathogenesis, clinic, chronicity of the disease. Microbiological diagnosis, treatment and prevention

- Hepatitis E virus. Virion structural features, genome, antigens, cultivation, persistence, ways of infection. Pathogenesis, clinic, complications of the disease (during pregnancy). Microbiological diagnosis, main markers of infection, specific treatment and prevention

- Hepatitis G virus. Virion structural features, genome, variability, epidemiology, clinical forms, complications. Microbiological diagnosis and treatment.

Purpose of the lesson:

- To acquaint students with the problem of viral hepatitis, to provide information about the morpho-biological characteristics of hepatitis A, B, C, D, E, G viruses, to introduce them to the methods of microbiological diagnosis of viral hepatitis.

INTRODUCTION

- **VIRAL HEPATITIS-** is a primary infection of the liver caused by heterogeneous group of ‘hepatitis viruses’.

- hepatitis viruses cause inflammation of liver producing identical histopathologic lesions similar to illness such as fever, nausea, vomiting and jaundice.

CLASSIFICATION

1. INFECTIVE OR INFECTIOUS HEPATITIS

- occurs sporadically or as epidemics.
- affects mainly children and young adults.
- transmitted through faecal-oral route.

2. HOMOLOGOUS SERUM JAUNDICE/SERUM HEPATITIS/TRANSFUSION HEPATITIS

-transmitted mainly by inoculation or blood transmission.

-associated with human or homologous antisera used for prophylaxis or therapy

TYPES OF HEPATITIS VIRUSES

- Hepatitis A and B
- Non-A non-B hepatitis (NANB): C, D, E and G.
- All are RNA viruses except type B a DNA type.

- HAV: causes infectious hepatitis.
- HBV: causes serum hepatitis.
- HCV: causes post-transfusion hepatitis.
- HDV: is defective virus, need HBV for replication.
- HEV: agent of enterically transmitted Non-A, Non-B hepatitis.
- HGV.

Hepatitis A

- CLASSIFICATION:

  Group: Group IV ((+) ssRNA)

  Family: Picornaviridae

  Genus: Hepatovirus

  Specie: Hepatitis A virus

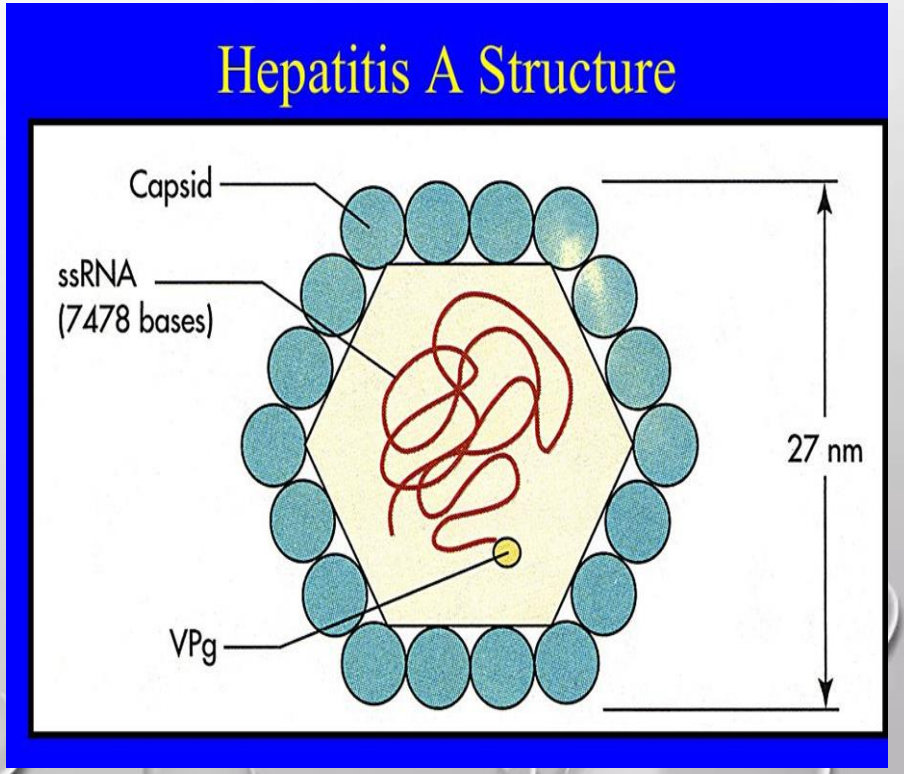
- Hepatitis A infection does not cause chronic liver disease and is rarely fatal, but it can cause debilitating symptoms.

HEPATITIS A VIRUS -HAV

- belongs to the family **PICORNAVIRIDAE**.
- GENUS: *Hepatovirus*.

MORPHOLOGY

- Size: 27-32nm
- Shape: spherical particles with icosahedral symmetry-with linear ssRNA.
- Has one serotype
- Does not cross-react with other hepatitis viruses.
- Genotypes: about seven are known.



Epidemiology

- Most common Viral Hepatitis
- Source of Infection: **patients**.
- Route of spread : Faeco-oral
- Patient remains infectious 2 weeks prior to & for upto 1 week after onset of illness
- Overcrowding & Poor Sanitation
- No carrier state

CLINICAL MANIFESTATION

- Incubation period: 15 to 45 days (mean 30)
- Onset is subacute.

Characteristics of infection

-**Pre-icteric phase**: mainly GIT symptoms such as nausea and vomiting

-**Icteric phase or jaundice**: dark urine, yellowish sclera and mucus membrane.

- **Complete recovery**: occurs in 98% cases.
- No chronic or carrier cases.

LABORATORY DIAGNOSIS OF HAV

- **Specimen:** Stool and serum**.**

- **Direct demonstration**: virus can be visualized by immunoelectron microscopy (IEM) in fecal extracts during late incubation period and in the preicteric phase.

- **Serology**: diagnosis is usually by antibody demonstration.

- **Anti HAV antibody detection by ELISA.**

-Appearance of IgM during acute phase

- Liver enzymes elevated after 2 weeks- rise in IgM abs

-IgM disappears within 3-6 months

-IgG antibodies appear after a week after IgM appears, it persists for years.

Interpretation

- Anti-HAV IgM positive: indicates acute infection with HAV.
- Anti-HAV IgG antibody detection-in absence of IgM: indicates past infection or recovery.

- **Detection of HAV particles**

-HAV appears in stool from -2 to +2 weeks of jaundice.

-HAV is also detected in liver, bile and blood by immunoelectron microscopy.

- **HAV antigen detection**

-ELISA is used to detect HAV antigen from stool sample from -2 to +2 weeks of jaundice.

Isolation

- HAV is difficult to grow in cell line.
- Non-specific findings: elevated liver enzymes and serum bilirubin level.

TREATMENT

- No specific antiviral drug available.

Prevention

- Improving hygiene

-Hand hygiene before and after use of toilet.

- Sanitary disposal of infected faecal material b (disinfection with 0.5% hypochlorite).

-Purification of drinking water by effective filtration and adequate chlorination with 1 mg/l chlorine.

-Use of boiled water during outbreaks.

VACCINES

- **Formaldehyde inactivated vaccine**:

-Prepared from human fetal lung fibroblast cell lines such as MRC5 and WI 38.

-Given to children after 12 months of age.

-Single dose given intramuscularly

-booster dose: given at 6-12 months gap.

- **Live attenuated vaccine**

-Is a single dose given subcutaneously

- Uses H2 and L-A-1 strains of HAV
- Prepaid in human diploid cell line

- **Human immunoglobulin (HAV-Ig)**

-Used for post-exposure prophylaxis of intimate contacts of persons with HAV or to travellers.

Dosage: 0.02ml/kg- gives protection for 1-2 months.

HEPATITIS B

DNA virus

Family- Hepadnaviridae

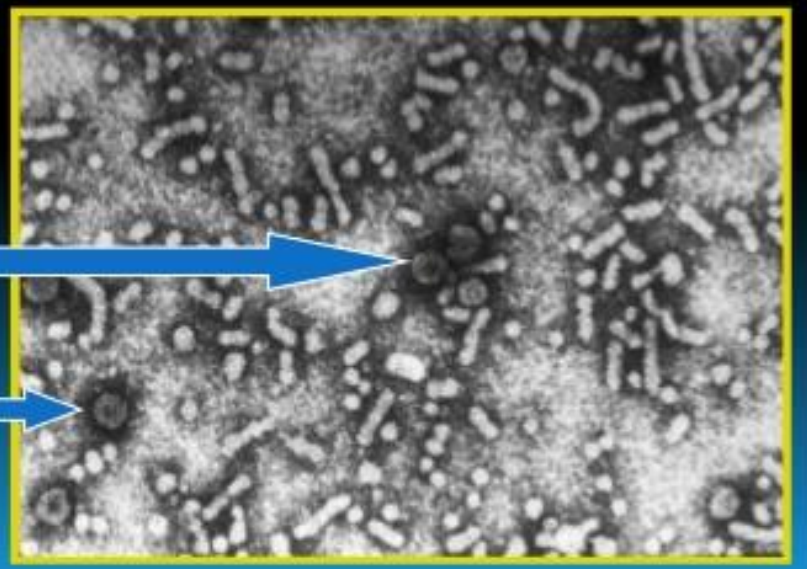
Genus – Orthohepadnavirus

Hepatitis B virus is 30-42 nm in diameter.

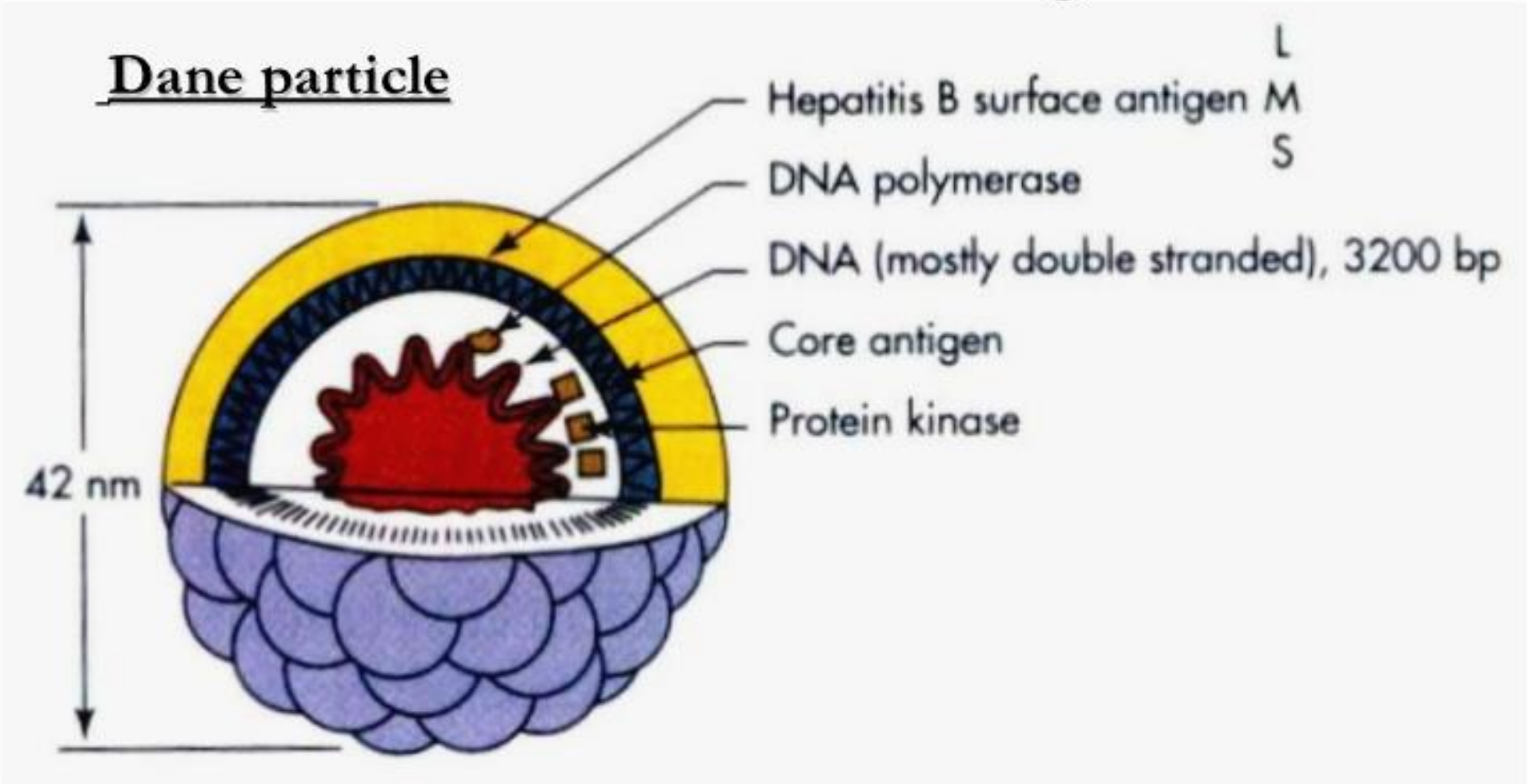
Hepatitis B virus (HBV)

- DNA virus (hepadnavirus).
- Complete virus particle is named Dane-particle,
- Genome is composed of incomplete double-stranded DNA.

Dane's particle



HBV Structure & Antigens



HBsAg = surface (coat) protein (**4 phenotypes : adw, adr, ayw and ayr)**
HBcAg = inner core protein (**a single serotype)**
HBeAg = secreted protein; function unknown

Route of transmission

Body fluids contain viral particles

- Semen
- vaginal secretions
- Blood
- Saliva

Route of transmission

- HORIZONTAL TRANSMISSION
  - Parenteral: Blood & blood products

    Injections

    Acupuncture needles
  - Sexual
- VERTICAL TRANSMISSION
  - Hbs Ag – positive mothers

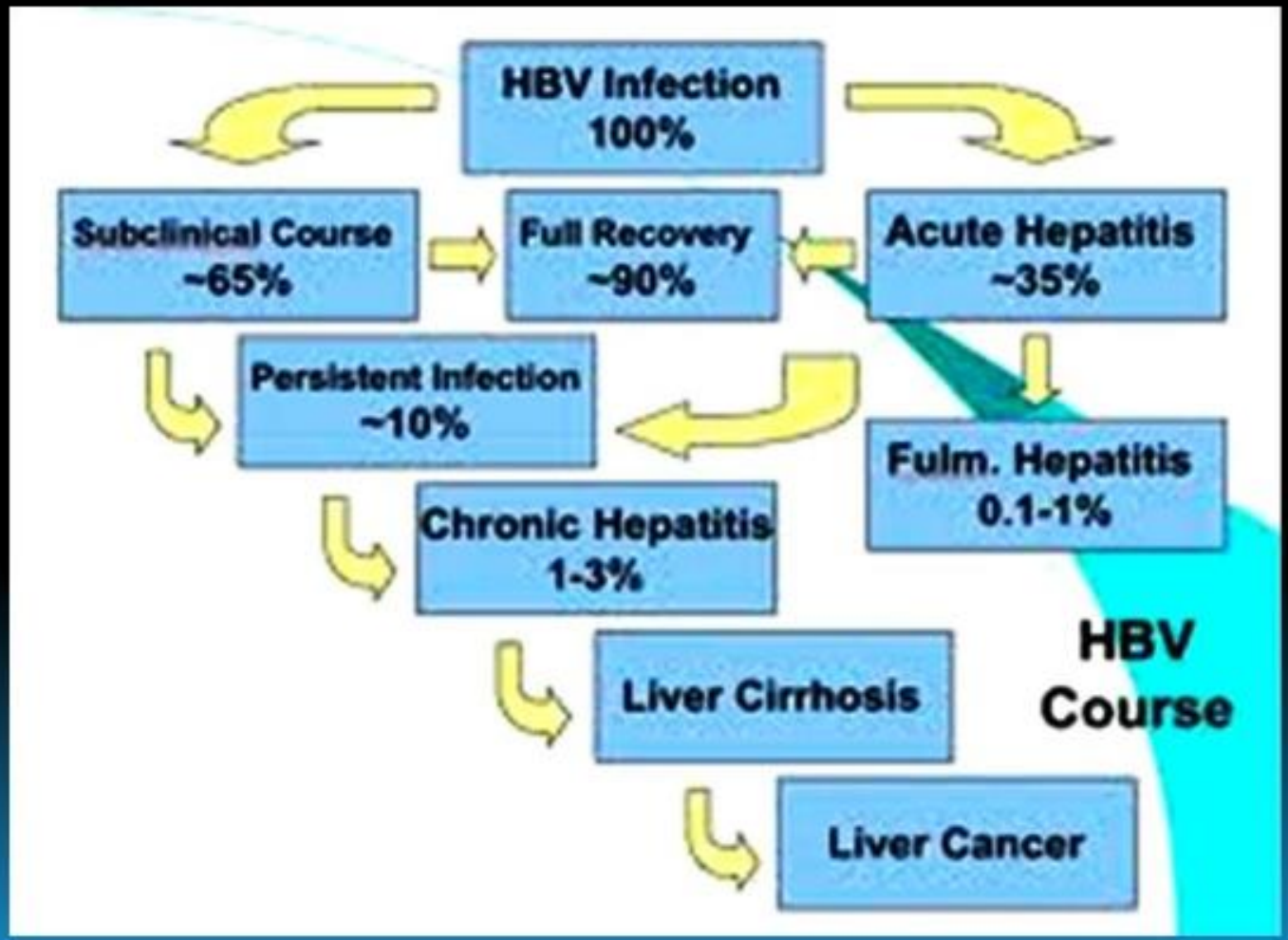
Risk Groups

- Multiple sex partners
- IV drug abusers
- Hemodialysis patients
- Patients requiring repeated blood transfusions
  - Hemophilia
  - Thalassemia
- Health care workers

Factors associated with the severity of hepatitis

- Infecting dose
  - Higher the dose of HBV, shorter is incubation period and more severe hepatitis.
- Age
  - Young age: mild initial hepatitis & more chance of chronicity.
- Immunological status
  - Immunological impaired hosts: Milder initial disease.

CLINICAL COURSE



ACUTE ICTERIC HEPATITIS

- Incubation period - 70 days (30 - 180 days);

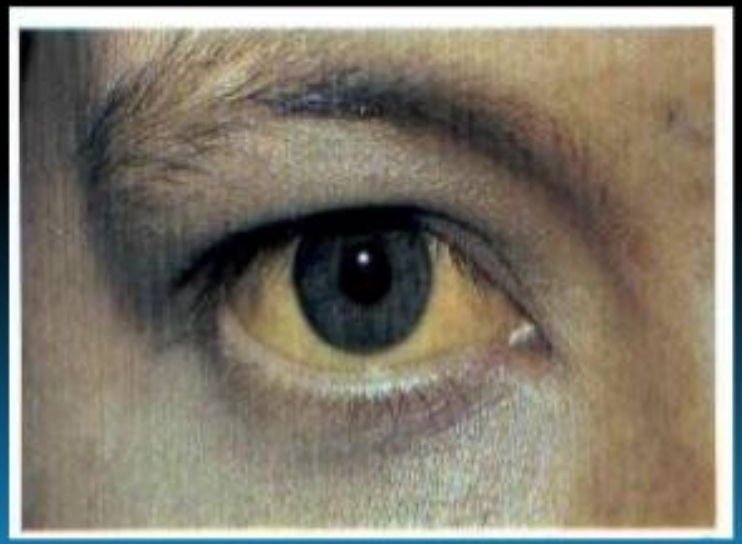
- Four clinical stages:
  - incubation period,
  - prodromal phase (pre-icteric phase),
  - icteric phase
  - convalescence.

Pre-icteric phase

- Symptoms are nonspecific;
  - Moderate fever
  - Headache
  - Malaise and weakness
  - Anorexia, nausea, vomiting
  - A vague, dull, right upper quadrant pain.

Icteric phase

- Clinical features of icteric phase:
  - Symptoms of pre-icteric phase being mild;
  - Jaundice (dark urine, skin and scleral icterus);
  - Stool light or clay colored;
  - Hepatomegaly
  - Liver function abnormalities
    - ALT and AST ⇧
    - Bilirubin (direct & indirect) ⇧



Acute anicteric hepatitis

---No jaundice otherwise similar to acute icteric hepatitis,.

---The symptoms are less severe than that in acute icteric hepatitis.

COMPLICATIONS

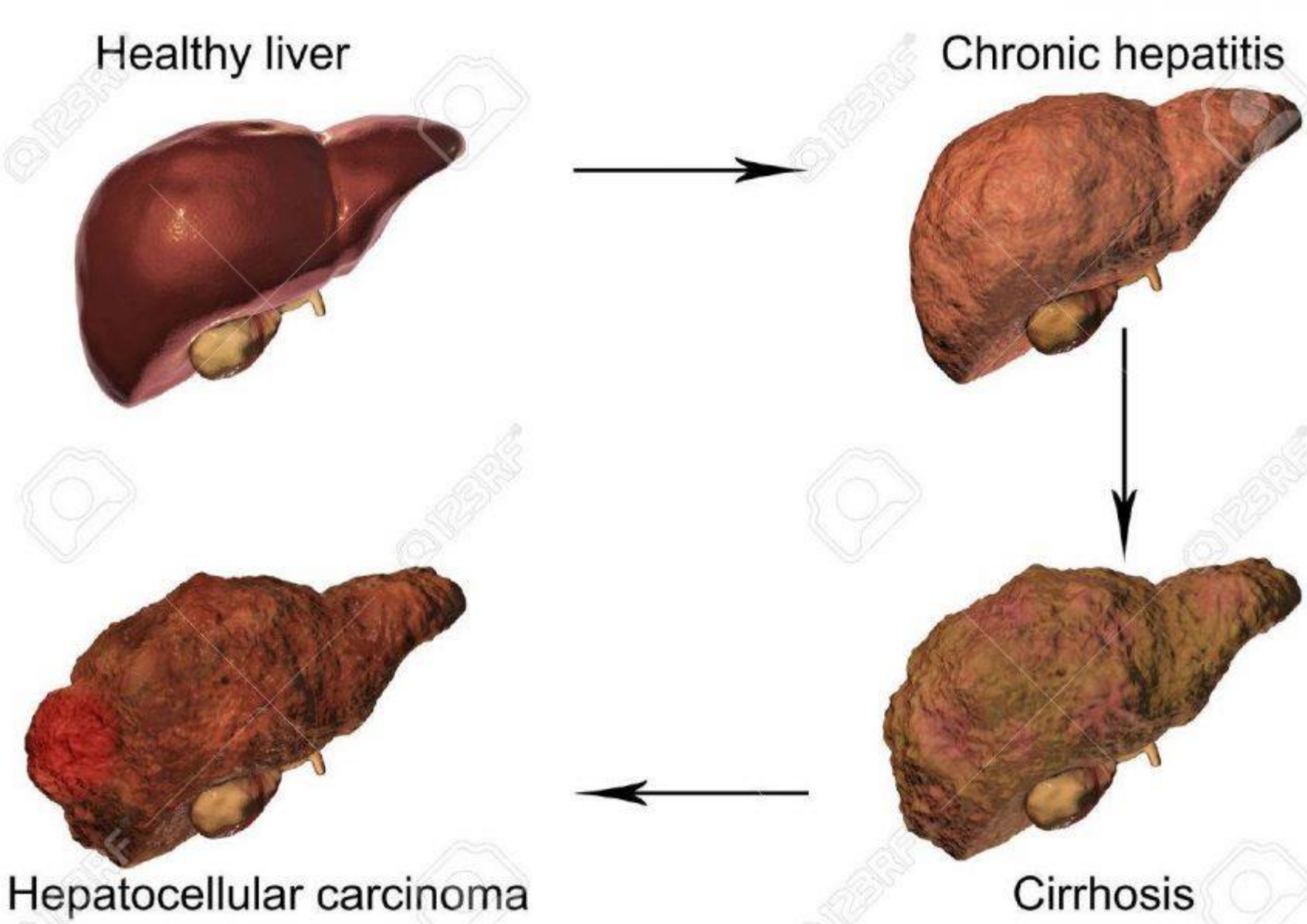
- Fulminant Hepatitis
- Chronic Hepatitis
- Rare complications:
  - Pancreatitis,
  - Myocarditis
  - Atypical pneumonia
  - Aplastic anemia
  - Transverse Myelitis

CHRONIC HEPATITIS

- Chronic viral hepatitis: Inflammatory disease of the liver > 6 months.

CLINICAL FEATURES

- Fatigue, anorexia, abdominal distension, diarrhea are common, but they are fluctuant.
- Hepatomegaly, splenomegaly, hepatic facies, liver palms, spider angioma can be seen.


Healthy liver
Chronic hepatitis
Cirrhosis
Hepatocellular carcinoma

DIAGNOSIS

SEROLOGY

- HBsAg
- Anti HBcAg IgM
- HBV DNA by PCR is most sensitive test

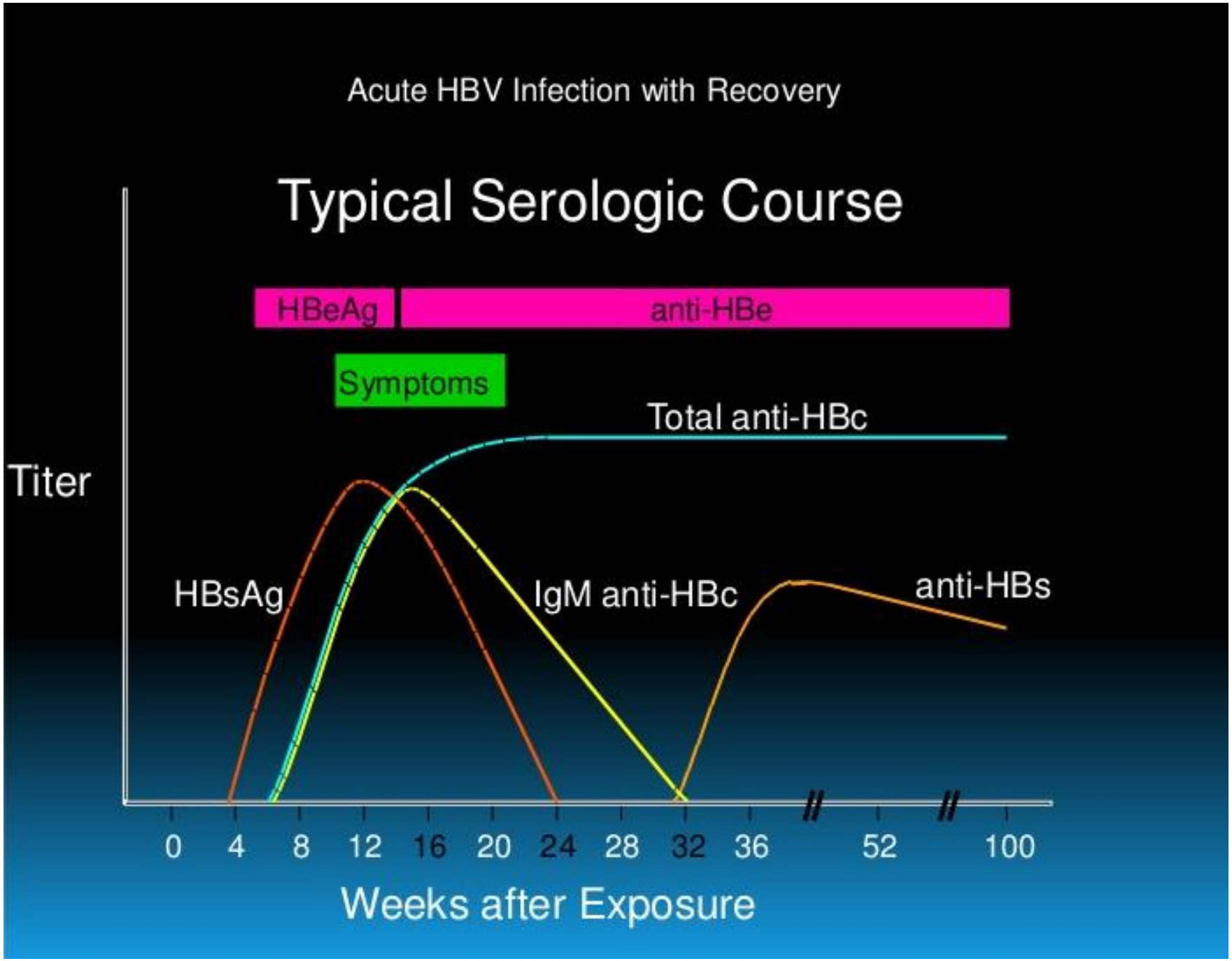
DIAGNOSIS

SEROLOGY

- HBsAg positive > 6 months
- Anti-HBc IgG in blood
- Serum HBV DNA > 10^5 copies/ml (20,000 IU/ml)
- HBeAg or Anti HBeAg may be present

Serological Markers of HBV

- **HBsAg**: Marker of infectivity
- **Anti-HBs**: Marker of immunity
- **HBcAg**: No commercial test available.
- **Anti-HBc**: Marker of past or current infection.
  - **IgM anti-HBc:** Recent infection.
  - **IgG anti-HBc**: Older infection.
- **HBeAg**: Marker of high degree of infectivity.
- **Anti-HBe**: May be present in infected or immune person.


Acute HBV Infection with Recovery
Typical Serologic Course
HBeAg
anti-HBe
Symptoms
Total anti-HBc
Titer
HBsAg
IgM anti-HBc
anti-HBs
0
4
8
12
16
20
24
28
32
36
52
100
Weeks after Exposure

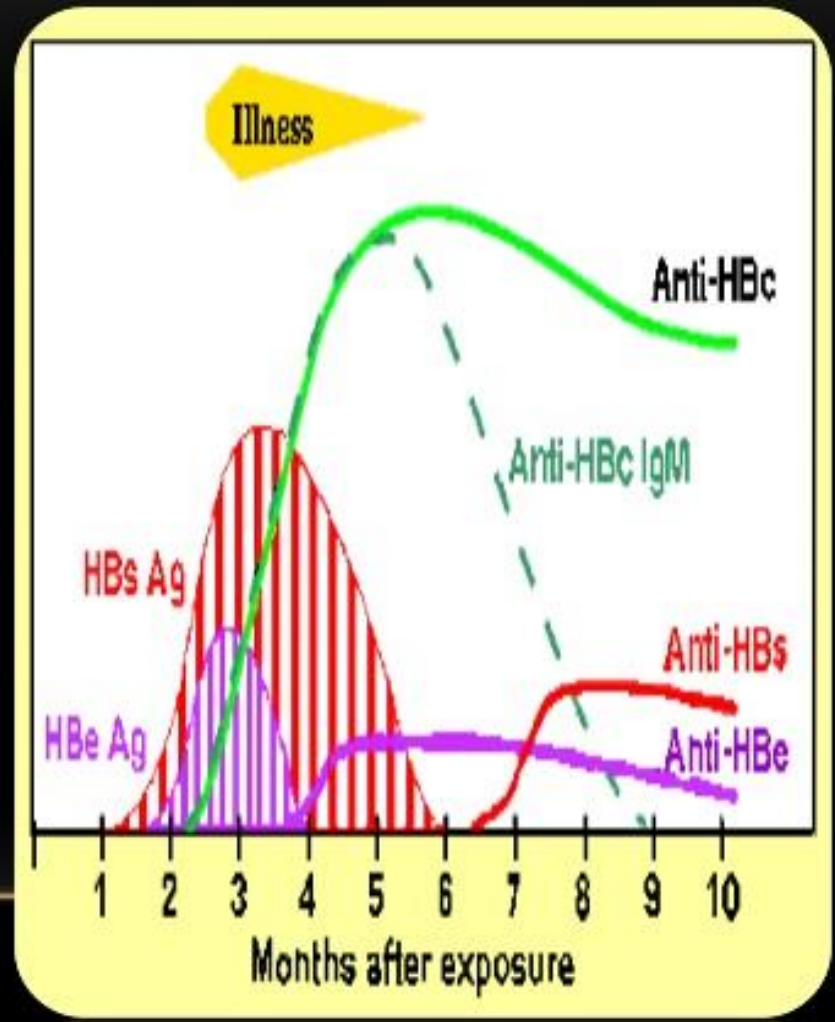
HBV Markers in different Stages:

Stage of infection	HBV DNA	HBsAg	HBeAg	Anti-HBc Ig M	Anti-HBc Total	Anti-HBe	Anti-HBs
Susceptible	–	–	–	–	–	–	–
Early incubation	+	–	–	–	–	–	–
Late incubation	+	+	+/–	–	–	–	–
Acute infection	+	+	+	+	–	–	–
Recent infection	–/+	–	–	+	+	+	+++

Stage of infection	HBV DNA	HBsAg	HBeAg	Anti-HBc Ig M	Anti-HBc Total	Anti-HBe	Anti-HBs
Remote infection	_ or very low	_	_	_	+	+/_	+
HBsAg-ve Acute infect	_	_	_	+	+	_	_
HBsAg variant infect.	_/+	_	_/+	+/_	+	_	_
Immune active carrier	++	+	_/+	_/+	+++	_	_
Healthy HBsAg carrier	_	+	_	_	+	+	_
Vaccination response	_	_	_	_	_	_	+

HBV – SEROLOGY INTERPRETATION

- **Acute infection**
  - HBsAg positive and anti-HBcAg IGM
  - Rarely, IgM anti-HBc only marker
    - Usually seen in acute fulminate Hep B
- **Chronic infection**
  - HBsAg positive and anti-HBcAg
- **Previous Infection**
  - HBsAg negative
  - anti-HBs positive
  - IgG anti-HBc positive



Treatment of Chronic Hepatitis B

- **DRUGS :** No specific therapy available
  - Interferon-alfa
    - IFN α → 6 month in Hbe +ve
    - Peg IFN α → 12 months in Hbe - ve
  - Lamivudine :100mg OD 48 weeks
  - Adefovir : 10 mg OD 48 weeks
  - Entecavir
  - Telbivudine
- **Liver Transplantation**

PREVENTION

- HBV Vaccine
  - DNA Recombinant vaccine
  - IM (deltoid but not gluteal)
  - 3 doses 0, 1, 6
  - Duration of protection – 5 to 10 yrs

❖ POST-EXPOSURE PROPHYLAXIS

- Combination of HBIG & HB vaccine (24 hrs)

❖ PERINATAL PROPHYLAXIS OF INFANTS

- HBIG 0.5ml IM in thigh immediately after birth
- Full course of HB vaccine started within 12 hrs of birth

Hepatitis C

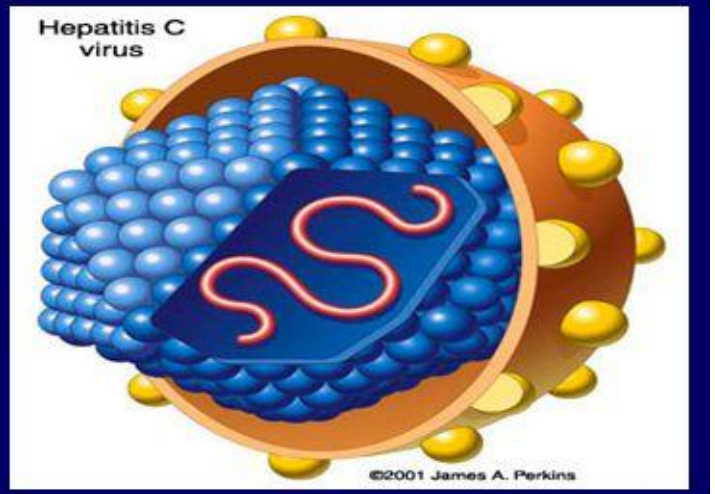
Hepatitis C virus: Classification & structure

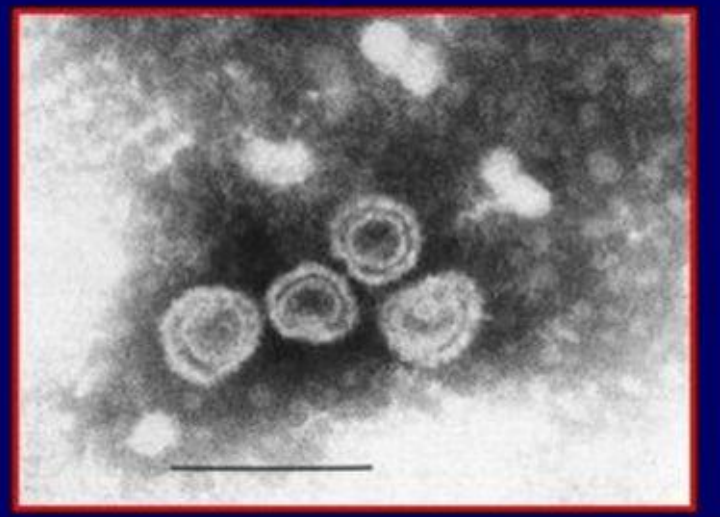
- Family: *Flaviviridae.*
- Genus: *hepacivirus.*

- The virus is small, 60 – 80 nm in diameter.

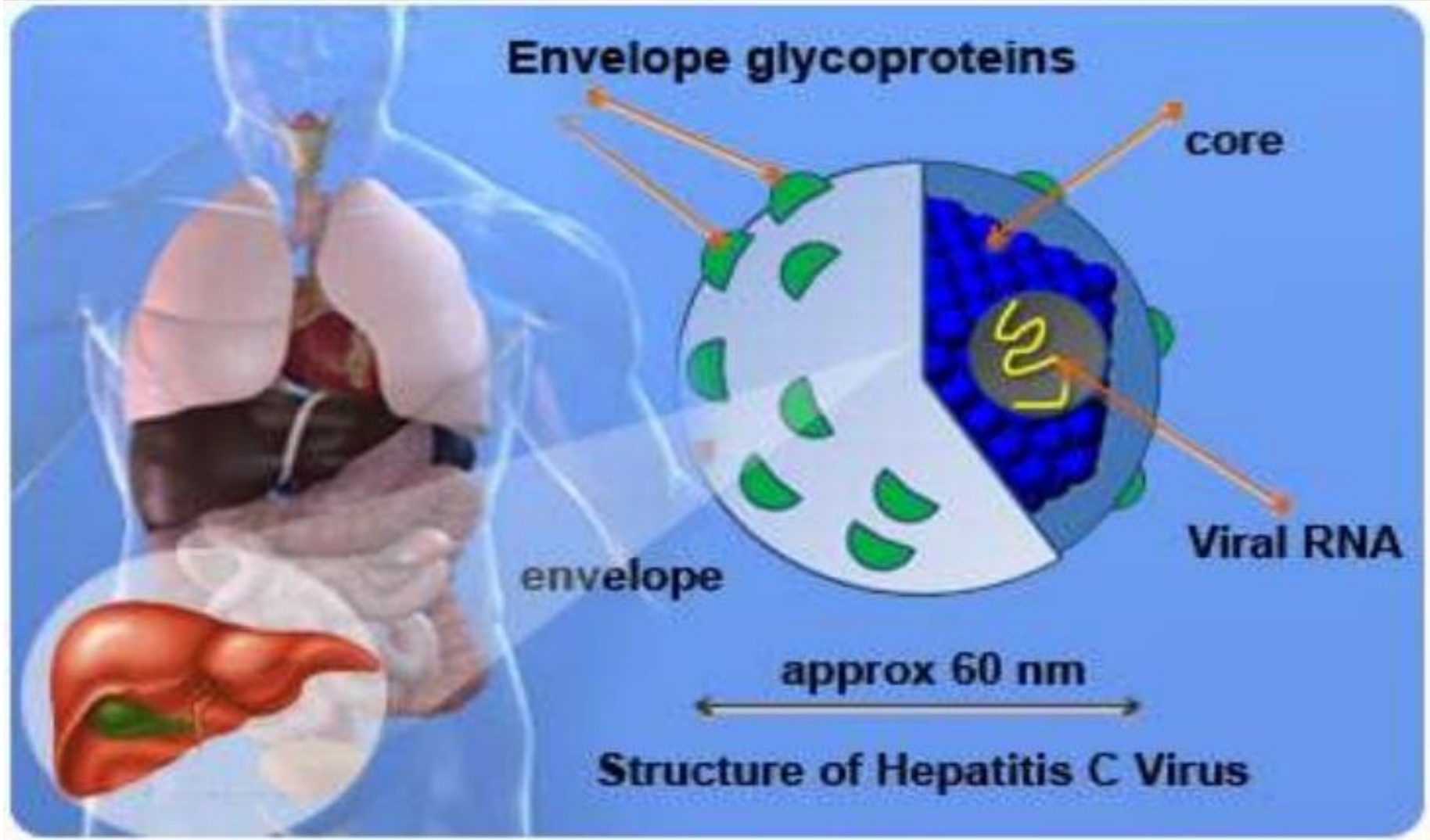
- Consists of an outer envelope, icosahedral core and linear positive polarity ss-RNA gemone.

- There are 6 major genotypes (1 – 6), genotype 4 is the dominant in Saudi patients.

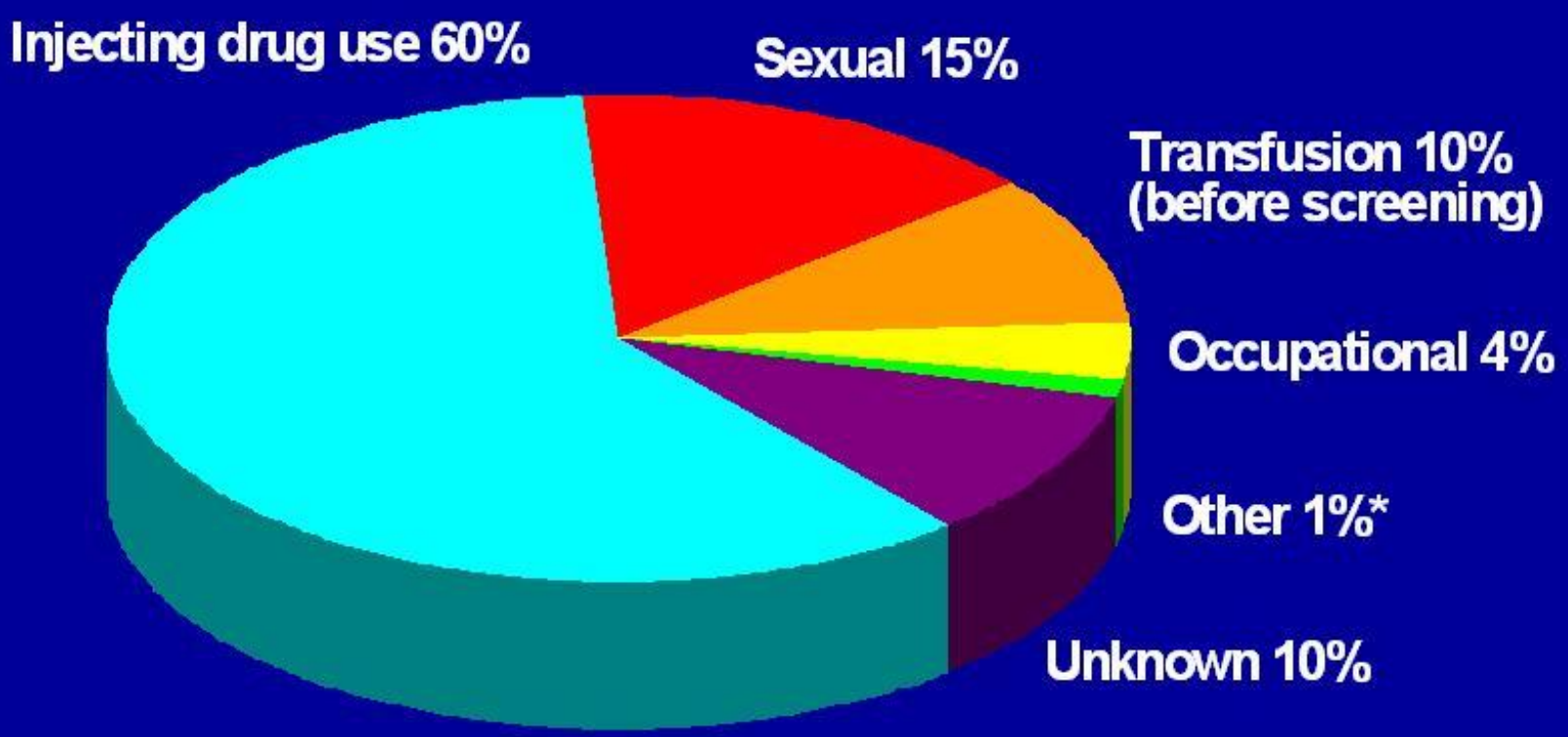




STRUCTURE OF HEPATITIS C



Routes of Transmission



* Nosocomial; iatrogenic; perinatal

CLINICAL FEATURES

- Incubation period: 50 days (15 – 150 days)
- 90% : Asymptomatic
- 10%: Mild flu like illness with jaundice & raised serum amino transferrases
- Extrahepatic Manifestation : Arthritis

  Glomerulonephritis

Clinical Course

- 85% : Chronic liver disease
- 15 – 20% : Cirrhosis in 10 – 30 yrs
- 7 – 15% of cirrhosis patient: Hepatocellular carcinoma

DIAGNOSIS

- HCV RNA detected in 1 – 2 weeks after infection
- Anti HCV is + ve 6 weeks after infection

Treatment for Chronic HC

- For CHC: IFN-α + Ribavirin 6 - 12 months

  Side Effects

  - Ribavirin: Hemolytic anemia
  - Interferon: Flu like symptoms

- Liver Tranplantation in Cirrhosis

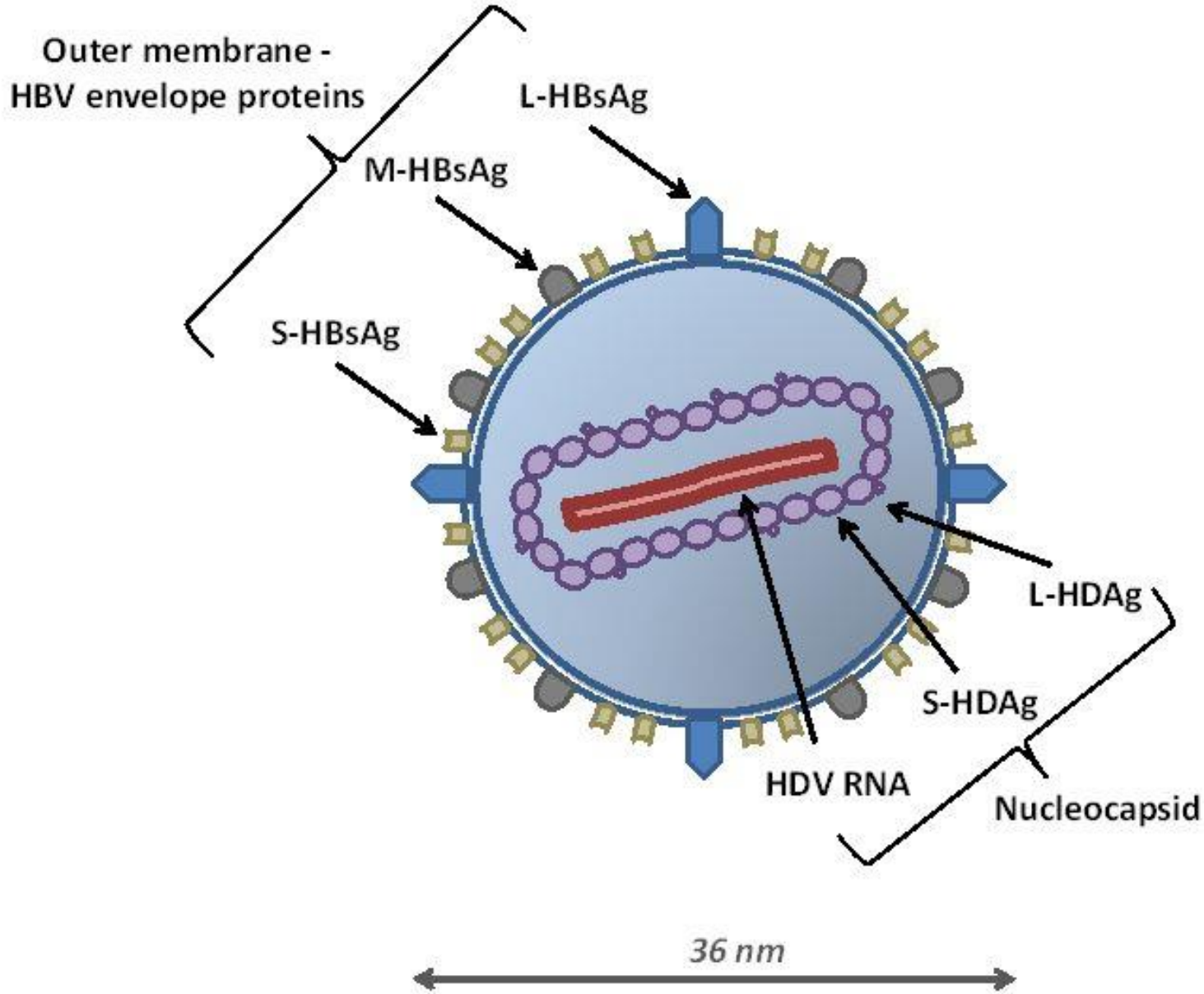
HEPATITIS D VIRUS

- Is a defective virus
- Cannot replicate by itself- depends on HBV for survival.

Morphology

- Resembles viroids
- Small size: 35nm
- Consists of circular, negative-sense ssRNA.
- Protein coat made of single protein-hepatitis d antigen (HDAg).
- Surrounded by envelope protein derived from HBsAg from HBV-hence called defective virus.

HDV virion


Outer membrane -
HBV envelope proteins
L-HBsAg
M-HBsAg
S-HBsAg
L-HDAg
S-HDAg
HDV RNA
Nucleocapsid
36 nm

TRANSMISSION

Parenteral route, sexual and vertical routes.

→**Co-infection:** occurs when person is exposed to serum containing both HDV and HBV.

→**Super-infection:** occurs when a chronic carrier of HBV is exposed to serum with HDV.

-Results in disease 30-50 days later with two phases

→Acute phase- HDV replicates actively with high transaminase levels with suppression of HBV.

→Chronic phase- HDV replication decreases, HBV replication increases, transaminase levels fluctuate, disease progresses to cirrhosis and hepatocellular carcinoma (HCC).

- Mortality rate higher (> 20%).

LABORATORY DIAGNOSIS

A. In co-infection: IgM against both HDAg and HBcAg are elevated

-IgM anti-HDV appears late and is short-lived.

B. In super-infection: HBV appears already as carrier, IgG anti-HBc is detected

-Anti-HDV would be IgM type initially

-In chronic state: mixture of IgM and igg persists for months or longer.

C. Anti HBc antibody differentiates between co-infection and super-infection.

-**IgM anti HBc + IgM anti-HDV**: indicates co-infection.

-**IgG anti HBc + mixture of IgM and IgG anti-HDV**: indicates super-infection.

- HDV RNA is detected in blood and liver before and early days of acute phase of both infections.

Epidemiology

- Globally- 15 million people are infected with HDV- where 5% of 350 million are infected with HBV.
- Infection occurs world wide.

Treatment

-IFN-a

Prevention

HBV vaccination and prophylaxis measures.

HEPATITIS E VIRUS (HEV)

- Causes an enterically transmitted hepatitis- in young adults.

Morphology

- Resembles caliciviruses.
- Size: small (30-32 nm), non-enveloped with icosahedral symmetry.
- Contains positive-sense, ssRNA and a specific antigen (HEV-Ag).

Genotypes

- HEV has a single serotype, five genotypes.
- Genotypes: -four are detected in humans.
  - Type 1 and 2 appear to be more virulent.
  - Type 3 and 4 are more attenuated and account for subclinical infections.

CLINICAL MANIFESTATION

- **Incubation period**: 14-60 days.
- Mostly present as self-limiting acute hepatitis lasting for several weeks.
- Fulminant hepatitis- occurs in 1-2% of cases; except for pregnant women at higher risk (20%).
- No chronic infection or carrier state.

EPIDEMIOLOGY

- HEV is a **zoonotic** disease- affects monkeys, cats, pigs and dogs.
- **Transmission:** faecal-oral route via sewage contamination of drinking water or food.
- **Epidemics:** most common in India, Asia, Africa and Central America.
- **Age:** young adults (20-40 years).

LABORATORY DIAGNOSIS

- Specimens: stool, serum.
- HEV RNA is detected by reverse transcriptase PCR.
- HEV virions- detected by electron microscopy.

- **Serum antibody detection-** by ELISA.

→IgM anti-HEV: appears in serum same time with appearance of liver enzymes- indicates **acute infection**.

→ Igg anti-HEV: replace IgM in 2-4 weeks- indicates recovery or past infection.

TREATMENT

- No specific antiviral drugs available.

Prevention

- Same prevention measures like for HAV.

HEPATITIS G VIRUS (HGV)

- Also referred as GB virus C.
- Related to hepatitis c.
- Family: flaviviridae, genus: *Pegivirus*.
- Transmission: contaminated blood or blood products via sexual contact.
- Does not cause hepatitis- replicates in bone marrow and spleen.
- Classified into 6 genotypes.
- Commonly co-infects people with HIV.