Hepatitis A
Hepatitis A
- The causative agent is hepatitis A virus (HAV)
- Earlier referred to as infectious hepatitis.
- Major public health problem, 1.4 million cases each year worldwide.
- In developing countries, pediatric disease; in developed nations, disease of adults.
- Prevalence of hepatitis A is inversely related to socioeconomic status.

Transmission
- Fecal-oral route.
- Person-to-person contact.
- Inadequately cooked food.
- Blood-products-associated transmission noted in Europe.

Disease
Ninety percent infections in children lead to subclinical presentation. Proportion of clinical cases increases with age. Fulminant hepatic failure is a rare serious complication. Does not lead to chronicity.

The virus
- Picornaviridae, genus Hepatovirus.
- 27-30 nm diameter particles.
- Plus-stranded 7.5 Kb RNA genome.
- Seven genotypes / Single serotype.
- HAV grows slowly in cell culture without cytopathic effect.
- Available animal models: Non human primates.

Laboratory diagnosis
- IgM-anti-HAV is the diagnostic marker for hepatitis A.
- At NIV, Pune, IgM anti HAV capture ELISA and Blocking ELISA tests have been developed and are routinely used to provide diagnosis of recent and past infection of hepatitis A virus.
- ELISA based hepatitis A diagnostic technology has been transferred to Hyderabad based industry. This is the first such transfer from NIV, Pune.
- Urine was found to be an alternative test specimen for diagnosis of hepatitis A, useful especially in children.
- Blood collection on filter paper disc proved to be satisfactory for anti-HAV detection.

Studies in Pune (1982-98)
The disease is hyper endemic and the pattern of HAV infection in urban lower middle and rural lower middle classes has remained unchanged. Better hygiene and non-exposure to HAV in higher middle and upper class young population renders them susceptible. Thus the possibility of HAV epidemic amongst them exists. Hepatitis A appears as an emerging infection in adults. Substantial increase in number of hepatitis A cases in higher age group from high socio-economic status has been recorded.

Epidemiology
In India, Hepatitis A virus is the main causative agent of acute hepatitis in children. It accounts for significant number of FHF cases in children.
Prolonged fecal excretion and viremia observed in Indian patients and experimental monkey.

Several animal species shown to be anti-HAV positive.

**Genotyping of HAV strains in sporadic cases from Pune**

Co-circulation of subgenotypes IB and IIIA with predominance of IIIA detected. Mixed infection with IB and IIIA identified in individual hepatitis A patients.

**Emergence of epidemic hepatitis A**

An outbreak of hepatitis A in day-care centers for children in Pune emphasizes importance of such centers in the transmission of HAV. From 2003, hepatitis A is assuming epidemic proportions, especially in rural and semi-urban settings. Source remains contaminated water supply.

Thirty percent HAV RNA positivity in neat sewage samples documents extremely high viral load in the environment. This also represents a potential threat for contamination of drinking water leading to endemicity and extensive outbreaks.
Vaccines
- Both killed and attenuated hepatitis A vaccines are available. Most countries do not have definite policies for hepatitis A vaccination.
- NIV studies suggested that 9 months is the appropriate age for hepatitis A vaccination.
- Isolated an Indian strain of HAV in tissue culture and transferred to industry for vaccine preparation.

Prevention
- Supply of safe potable water.
- High standards of public and personal hygiene.
- Education of food handlers.
- Vaccination of high-risk individuals:
  - Children from high socio-economic status.
  - Young food handlers.
  - Siblings of hepatitis A patients.
  - HBV/HCV carrier children.

Patent
A patent on “Novel process of Hepatitis A vaccine preparation” has been filed.
Hepatitis B

- The causative agent is hepatitis B virus (HBV).
- Earlier referred as Serum Hepatitis.
- 350 million carriers of the virus worldwide.
- 34 million HBsAg carriers in India.
- 200-fold higher risk of primary hepatocellular carcinoma.
- Wide spectrum of clinical presentations.
- Several transmission modes.
- Efficacious vaccine available since 1982.

Infection with HBV may lead to

- Subclinical infection.
- Acute self-resolving hepatitis.
- Fulminant hepatitis.
- Asymptomatic carrier state.
- Chronic hepatitis.
- Cirrhosis.
- Hepatocellular carcinoma.

The risk factors

- Transfusion of unscreened blood.
- Improperly sterilized syringes / needles / dental / other equipments.
- Close contact with HBV positive individual.
- Shared razors, toothbrushes with carrier.
- Tattooing.
- Sexual contact with HBV positive individual.
- Multiple sex partners.
- Infected mother to infant.

Transmitting routes

- Parenteral
- Inapparent parenteral
- Sexual
- Vertical
- Horizontal

Hepadnavirus

Electron micrograph of 42 nm HBV from a blood donor in Pune

Transmission routes

Hepatitis B vaccination introduced for tribals of Andaman and Nicobar Islands

Hepatitis B prevalence in Pune was determined according to socio-economic status viz. lower middle class status (LMS) and higher socio-economic status (HS).

HBV Epidemiology in and around Pune (1982-1998)

Based on transmission modes, several high-risk groups have been identified.

These high risk individuals must be considered for vaccination

Cultural practices make tribals a high-risk category
HBV Pre-Core mutant and clinical presentation

Asymptomatic HBsAg Carriers  
- Chronic Hepatitis B
- Acute Hepatitis B
- Fulminant Hepatitis B

- Risk of Chronic hepatitis 4.3-fold higher with HBV pre-C wild
- 3.2 fold higher chances of asymptomatic carrier state with pre-C mutants
- Pre-C mutant not associated with fulminant hepatitis

HBV Genotypes

- Genotype D predominant in western India.
- Did not influence outcome of HBV infection.
- Genotype D highly prevalent in tribes from Andaman and Nicobar islands

Diagnostics

As early as 1980, highly sensitive and specific ELISA for the detection of HBsAg was developed at NIV. This represents the first indigenously developed HBsAg ELISA in India. These ELISA reagents were certified by WHO.

In 1981, again for the first time, ELISA for the detection of anti-HBs antibodies was developed.

- To assess the presence of replicating virus, PCR for the detection of HBV DNA was standardized in 1990.
- An important development was standardization of Quantitative Real Time HBV DNA PCR assay employing primers and probes designed at NIV.

HBV DNA quantitation is a national facility available at NIV

Assessment of screening tests / vaccines

- Evaluation of commercially available assays as part of WHO-SEARO designated national reference center.
- From 1985, worked closely with blood banks from Pune in verifying efficiency of the tests used for donor screening.
- Immune response to several plasma-derived and recombinant vaccines evaluated in Indian population.
- Follow up of vaccinated persons for 14 years.

Delta Rn vs Cycle No HBV Standard

Cycle Number

HBV Genotypes

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Creation of awareness about hepatitis B

- Children from several urban and rural schools.
- Family contacts of HBsAg carriers.
- Several family contacts immunized
- Several HBsAg carriers counselled at a young age
- Immunized would-be spouses before marriage
- Immunized children at birth, born to HBsAg positive mothers

Based on NIV results immunization of dental students and dentists was made mandatory in Maharashtra

Orphans screened for HBsAg before adoption

One orphan selected by needy parents and born to HBsAg carrier mother was vaccinated, followed for seroconversion and finally adopted

Hepatitis C
Hepatitis C
- Earlier known as post-transfusion non-A-non B hepatitis (PT-NANB).
- 170 million hepatitis C virus (HCV) infected individuals worldwide.

The disease
- Mostly subclinical.
- High chronicity potential (>70%).
- 50-70% of chronically infected individuals develop chronic liver disease.
- Not a major cause of acute or fulminant hepatitis.
- Important cause for primary hepatocellular carcinoma.

The virus
Belongs to family Flaviviridae; positive sense, ssRNA genome (~9.4 kb). Classified into 6 genotypes.

Diagnosis
- No marker distinguishes between acute and chronic infections.
- Detection of anti-HCV antibodies by 3rd generation screening ELISAs.
- At present, no confirmatory immunoassays are obligatory.
- Presence of HCV RNA by nested RT PCR employing primers from 5’NTR.
- Nested RT-PCR assay (1990) was standardized at NIV. Screening of clinical samples from all over India for HCV RNA is ongoing.
- Quantitation of HCV viral load is a very important parameter in disease staging and response to antiviral therapy. Quantitative real time PCR-based assay was standardized in 2003 on the basis of viral genotypes circulating in India.
- First, 2nd and 3rd generation ELISAs evaluated in Indian population immediately after availability in the market.
- At present, several host and viral factors are being investigated for chronicity potential and success of interferon therapy.

Experimental transmission
- So far, chimpanzee is the only animal model for HCV.
- No convenient cell-culture system is available.
- At NIV, attempts to infect rhesus monkeys and insect cell lines susceptible for other flaviviruses, were not successful.

Genotypes
Samples from 149 HCV RNA positive patients from different parts of India were genotyped on the basis of phylogenetic analysis.

Transmission
- Parenteral transmission is the important mode.
- Other modes like sexual, vertical and intrafamilial are infrequent.
- In dialysis units, a new patient usually gets infected with HCV within six months, mainly through nosocomial spread.

Epidemiology
- Anti-HCV prevalence among age stratified general population is low.
- Prevalence was low in rural and tribal populations.
- Anti-HCV positivity in commercial blood donors was high.
- Dialysis patients and hemophiliacs are at higher risk of getting infection.
- Blood donors from a commercial plasmapheresis unit had shown 90% anti-HCV positivity, most antibody positives being HCV RNA positive.

Blood products being imported in India are screened for HCV RNA at NIV.

A new subtype of HCV, 3i, first identified in western India, was later found in other parts of the country.

HCV Genome Organization

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Structural proteins
Nonstructural proteins

Electron Micrograph of HCV

Death Rn vs Cycle

Delta Rn vs Cycle

Commercial blood donors

Percentage

Year

1982
1983
1986

14
12
10
8
6
4
2
0

WEST
NORTH
SOUTH
EAST

52.11%
63.33%
25.00%
75.00%

2.82%
3.33%
38.89%
25.00%

45.07%
33.33%
38.89%
25.00%

Genotype 1
Genotype 2
Genotype 3
Genotype 4
Nontypable

Genotype 1
Genotype 2
Genotype 3
Genotype 4
Nontypable

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**Other studies**

**Association with blood banks**
As serological tests for HCV were still evolving (1st, 2nd & 3rd generations), the NIV together with local blood banks sorted out the problems of specificity and sensitivity, based on the use of confirmatory Recombinant Immunoblot Assay (RIBA) and PCR tests.

**Interferon treatment**
A national facility for the detection of HCV RNA, employing nested RT PCR was set up in July 1995. Samples from different parts of India were screened as a prerequisite for the initiation of interferon therapy as well for the assessment of success of therapy.

**ICMR’s multi-centric trial for combination therapy**
NIV is responsible for all the virological aspects of this trial, including detection and quantitation of HCV RNA and sequencing-based genotyping.

**Expression of recombinant core antigen**
Highly immunoreactive recombinant HCV core antigen was expressed in baculovirus system.

**Prevention**
In the absence of possibility of vaccine for hepatitis C in the near future, strict adherence to universal precautions in relation to parenteral transmission mode seems the best available choice.
The disease
Acute, self-limiting; occasionally leads to fulminant hepatitis. No chronicity recorded. Usually affects young adults; high mortality among pregnant women, especially in the third trimester.

Diagnosis
Detection of IgM-anti-HEV by ELISA. In very early acute cases, IgM antibodies may not be detectable, HEV RNA by nested RT PCR may be the method of choice.

Genotypes
HEV strains are classified into 4

Indian strains:
All human strains - genotype 1
All swine strains - genotype 4

Epidemiology
The disease was believed to be restricted to developing countries wherein both epidemic as well as sporadic forms exist. However, recent studies have documented hepatitis E among persons from several developed countries without any history of travel to endemic countries.

Studies conducted at NIV and in different countries have shown that HEV has predilection for young adults. However, an outbreak of hepatitis E among residential school children at Talegaon, India (1988) was recorded. Moreover, ~ 70% anti-HEV positivity among children from Shompen tribe from Andaman and Nicobar Islands was also documented.

Hepatitis E is the major cause of epidemic hepatitis in India. NIV has investigated over 100 outbreaks of hepatitis E.
Initial studies conducted by NIV showed that ~ 60% of the sporadic hepatitis cases among adults are of NANB type. With the availability of ELISA for IgM-anti-HEV detection, over 40% of sporadic cases among adults were diagnosed as hepatitis E.

Though high mortality in pregnant women is the characteristic feature of hepatitis E, in sporadic situation, non-pregnant women as well as men were shown to succumb to fulminant hepatitis E.

During epidemics, the estimated ratio of clinical:subclinical infections based on serology was shown to be 1:26 in pregnant women.

**Transmission**

**Water contamination**

Leaky water/drainage pipelines running in close proximity or other means of contamination at the source of water reservoirs results in explosive outbreaks.

**Parenteral transmission**

- 1.5% (3/200) voluntary blood donors from Pune positive for HEV RNA.
- 2/37 anti-HEV negative transfusion recipients sero-converted, 4 and 5 weeks post-transfusion.
- Transfusion associated hepatitis E may occur in countries endemic for HEV.

**Intra-familial spread**

Based on the study of 49 families during an epidemic of hepatitis E, the intra-familial spread was shown to be negligible.

**Risk factors**

Multivariate analysis showed that age > 15 yrs (5.7 fold), lower middle socioeconomic status (2.4 fold) and well water usage (1.9 fold) are important risk factors for contracting infection.

About 15% of neat sewage samples collected year round were HEV RNA positive, 7-fold higher risk of infection in sewage workers was documented.

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HEV as zoonosis

- In 1994, wild caught rhesus (36.7%) and bonnet (19.1%) monkeys from India were shown to be anti-HEV positive.
- In 1997, swine HEV was discovered in the US and strong possibility of zoonotic spread to humans was suspected. Wherever examined, all countries showed anti-HEV positivity in pigs.
- In 2001, anti-HEV antibodies were detected in pigs, dogs, cattle, rodents, and chickens from India. Goats were negative.

**Swine HEV**

Anti-HEV positivity documented in pigs from states of Maharashtra, Karnataka and Andaman & Nicobar Islands. Mean age of pigs for seroconversion was 4.8 ± 1.6 months.

In contrast to other countries, different genotypes circulate in humans (genotype-I, 1976-2004) and pigs (genotype IV, 1985-2000) in India.

The complete genome of Indian swine HEV was sequenced.
Delta & other Hepatitis Viruses
Hepatitis Delta Virus (HDV)

- Defective RNA virus.
- Requires HBV replication for its multiplication.
- Occurs as co- or super-infection with HBV.
- Leads to severe course of liver disease.
- Though HDV replication is HBV dependent, its prevalence is not a simple function of HBV prevalence.
- Parenterally transmitted.

- HDV is not an important cause of viral hepatitis in western India.
- In 1987, normal immunoglobulin preparations were shown to be anti-delta positive.

Hepatitis G Virus (HGV)

- Discovered in 1995.
- Disease potential yet to be confirmed.
- HGV does not contribute to sporadic Non-A, Non-E hepatitis in India.
- Not an important cause of chronic or fulminant hepatitis.
- The virus belongs to the family Flaviviridae.

TT virus

- Discovered in 1997 in Japan.
- Disease potential questionable.
- Parenteral and enteric modes of transmission.

The virus

DNA virus, belong to family Parvoviridae.

Phylogenetic analysis showed presence of genotypes 1, 1a and 2.
TTV DNA positivity

- Though high prevalence of TTV DNA was recorded, no disease association could be shown.
- Ten percent neat sewage samples were TTV DNA positive.
- Sewage treatment did not reduce TTV DNA positivity.

Non-A to E agents

- Despite use of sensitive and specific serological and molecular assays, all acute viral hepatitis cases cannot be diagnosed.
- Non-B, non-C chronic hepatitis cases continue to occur.
- An epidemic of enterically transmitted non-A, non-E hepatitis in a tribe of Andaman and Nicobar islands in December 1987 was investigated by NIV. Subsequently shown to be TTV DNA and HGV RNA negative. So far, no etiologic agent identified. Transmission experiments in rhesus monkeys were unsuccessful.