Hepatitis C Viruses

*You don't need to refer to the slides

HCV History

-1970s: many cases were labeled as non-A, non-B hepatitis.

→ In 1970s, Hepatitis was classified into 2 types:

1-**infectious hepatitis**

2-serous hepatitis: which was thought to be transmitted by blood & blood products only.

But this classification wasn't sufficient to include all cases of hepatitis.

For example, some cases of hepatitis were acquired by **parenteral** route weren't caused by Hepatitis B and other cases that were acquired by the **oral-fecal** route weren't Hepatitis A. Those cases were grouped together into **non-A,non-B hepatitis**.

Most of the non-A,non-B hepatitis category that were by **parenteral** route, turned out to be **Hepatitis C**.

Most of the non-A,non-B hepatitis category that were by **oral-fecal** route, turned out to be **Hepatitis E**.

Still, there were cases of non-A,non-B hepatitis that weren't caused by hepatitis C, and thought to be caused by hepatitis GB and other types of hepatitis.

- 1988: hepatitis C virus was first isolated & identified (very recent)

-1990: antibody screening tests were available

They weren't very sensitive to be wildly utilized to screen blood for hepatitis C

-1992: better tests to insure safety of blood supply

The first acceptable tests by FDA standards were used to screen blood. Since then, the kits were improved in terms of specificity & sensitivity to detect more cases, and we have now the 3rd generation of these tests which detect almost all cases of hepatitis C, by inclusion more antigens of the virus in the kit.Because in the beginning the kits didn't include all the antigens and few antigens were included later on. *(Few antigens=non-structural proteins; this will be explained later in the lecture)*

So, the screening for hepatitis C in donated blood started in 1992 -just recently-, that's why those, who were acquired infection in the 70s, 80s and early 90s because there was no method to screen the transported blood or the kits weren't so sensitive at that time, are still having chronic hepatitis C infection and didn't die of hepatocellular carcinoma if they are still alive.

-1994: the oldest blood sample stored since 1948 contain HCV antibodies (earliest sample known).

The virus is present long ago before that sample but this is the oldest sample known for the virus.

-Hepatitis C is a member of Flaviviridae family, and is classified in the genus Hepacivirus (which consist of HCV and GBV-C).

These two viruses are the only viruses of Flaviviridae that aren't transmitted by arthropods, all other Flaviviridae are transmitted by arthropods.

-Hepatitis C is an enveloped virus

-The virus is 37-60 nm in diameter (wide range of size)

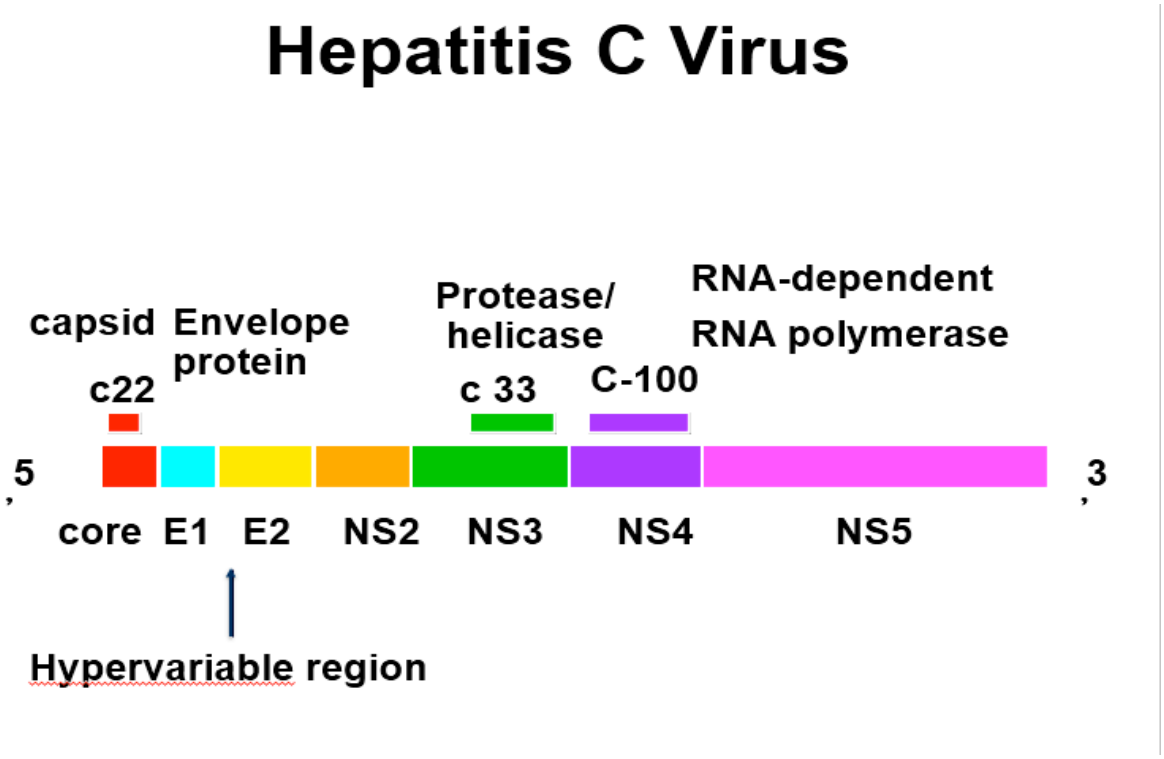
-It has + ss RNA genome (A single stranded RNA genome with a positive polarity) of **9.4 kb length** (genome has 9400 nucleotides).

- The genome comprises a single large ORF (OPEN READING FRAME)

This means that the genome after being translated forms a poly-protein that later being cut into individual proteins which are **9 in number (3 are structural & 6 are non-structural proteins).**

Those 6 non-structural proteins weren't included in the kits at the beginning, that's why those kits weren't so sensitive for the virus and couldn't detect every case, but with time and improvement the non-structural proteins were included.

- Over 98% of the genome contains protein coding sequence.



-The 3 structural proteins are:

1- Core protein (capsid protein) (C22)

2&3 are Envelope proteins E1 (gp76) and E2 (gp35).

This is the most variable part of the genome and the hyper-variability exists in the E1 & E2 region which codes for the envelope proteins, this region is the target for naturalizing antibodies.

HCV is capable of antigenic change because of the variation in this sequence. HCV has 6 genotypes & several subtypes because of these variations.

In a single infection, HCV changes itself frequently like HIV and this creates what is called **Quasi-Species** of the virus, and that's why HCV is capable of establishing chronicity because the host immune response is overwhelmed by the ability of HCV to change its structure. So the immune response which is created against the virus clears virus with a certain structure but the structure changes and new immune response generates and so on.

That's also why we don't have vaccines because HCV keeps changing itself.

-The 6 nonstructural proteins are: NS2, NS3, NS4A, NS4B, NS5A, and NS5B.

-HCV has circumvented the cap requirement by evolving an Internal Ribosome Entry Site (IRES) at its 5' end.

Explanation:

The virus genome is a +ssRNA that can act as mRNA and then is translated into a poly-protein which is cleaved into individual proteins. This whole process requires that the genome or RNA to be polyadenylated at 3' end and has a cap at 5' end, but this isn't the case here.

So, instead of the cap the virus has IRES at its 5' end. This feature permits the virus genome to be translated immediately after entry into the cell.

Genetic heterogeneity

Genetic heterogeneity is remarkable for HCV and the genetic heterogeneity is reflected in the response or susceptibility of the virus to chemotherapy. For example, certain genotypes require 24 weeks of treatment but there are other genotypes - especially genotype 1 – require 48 weeks of treatment. So, in the countries where genotype 1 of the virus is prevalent, the treatment is difficult and relapse take place and requires more time for treatment. But in countries where other genotypes are prevalent, the treatment is easier.

-Marked genetic heterogeneity due to hypervariable region (Major neutralization epitope of HCV).
-Six major genotypes (1-6)
-HCV mutates under immunologic pressure
-Genotype dictates length of therapy and predicts therapeutic response
For example, genotype 1 requires longer therapy and has lower response.

Pathogenesis and Pathology

Hepatitis C infections are similar to other hepatitis in terms of chronicity, clinical feature and pathology.

-HCV used to cause about 90% of transfusion associated hepatitis.

Now, there is no more cases because the transported blood - which was the major cause of transmission of HCV - is screened.

-The percutaneous route is the most efficient in transmission.

Infections are usually associated with parenteral route of transmission.

-Most risk groups are recipients of blood or blood products, IV drug users, renal dialysis patients and needle stick victims - especially in hospitals -.
-Sexual and transplacental spread is less efficient but it may take place.

70-80% of HCV infections results in chronicity.

HCV is less efficient as infectious agent when compared to HBV. But once infection with HCV virus it tends to be chronic

-Chronic HCV is commonly associated with:

1-The appearance of lymphoid follicles and/or aggregates within portal tracts.

2- Large droplet fat vacuoles in the cytoplasm of infected hepatocytes (steatosis)

3- Activation of lobular sinusoidal inflammatory cells.

4-There might be bile duct damage.

5-The level of necrosis, scarring and fibrosis can vary widely.

That's why it ranges from totally asymptomatic to form hepatitis in some cases.

6-Cirrhosis is more common with these patients and seen in about 20% of cases and is commonly followed by PHCC within a period of 6-30 years.

PHCC: **P**rimary **H**epato-**C**ellular **C**arcinoma.

Only Hepatitis D leads to more chronic Cirrhosis than Hepatitis C, but Hepatitis D isn't a major cause of Hepatitis because number of infected individuals is less than those who is infected with HCV.

But if we compared the infections with these 2 viruses as absolute number of patients who have Cirrhosis, HCV will have greater number because HCV is most common cause of hepatitis and 70-80% of these infections will be chronic and in 20% of chronic individuals will suffer from Cirrhosis and this number is still greater than Cirrhosis with HDV.

Clinical Features

-Incubation period of 40-120 days.

Incubation period is long & similar to that of HBV

-Commonly asymptomatic.

-25% of cases are icteric (associated with jaundice) **but milder than HAV or HBV.**

-Fatigue is the most common symptom but weakness, wasting, edema and ascites also occur.

Extrahepatic Manifestations

They are immune mediated manifestations similar to the case of HBV and they are almost similar in the organs involved in both infections.

These manifestations include:

- Mixed cryoglobulinemia (vasculitis)

- Membranoproliferative glomerulonephritis

- Porphyria cutana tarda (skin manifestation)

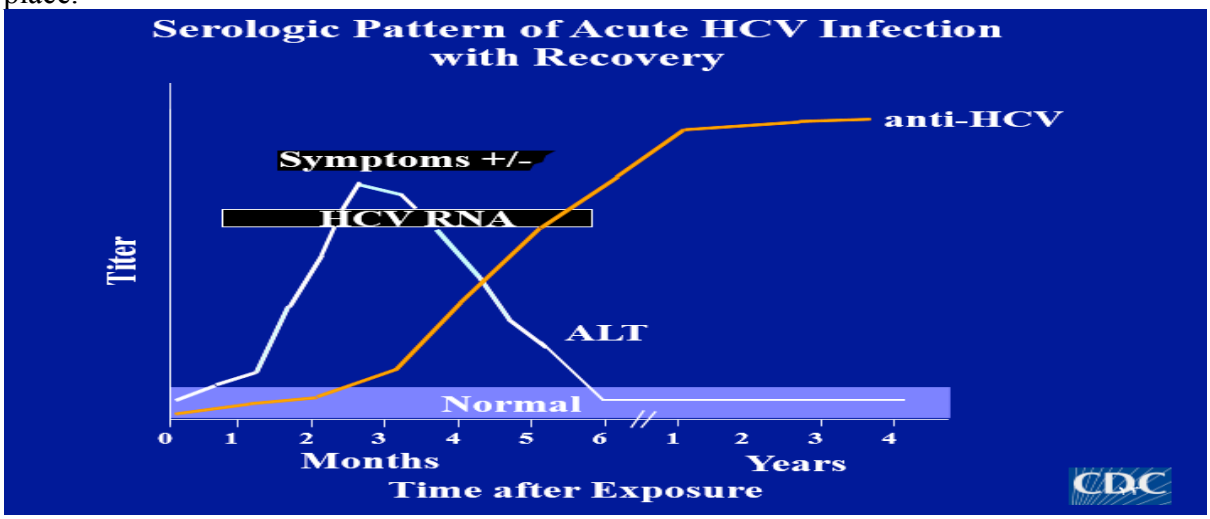
Characterized by:

- **photosensitivity**
- **increased skin fragility**
- **hypopigmentation**
- **sclerodermoid plaques.**

Conditions Linked to HCV

- **Arthritis** (who has chronic hepatitis C develop Arthritis)
- **Autoimmune hepatitis**
- **Sjogrens Syndrome** (connective tissue disease)
- **Cryoglobulinemia**
- **Kidney disease**
- **Liver Cancer**

Infection with HCV in the minority of cases can be acute, that means the individual can resolve infection & develop immunity (which isn't long lasting) to that type of virus and reinfection can take place.



In the picture above notice that:

-The individual can be infected with acute HCV for few months.

- Symptoms persist for few weeks

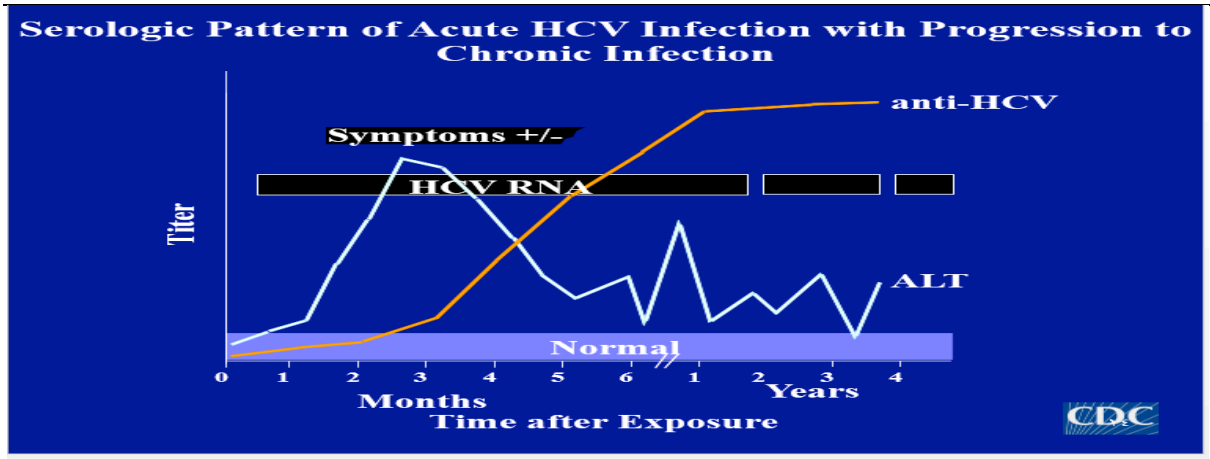
-We can notice that HCV RNA are defected over a period of time.

-Antibodies are produced whether the virus is cleared or not. So, antibodies aren't markers for recovery because antibodies can exist with the antigens of the virus.

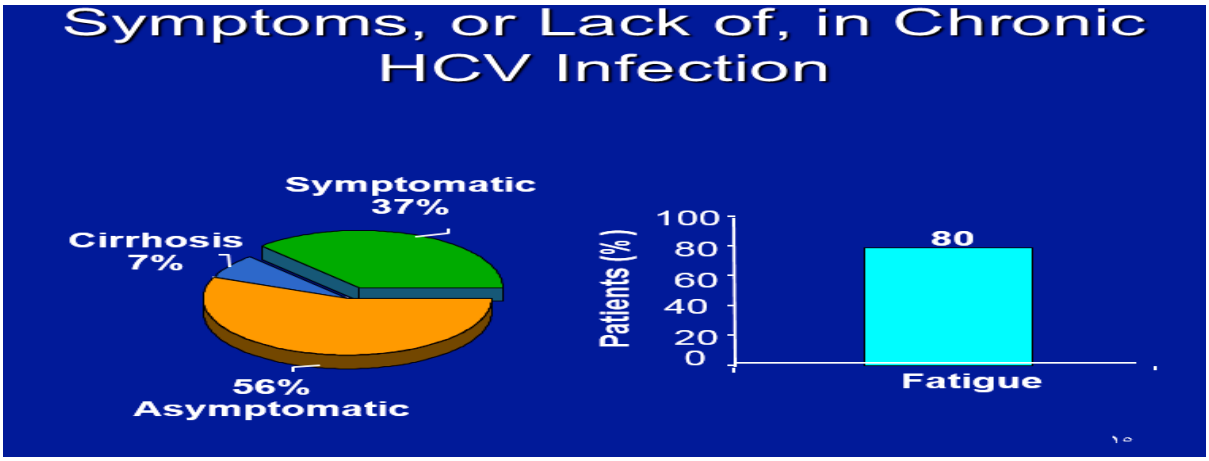
-The individual has elevation in ALT (liver enzyme) for a while then it backs to normal and the only marker that remains is the antibody.

-Acute infection is characterized by the lack of the virus after it has been resolved. So, we can't detect the virus or its genome by PCR. Whereas in chronically infected individuals the virus remains in the circulation. So, we can detect the virus and the antibodies in chronic cases.

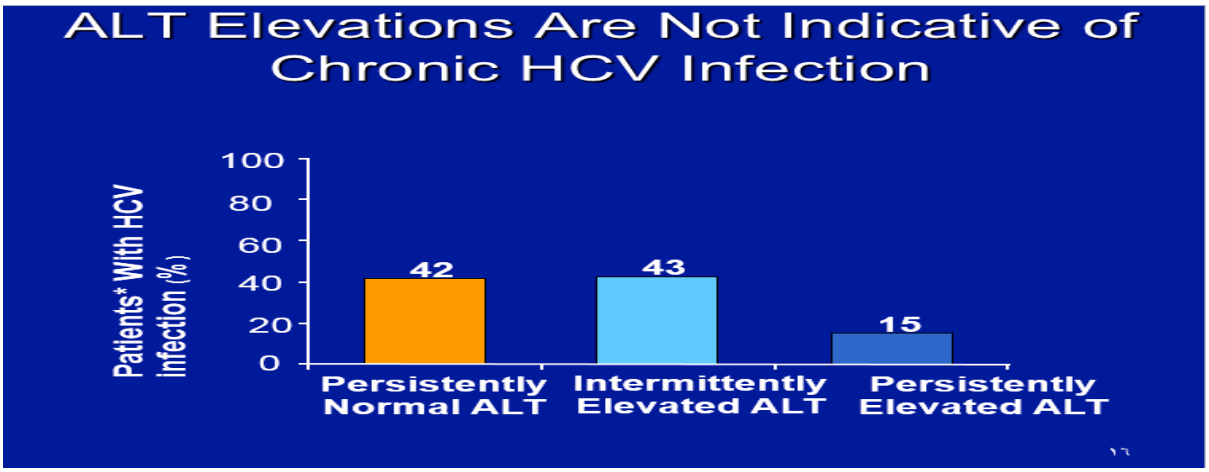
-For detection of exposure to the virus we look for antibodies, while for detection of recovery or if he still infected we look for the virus RNA.



-In this case the individual doesn't have normal liver function because we have variation and spikes of levels of ALT depending on viral activity and he remains infected for a long period of time.



-In 80% of cases of infected individuals with HCV, fatigue is the sole manifestation.
-In 37% of infected individuals may develop symptoms.
-In 56% (majority) of infected individuals are asymptomatic.
-Cirrhosis may develop in infected individuals over time and Cirrhosis is associated with the development of PHCC.



In the past time, one of the diagnostic technics to especially detect whether the blood donor has chronic hepatitis or not, was the measurement of the lever enzyme (especially Alanine AminoTransferase -ALT-) which was called **Surrogate Test**, but this was seem to be non-reliable measure because the elevation of the enzyme was seen in fraction of patients and the fraction was the same fraction as those without any elevation in the enzyme although both of them are infected with HCV, as demonstrated in the figure.

So, the only reliable measure is the detection of the RNA of the virus.

Factors Promoting Progression or Severity

These factors are associated with increased severity of the disease and they include:

-increased alcoholic intake

-Age above 40 years

- Co-infection with HIV (because they have the similar modes of transmission)

-Other factors include:

Male gender (HCV is similar to HBV in this case)

Chronic HBV co-infection

So, the infected individual with HCV can develop a co-infection with HIV (HIV co-infect with HCV more than HBV) or HBV and this is a challenge for chemotherapy.

Laboratory diagnosis

The reliable way for making diagnosis relies on 3 categories:

-HCV antibody: generally used to diagnose hepatitis C infection. Not useful in the acute phase as it takes at least 4 weeks after infection before antibody appears.

These antibodies are marker for infection only and not markers for recovery

-RNA of HCV: various techniques are available to detect the RNA, e.g. PCR and branched DNA. This test may be used to diagnose HCV infection in the acute phase. However, its main use is in monitoring the response to antiviral therapy.

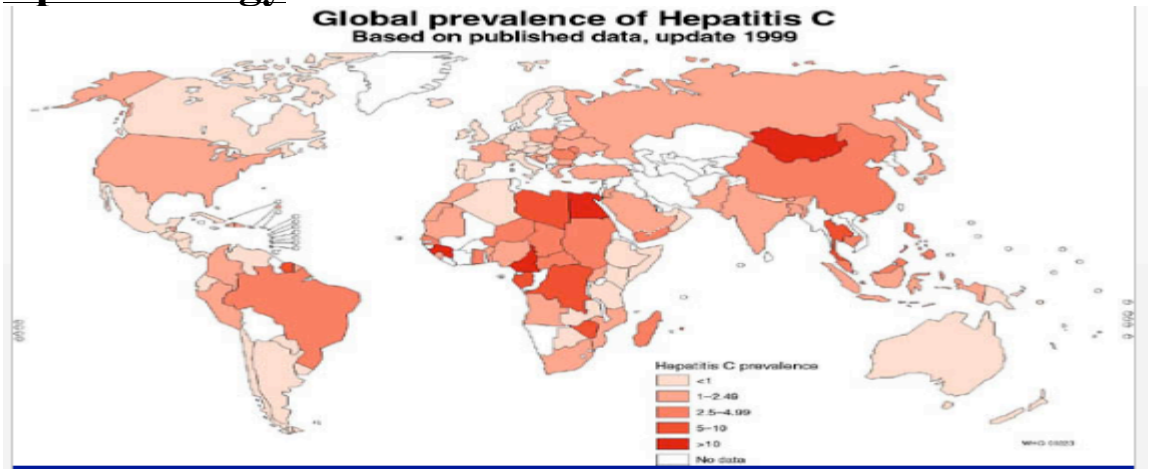
-HCV antigen: can be detected by EIA (enzyme-linked immunoassay) **or other methods. It is used in the same capacity as HCV-RNA tests because it has the same specificity but it is much easier to carry out.**

****You should study this table****

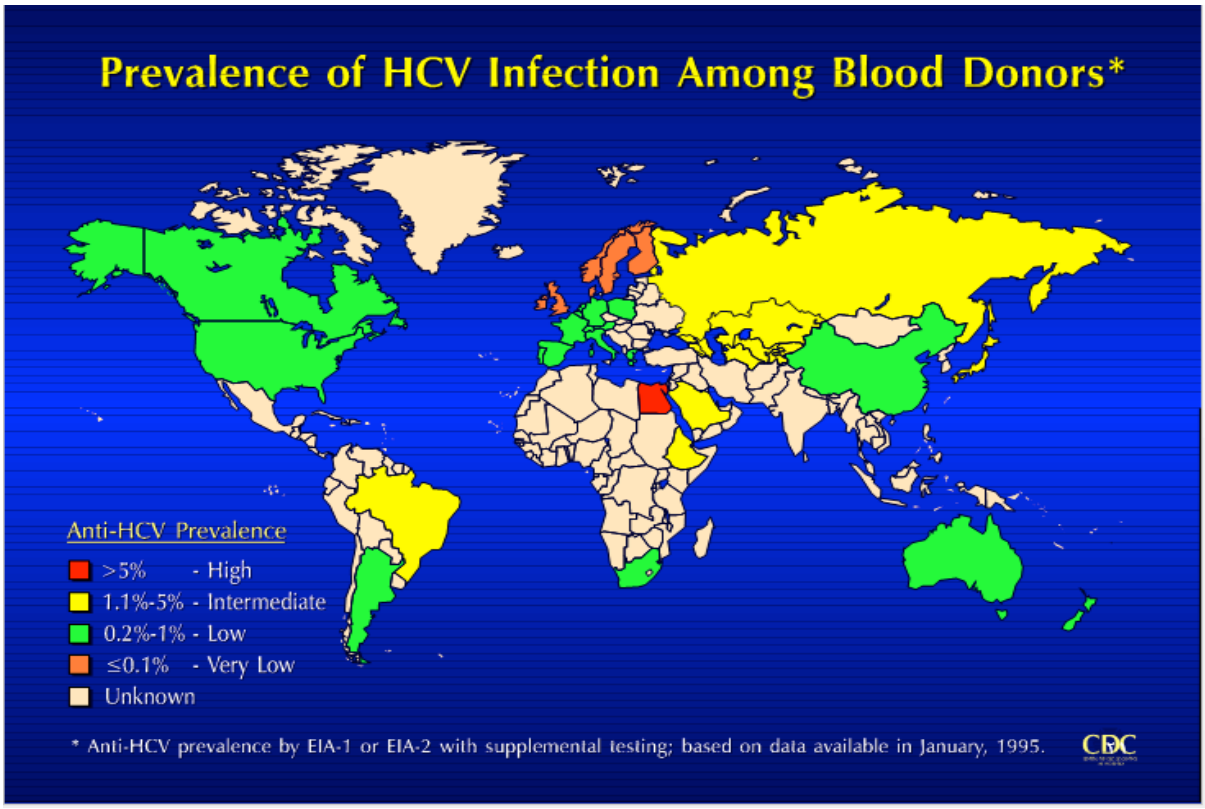
Diagnostic Tests for HCV Infection		
	Diagnostic Test Type	
Specifications	**Serologic**	**Virologic**
Mode of detection	Antibodies	Virus
Sensitivity	> 95%	> 98%
Specificity	Variable	> 98%
Detection postexposure	2-6 mos	2-6 wks
Use	Screening	Confirmation

-Here we have a comparison between the serologic(Antibodies) methods and the virologic (Antigen & RNA of the virus) methods of diagnosis.
-The specificity of serologic methods is variable depending on the kits and on the inclusion of the antigens of the virus.
-Detection postexposure in serologic methods needs 2-6 months, which means sero-conversion may be delayed for 31 weeks which is a problem for detection because the individual tests will be negative for the antibody for a period can be up to 31 week, and these individuals can donate blood and cause infection of HCV in others. *(***the number 31 weeks will be explained later as it is the time needed for antibody production***)*
-**Serologic method** is used for **screening** of blood and **Virologic methods** are used for **confirmation** of the infection.

Epidemiology



The map above shows the global prevalence of HCV. Egypt is one of the countries with highest prevalence (>10 infected patients per 100,000 population).



In this map we take particular group which is the blood donors. Also, here the prevalence varies and Egypt is still has the highest prevalence among the blood donors. (>5% of blood donors have HCV infection).

-The high incidence of chronic asymptomatic infection and the inadequacy of screening tests resulted in promoting the spread of the HCV via blood supply.

Also, the screening methods take part in promoting the spread of HCV because they weren't sensitive or they were used in the period in which the patient is negative for the antibodies' presence because antibody production may be delayed.

-Viremia can be detected (by immunological test not by antibodies tests) **within 3 weeks of infection and lasts for 4-6 months in acute infection and for up to 10 years or longer in chronic infections**.

In chronic patients the virus presents in their blood for about 10 years.

-Antibody production requires a period of 7-31 weeks of infection and its detection forms the basis for diagnosis but is not always present in viremic individuals.

This period will make the detection of HCV in blood donors very difficult, especially if they were in the early course of infection. So, they donate blood while they're asymptomatic and negative for antibody presence although they're infected.

-That's why detection of RNA is a better determinant of disease but RNA detection can't be used as screening method.

-It is estimated that nearly 200 million people are chronically infected with HCV worldwide.

-During HCV replication, hypervariable regions within the envelope genes are constantly undergoing mutation.
-Thus an infected individual carries not one unique virus but rather a whole population of related quasi-species.
-Such properties may allow these viruses to escape immune mediated clearance.

Risk Factors Associated with Transmission of HCV

-Transfusion or transplant from infected blood donors (during the window period or if the test was delayed)
-Injecting drug use
-Hemodialysis (in patients with years of treatment with hemodialysis)
-Accidental injuries with needles/sharps in hospitals
-Sexual/household exposure to anti-HCV-positive contact
-Multiple sex partners
-Birth to HCV-infected mother

HCV can't spread by

-BREAST FEEDING
-SNEEZING
-COUGHING
-HUGGING
-FOOD OR WATER
-SHARING EATING UTENSILS OR DRINKING GLASSES
-CASUAL CONTACT

- HCV is similar to HIV in this term which also can't spread by these methods.

Occupational transmission

-Inefficient by occupational exposures
-Average incidence is 1.8% following needle stick from HCV-positive source
Usually associated with hollow-bore needles
-Case reports of transmission from blood splash to eye; one from exposure to non-intact skin

-Prevalence 1-2% among health care workers
Lower than adults in the general population
10 times lower than for HBV infection

Prenatal Transmission of HCV can take place

-Transmission only from women who have HCV-RNA positive at delivery
Average rate of infection 6%
Higher (17%) if woman is co-infected with HIV
Role of viral titer unclear
-No association with:
Delivery method
Breastfeeding
-Infected infants do well but in rare cases they may develop Severe Hepatitis

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Treatment of HCV

The treatment is achieved by:
-Pegylated interferon alfa & ribavirin
Ribavirin is a broad spectrum antiviral agent that can be used to treat RNA & DNA Viruses

-Treatment duration is 24-48 weeks depending on the genotype (genotype 1 requires more period)
-Overall success rates is 50% which is decreased by genotype 1, HIV positivity and by old age
-We can try longer treatments for 48 weeks for genotype1
-The individual is followed by serum HCV viral load or liver biopsy

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Telaprevir (*Incivek*)

-There're new treatments for HCV which consist of new protease inhibitors and Lysins for HCV. Telaprevir is an example of this new treatment which is added to (Pegylated interferon alfa & ribavirin) and doesn't replace that old treatment.

Approval
- FDA Approved May 23, 2011

Indications
-It is used in combination with Peginterferon-alfa and Ribavirin (PR)
-Treatment of chronic HCV genotype 1 infection that doesn't respond well to (Ribavirin and interferon)

-Treatment of adults (≥ 18 years of age) with compensated liver disease including cirrhosis which isn't treated by old treatment.
- Treatment-naïve or prior interferon-based treatment

-About 80% of those receiving Telaprevir experience a kind of response to antiviral therapy which is known as **sustained virological response**. Sustained virus response means absence of the virus from bloodstream of these individuals or the virus exist below a certain level.
- On the other hand, just about 50% of those receiving the old treatment (interferon & Ribavirin) develop sustained virological response. So, the new treatment is more efficient in inducing this response.
-Rate for patients treated with Telapriver, across all studies and across all patients, are 20-40% higher than those who use the old treatment. And the course of treatment with Telaptiver is 24 weeks only.

Dosing

- 750 mg (two 375-mg tablets) three times daily with food
- Treat with PR for 12 weeks (followed by additional 12 or 36 weeks PR)

Adverse Effects

The doctor said it has adverse side effects that will be covered in pharmacology
- Rash, anemia, nausea, fatigue, headache, diarrhea, pruritus, and anal or rectal irritation and pain

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Clinical Significance of HCV Genotypes

-HCV has great genetic diversity:
6 genotypes (1 through 6)
Multiple subtypes within the genotypes: a, b, c, etc
-Genotype is best pretreatment predictor of response
For example, genotype 1 has the least responsive to therapy
-Determines dose and duration of therapy
Genotype 1: 48 weeks of peg-IFN alfa + RBV 1000-1200 mg
Genotype 2/3: 24 weeks of peg-IFN alfa + RBV 800 mg
-All patients should have genotype determined prior to initiating therapy

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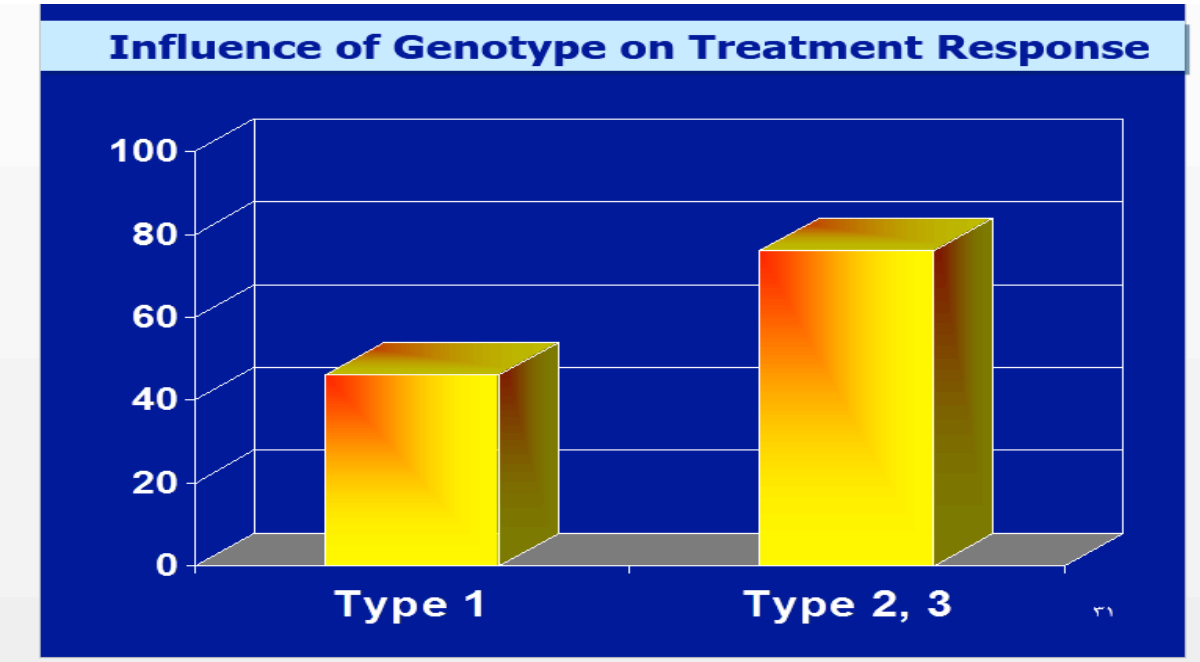
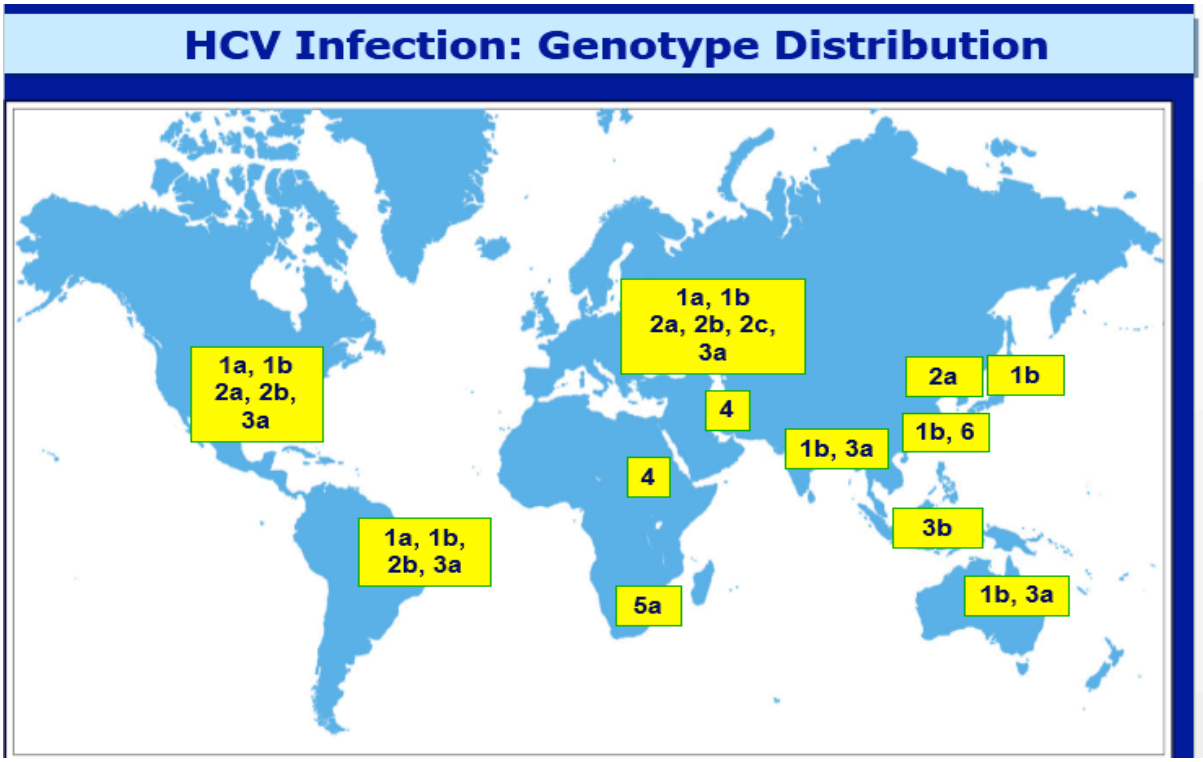


-Genotype 1 treatment:

Related to complications, side effects, relapse and partial or no response for the treatment.
More period of time. It needs 48 weeks.

-Genotypes 2&3 treatment:

More response to treatment than genotype 1
Less period of time. It needs 24 weeks



-Genotype 4 is prevalence in our region, but genotype 1 isn't.
-Genotypes 1&2 are present in Europe.

Prevention of Hepatitis C

Achieved by:

-Screening of blood, organ, tissue donors (in tissue transplantation)
-High-risk behavior modification (especially in drug use, homosexuality etc)
-Blood and body fluid precautions in hospitals

-Vaccine development has been hampered because of 2 factors:
1-The presence of multiple genotypes and numerous subtypes
2-Sequence diversity of envelope glycoproteins.
-Consistent with this is the fact that patients who appear to have cleared an HCV infection have no protective immunity to future infections.

Even if the vaccines are available, that doesn't mean protection from infection because of the hypervariable region in the virus genome.

Hepatitis GB Virus

-There're three types of this virus: A, B and C .
-The genome is ss RNA of positive polarity. It is related to HCV virus and is enveloped.
-It is transmitted by the parenteral route and it is present in 1-2% of eligible blood donors .
-There has been a large difference between exposure rates (80-90%) and active infection (15-20%) meaning that this virus isn't efficient in establishing of infection

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-10-20% of HCV infected individuals are co-infected with HGBV, because of similarity in the mode of transmission.
-There is no clear association with disease but it is believed that it causes both acute and chronic infections.
-Best diagnosed by PCR detection of the RNA genome
-Antibody detection isn't a reliable method for detecting HGBV because anti-E2 is a recovery phase antibody that is detectable only when HGBV-RNA has been cleared.

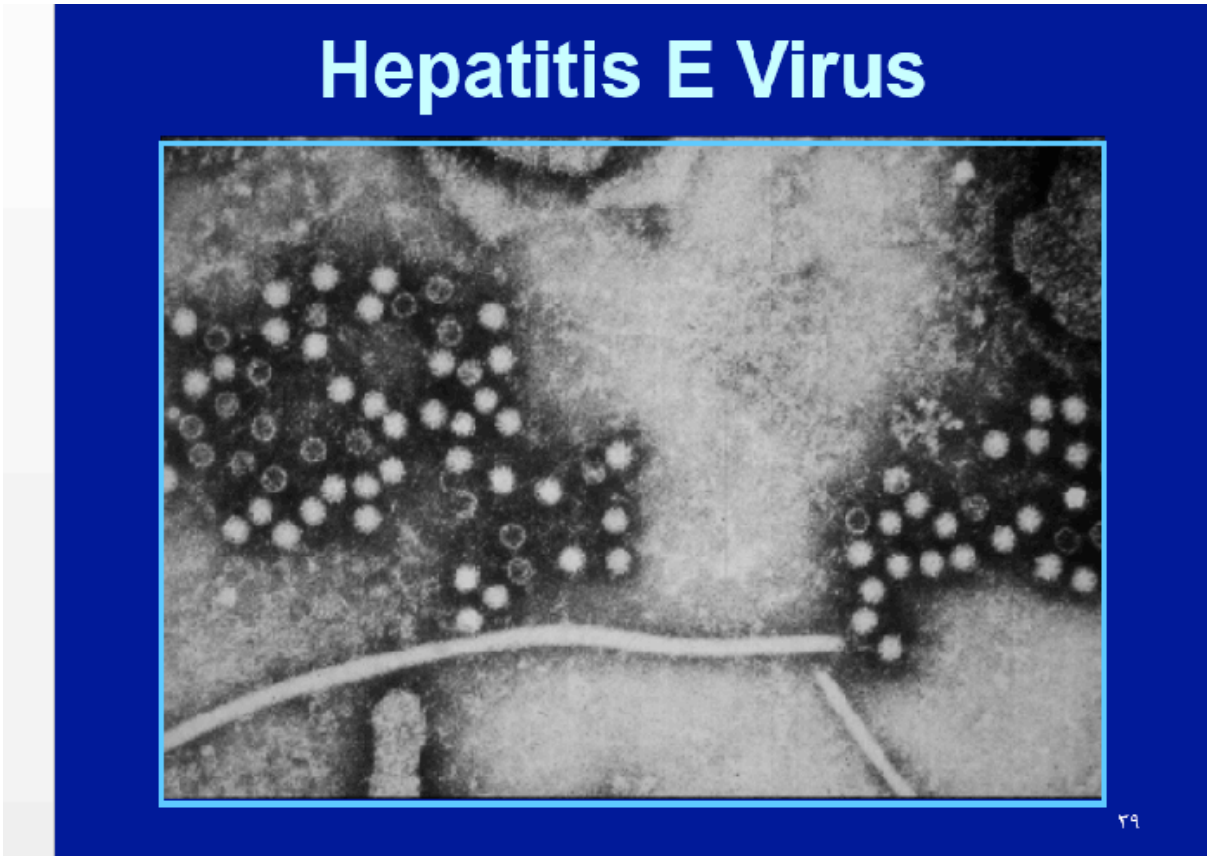
So, the antibody isn't present while the patient is infected, and it is only a marker for previous infection.

Hepatitis E Virus

-Recognized as a distinct disease in 1980.

Because at that time (1980), the first kit for diagnosis of hepatitis A was available. So hepatitis A antibody detection was available in 1980. But, cases that were labeled as hepatitis A were retested after improvements of the kits and it tested negative for hepatitis A. Then, these non-A hepatitis that were transmitted by oral-fecal route was known to be caused by new virus; hepatitis E virus.

-It is classified as a Calicivirus (HEV is similar to Norovirus that is classified in this family**)**
-Virion is 32-34 nm in diameter, nonenveloped, has an icosahedral symmetry
-Genome is +ss RNA 7.5kb in length .
-It encodes only for 3 proteins, a structural one; a nonstructural one and a third of unknown nature.

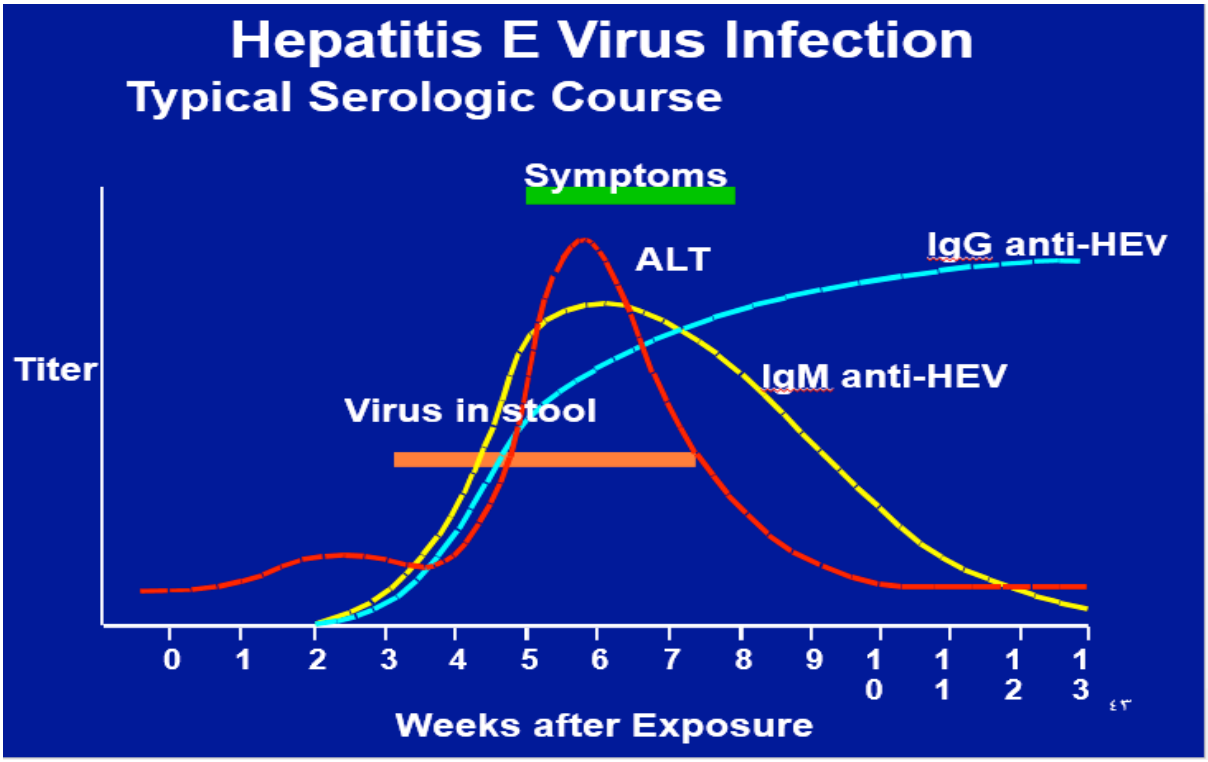


This is an electro micrograph of the virus showing small icosahedral naked virus.

-There is only one serotype exists with many strains (that's why infection with HEV is followed by immunity)
-The pathogenesis of HEV is poorly understood .
-Infection is acquired by the fecal oral route of transmission as a consequence of food/drink contamination (Similar to HAV in this term) **or by person-to-person contact** (Similar to other Caliciviruses in this term)
-Primary site of replication is believed to be the GI tract and the liver is then invaded probably via the portal vein .
-Virus is released to bile and blood after replication in & damaging hepatocytes.

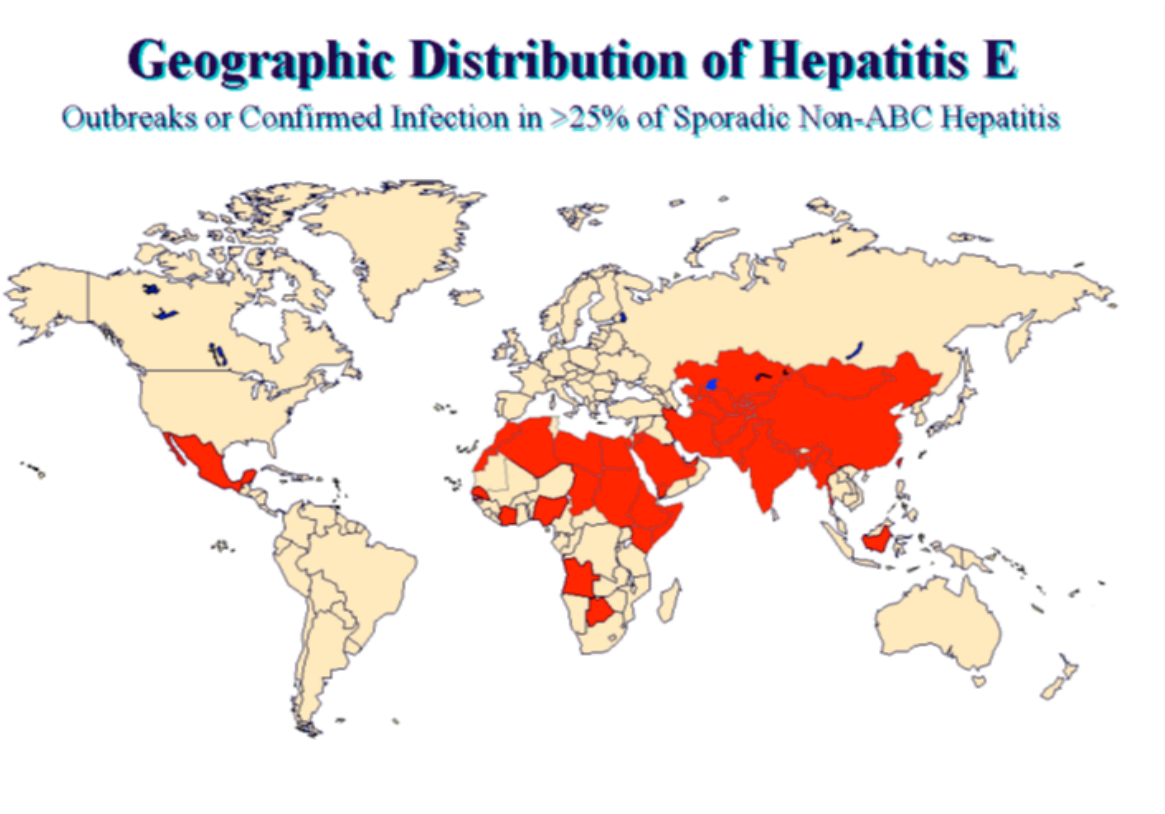
-The incubation period is 10-40 days. (Similar to HAV)
-Pathologically, there is focal necrosis with minimal infiltration but no localization to a particular zone of the lobule in the liver.
-Cholestatic hepatitis is often present in HEV infections and is characterized by ballooning hepatocytes.
-Clinical manifestations are similar to those of hepatitis A but with higher mortality (1% for HEV Vs 0.1% for HAV) (10 times more mortality for HEV)

-HEV infection is particularly severe among pregnant women and mortality increases with gestational age to reach up to 20% in the 3rd trimester (because of fluminant hepatitis).
-Water borne outbreaks especially in the developing countries especially India in 1955 which was thought in the first as HAV cases.
-Epidemics of HEV were first reported when diagnosis was available in 1980 from the Indian subcontinent but outbreaks involving tens of thousands cases have also been documented in the previous USSR, Southeast Asia, Northern Africa and Mexico.



-Pathogenesis is similar to HAV in that the infection is acquired and few weeks later the virus can be detected in stool. There's also elevation of the liver enzyme (ALT) and the virus also is present in blood.
-Antibodies to the virus first develop as IgM for few months then IgG appears.

-HEV is also similar to HAV in cure. Because the acute hepatitis is followed by full recovery & immunity without any chronicity but mortality varies significantly from HAV.



-Notice that the high prevalence of HEV in South East Asia & Africa.

Sorry for any mistakes
Done by: Oday Halhouli