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The Crux

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CONTENTS

Editorial

Hepatitis is a general term meaning inflammation of the liver and can be caused by a variety of different viruses such as hepatitis A, B, C, D and E. Since the development of jaundice is a characteristic feature of liver disease, a correct diagnosis can only be made by testing patients' sera for the presence of specific viral antigens and/or anti-viral antibodies. We have thus far covered Hepatitis, A, B and C. This issue delves deep into the clinico-diagnostic aspects of Hepatitis E. Hepatitis E was not recognized as a distinct human disease until 1980, when specific tests for antibody against hepatitis A were first applied to the study of epidemic waterborne hepatitis in India. Hepatitis E is caused by infection with the hepatitis E virus (HEV), a nonenveloped, positive-sense, single-stranded RNA virus. People who never have contracted HEV are at risk of infection. Hepatitis E has a restricted distribution: epidemics of hepatitis E have been found in much of Central and South-East Asia, North and West Africa, and in Mexico, confined to geographic areas where faecal contamination of drinking water is common. Hepatitis E is usually a self-limiting disorder, except in pregnancy where it can potentially become a life threatening disease. Go on, read the whole article inside to get a deeper understanding of this form of Hepatitis.

INTERPRETATION segment may evoke an allergic response! Not really, it just talks about immunoglobulin E. Immunoglobulin E (IgE) is a class of antibody (or immunoglobulin "isotype") that has only been found in mammals. It plays an important role in allergy, and is especially associated with type 1 hypersensitivity. Equally, presence of most parasites in the human body also evokes an IgE response. Flip a few pages to know more about this immunoglobulin.

It was a couple of decades back when a few Australian pathologists accidentally discovered *Helicobacter pylori* in the stomach biopsy specimens. Since then, these bacteria have found a significant place as the aetiological organisms responsible for causing gastritis, peptic ulcers and duodenal ulcers. Fortunately they are easy to eliminate just by consuming the effective antibiotics as prescribed. The diagnosis can be made by visualising the bacteria in the endoscopic biopsy specimens or by assessing the presence of antibodies in the blood sample. All relevant diagnostic aspects / methodologies available are considered under the TROUBLESHOOTING segment.

Amidst the above-mentioned serious presentations, somewhere, you'll find the lighthearted BOUQUET too. Have a nice and healthy life yourself. Trust this endeavour of ours does to an extent drives away at least a few of your diagnostic worries.

DISEASE DIAGNOSIS

Hepatitis E

Introduction: Hepatitis is a general term meaning inflammation of the liver and can be caused by a variety of different viruses such as hepatitis A, B, C, D and E. Since the development of jaundice is a characteristic feature of liver disease, a correct diagnosis can only be made by testing patients' sera for the presence of specific viral antigens and/or anti-viral antibodies. Hepatitis E was not recognized as a distinct human disease until 1980, when specific tests for antibody against hepatitis A were first applied to the study of epidemic waterborne hepatitis in India. The results showed that the epidemics were not epidemics of hepatitis A. Actually, very few epidemics of waterborne disease in developing countries of Asia and Africa have been linked to hepatitis A. The first experimental evidence for the existence of an additional waterborne hepatitis agent was reported in 1983. This form of non-A, non-B hepatitis came to be known as Enterically transmitted non-A non-B hepatitis (ET-NANB), Epidemic non-A non-B hepatitis (ENANB), or Faecal-oral non-A non-B hepatitis, and the agent of this disease was subsequently found to be the major cause of sporadic hepatitis cases in regions where the epidemic form was known to exist. (hepatitis E should not be confused with hepatitis C, also called parenterally transmitted non-A non-B hepatitis (PT-NANBH), or B-like non-A non-B hepatitis).

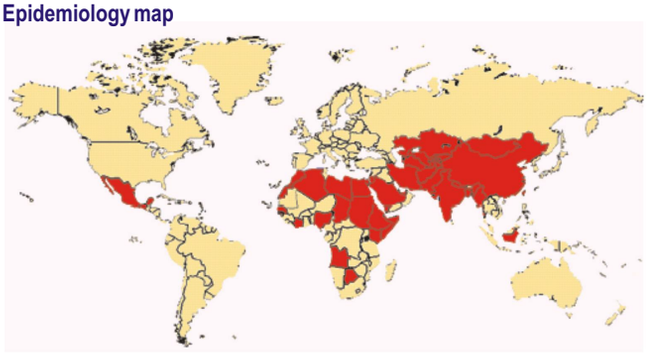
Causative agent: Hepatitis E is caused by infection with the hepatitis E virus (HEV), a nonenveloped, positive-sense, single-stranded RNA virus. Originally classified within the family of caliciviruses, HEV is now unclassified.

Transmission: HEV is spread by the oral-faecal route. This enterically transmitted virus has been implicated in several food and waterborne outbreaks. Consumption of faecally contaminated drinking water has given rise to epidemic cases, and the ingestion of raw or uncooked shellfish has been the source of sporadic cases in endemic areas. The low amount of intact HEV particles present in patient stools accounts for the generally lower rate of person-to-person transmission of hepatitis E when compared with that of hepatitis A. Naturally acquired HEV antibodies have been detected in primates, rodents and swine. Swine HEV cross-reacts with antibodies to the human HEV capsid antigen. Human hepatitis E has been transmitted under laboratory conditions to various species of primates, domestic pigs, lambs and laboratory rats. Species specific HEV has been demonstrated in pigs with the identification of swine HEV. Swine HEV is distinct, but closely related to human HEV strains. While specific-pathogen-free pigs can be experimentally infected with the US-2 strain of human HEV, it is still not known whether swine HEV can infect humans, although it can infect chimpanzees under experimental conditions. Until then, swine HEV raises a potential public health concern for zoonosis and xenozoonosis following xenotransplantation with pig organs. A zoonotic spread of HEV is not excluded, since monkeys, pigs, cows, rodents, sheep and goats are susceptible to infection with HEV (possible non-human reservoir of virus). Although hepatitis E is not endemic in the US and other developed countries, anti-HEV has been found in a significant proportion, up to 28% in some areas, of healthy individuals in these countries. Subclinical infection of humans with swine HEV (possible non-human reservoir of virus) might explain the relatively high prevalence of anti-HEV in healthy individuals in areas where hepatitis E is not endemic. Most cases of acute hepatitis E in the US, central and western Europe have been reported among travellers returning from high HEV-endemic areas, although a few have occurred in individuals who have not left their country. It appears, therefore, that some HEV is imported into industrialized countries and some is probably endemic, possibly as a zoonosis. The occurrence of sporadic HEV infections in humans may maintain transmission during inter epidemic periods. Regardless of whether HEV is endemic in the respective human population, hepatitis E is enzootic in pigs, probably worldwide. There is no evidence for sexual transmission or for transmission by transfusion.

Susceptibility: People who never have contracted HEV are at risk of infection. The risk factors for HEV infection are related to resistance of HEV to environmental conditions, poor sanitation in large areas of the world, and HEV shedding in faeces.

Historical Incidence / Epidemiology: Outbreaks of hepatitis E are more common in parts of the world with hot climates and are rare in temperate climates. Outbreaks are mainly associated with faecally contaminated drinking water; exceptions are food-borne epidemics (raw or uncooked shellfish). HEV was first identified in India, and has since been recognized in the Middle and Far East, in northern and western Africa, the central Asian Republics of the former Soviet Union, in China and Hong Kong SAR. Epidemic and sporadic cases have been reported from southeast and central Asia, the Middle East, northern and western Africa and North America (Mexico). 30000 cases were reported in New Delhi, India, (1955-1956) after the flooding of the river Yamuna and contamination of the city's drinking water. 20000 cases occurred in Mandalay, Myanmar, (1976-1977) with 18% case fatality rate in infected pregnant women. 52000 cases were reported in Kashmir, India, in 1978. 100000 cases were reported in China between 1986 and 1988. 11000 cases occurred in Somalia, and about 4000 cases were reported in Mexico between 1988 and 1989. Low incidence is reported in Italy and Spain (1995).

Epidemiology map



Trends: Hepatitis outbreaks occurring in Europe prior to the 20th century, and believed to be hepatitis A, had the epidemiologic characteristics of hepatitis E. HEV, more labile and shed in lower titres than HAV, may have disappeared from more industrialized countries in the recent past, just as HAV is currently diminishing in importance in these countries.

Hepatitis E distribution: Hepatitis E has a restricted distribution: epidemics of hepatitis E have been found in much of Central and South-East Asia, North and West Africa, and in Mexico, confined to geographic areas where faecal contamination of drinking water is common. However, the application of recently developed serologic tests has revealed anti-HEV in every country in which it has been sought, including developed countries like the United States (US), in which the disease virtually does not occur. It is not clear whether such antibodies represent missed diagnoses of hepatitis E, asymptomatic infections, infections with attenuated strains of HEV, antibodies that cross-react with an as yet unrecognized agent, or some type of nonspecificity of the existing assays. Possible reservoirs of HEV in the mentioned regions could be found in animals like monkeys, pigs, cows, rodents, sheep or goats. In fact, all these species are susceptible to infection with HEV.

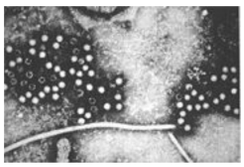
Prevalence: The highest prevalence of infection occurs in regions where low standards of sanitation promote the transmission of the virus. The prevalence of antibody to HEV in suspected or documented endemic regions has been much lower than expected (3 - 26%). Screening of blood donors in central Europe and North America has shown a prevalence of anti-HEV antibodies of 1.4 - 2.5%, in South Africa of 1.4%, in Thailand of 2.8%, in Saudi Arabia of 9.5%, and in Egypt of 24.0%. The prevalence of antibody to HEV in non endemic regions (like the US) has been much higher than anticipated (1 - 3%). HEV infections account for >50% of acute sporadic hepatitis in some high endemic areas.

Risk groups: Here is a list of groups of people who are at risk of contracting HEV: Persons residing in areas where extended community outbreaks exist. International travellers to regions of the world where HEV is endemic. Refugees residing in overcrowded temporary camps following catastrophies, especially in Sudan, Somalia, Kenya and Ethiopia. Persons who have chronic liver disease. Possibly persons working with non-human primates, pigs, cows, sheep and goats.

Life threatening circumstances: Hepatitis E is a mild to moderate disease in severity (mortality rate of 0.4-4.0%) except in pregnancy, where the mortality rate is progressively higher in each succeeding trimester and may reach 20%. HEV

infections are usually self-limited, and hospitalization is generally not required. No available therapy is capable of altering the course of acute infection.

The Hepatitis E virus:



Virus classification
Group: **Group IV ((+) ssRNA)**
Family: **Unassigned**
Genus: **Hepevirus**
Species: ***Hepatitis E virus***

TEM micrograph of hepatitis E virions.

HEV causes self-limited acute viral hepatitis in adults aged 15-40. HEV is a nonenveloped, spherical, positive-stranded RNA virus. Several different strains have been isolated, partially characterized and molecularly cloned (1990-92). Although originally classified within the family of caliciviruses, they are now unclassified. Replication in cell culture was first reported in 1993; yields of virus are generally very low. In natural infections, the virus replicates in hepatocytes. In vivo infected macaque hepatocytes support HEV replication after isolation and placement into tissue culture. Man is the natural host for HEV, but certain non-human primates, e.g. chimpanzees, cynomolgus monkeys, rhesus monkeys, pigtail monkeys, owl monkeys, tamarins and African green monkeys are reported to be susceptible to natural infection with human strains of HEV. Transmission of human strains of HEV to swine has been reported for the US-2 strain of human HEV, but could not be shown for the Sar-55 and Mex-14 strains. A swine strain of HEV has been isolated, identified and characterized and subsequently shown to infect rhesus monkeys and chimpanzees, experimental surrogates of humans. This ability to cross species barriers puts humans at risk for infection with swine HEV. In endemic areas, naturally acquired anti-HEV has been found in 42 - 67% of cows, sheep and goats. Recent studies show evidence of widespread HEV or HEV-like infection in rodents in the US (prevalence rate of 60% in rats), raising the question of transmission, reservoirs, and strains of HEV in developed countries.

Morphology and physicochemical properties: HEV is a small and structurally simple RNA animal virus. The virion is nonenveloped and, with a diameter of 27-34 nm, is composed entirely of viral protein and RNA. Electron microscopy (EM) analyses show spherical particles of possible icosahedral symmetry, with indefinite surface substructure, resembling the caliciviruses. Morphologically, HEV is similar to Norwalk virus, a member of the calicivirus family, although the sequence of HEV most closely resembles the sequence of rubella virus, a togavirus, and beet necrotic yellow vein virus, a plant furovirus. Full virions have a buoyant density of 1.29 g/cm3 in potassium tartrate/glycerol gradients and a sedimentation coefficient of 183 S in neutral sucrose gradients, empty capsids of 165 S under the same conditions.

Genome and proteins: The hepatitis E genome consists of a linear, single-stranded, positive-sense RNA (that is, mRNA) of approximately 7.5 kb containing a 3' poly(A) tail and short 5' and 3' noncoding (NC) regions. Three overlapping open reading frames (ORFs) exist, and all three coding frames are used to express different proteins. ORF1 (5 kb) is located towards the 5' end of the genome and encodes a polyprotein of about 1690 amino acids that probably undergoes post-translational cleavage into multiple nonstructural proteins required for virus replication, including a methyltransferase, a putative papain-like cystein protease, an RNA helicase and an RNA-dependent RNA polymerase. ORF2 does not overlap with ORF1; it is located at the 3'-end of the genome and encodes the principal and probably only structural protein. It is a capsid protein of 660 amino acids (71 kDa). ORF3 begins with the last nucleotide of ORF1; it overlaps extensively with ORF2 and is the shortest of the open reading frames, encoding a small immunogenic 123 amino acid phosphoprotein (14.5 kDa) which associates with the cytoskeleton, suggesting a possible role in the assembly of virus particles. The genomes of several HEV strains from different parts of the world have been sequenced and compared. Overall, they appear to fall into four major genetic groups: (1) South-East Asian (Burmese, some Indian strains), North and Central Asian (strains from China, Pakistan, Kyrgyzstan, and a few from India), and North African strains form one somewhat heterogeneous genotype, (2) the single North American (Mexico) isolate comprises a second, (3) the US and swine isolates comprise a third and (4) a subset of isolates from China and most isolates from Taiwan comprise a newly described fourth group. Genetically heterogeneous isolates from several European countries have been designated new genotypes, but, at this time, probably should be grouped with the US isolates into a large, heterogeneous group. Two novel isolates of HEV have recently been described in Argentina. Distinct from all previously described isolates, they represent two diverse subtypes of a new genotype of HEV. The genome of swine HEV, an animal strain of HEV, has recently been identified and characterized. The putative capsid gene (ORF2) of swine HEV shares about 80% sequence identity at the nucleotide level and about 92% identity at the amino acid level with that of human HEV strains. The small ORF3 of swine HEV has about 84% nucleotide sequence identity and about 80% amino acid identity with human HEV strains.

Antigenicity: All HEV strains appear to comprise a single serotype. Major epitopes appear to exist near the carboxyl ends of ORF2 and ORF3. Epitopes contained in ORF2 are more conserved (90.5%) than epitopes contained in ORF3 (73.5%) in different strains. Western blot data indicate that type-specific (virus-specific) epitopes exist and can be used to differentiate serologically different isolates. Serologic tests for anti-HEV based upon expressed ORF2 sequences are more sensitive for detecting IgM and IgG anti-HEV than are tests based upon antigens containing ORF3 sequences. In fact, proteins expressed from ORF2 measure antibodies that correlate with protection against hepatitis E, whereas no such correlation has been shown for antibodies to ORF3. Antibodies to swine HEV cross-react with capsid antigens from strains of human HEV. No serologic or hybridizing cross-reactivity between HEV and other viral hepatitis agents, including hepatitis A virus (HAV), has been observed.

Stability: HEV is extremely sensitive to high salt concentrations. HEV should be stored as cold as possible, although it is rapidly degraded when freeze-thawed. The virus is sensitive to degradation by proteolytic enzymes. HEV is excreted from the liver via the common bile duct into the duodenum of the small intestine. Survival in the gastrointestinal tract suggests relative stability to acid and mild alkaline conditions. The amount of infectious virions shed in the faeces during infection is low, consistent with the low rates of secondary spread during epidemics. Virions remain unaltered after exposure to trifluorotrichloroethane. Outbreaks of HEV have been successfully controlled by chlorination of water supplies. Iodinated desinfectants or autoclaving destroys the virus. For transportation, specimens containing HEV should be kept frozen in dry ice (solid CO_2, -70^{0}C), or preferably in liquid N_2 (-120^{0}C).

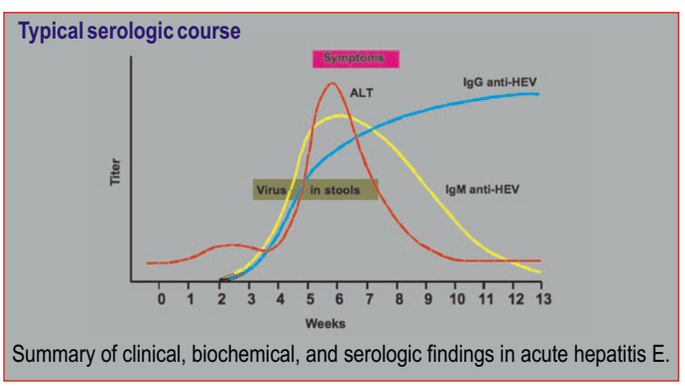
Pathogenesis: In monkeys, viral replication apparently causes liver damage. The immune response successfully eliminates viremia and shedding of virus in faeces, while not inducing much damage to the liver. Seroconversion marks the clearing of virus from faeces and blood and is correlated with resolution of disease. As with hepatitis A, virus is detected in bile, liver and faeces before the onset of liver function abnormalities. Severe or fulminant cases may show submassive and massive hepatic necrosis.

The Disease: Hepatitis E virus causes acute sporadic and epidemic viral hepatitis. Symptomatic HEV infection is most common in young adults aged 15-40 years and is uncommon in children. Although HEV infection is frequent in children, it is mostly asymptomatic and anicteric. The clinical presentation of hepatitis E is comparable to hepatitis A. The incubation period following exposure to HEV ranges from 3 to 8 weeks, with a mean of 40 days. Typical signs and symptoms of hepatitis include jaundice, anorexia, hepatomegaly, abdominal pain and tenderness, nausea and vomiting, and fever, although the disease may range in severity from subclinical to fulminant. Peak viremia and peak shedding of HEV into the faeces occurs during the incubation period and early acute phase of disease. Detection of HEV antigens in the liver generally parallels viremia and faecal shedding of virus. The highest rate of clinically evident disease is typically observed in young to middle-age adults. Lower disease rates in younger age groups may be the result of anicteric or/and subclinical HEV infections. The severity of an HEV infection is generally greater than the severity of an HAV infection. In general, hepatitis E is a self-limiting viral infection followed by recovery. Occasionally, a fulminant form of hepatitis develops, with mortality rates ranging between 0.5% - 4.0% of the overall population of patients. Fulminant hepatitis cases in pregnancy may reach a mortality rate of 20% in the 3rd trimester. Premature deliveries with high infant mortality of up to 33% are also observed. The reason for this high mortality is not clear yet. Some of the complications of pregnancy are toxemia with hypertension, proteinuria, edema,

and kidney lesions. By directly or indirectly affecting the kidneys, HEV might precipitate eclampsia and lead to increased mortality in pregnant women. Common cholestatic jaundice can persist for several weeks. No evidence of chronic inflammation or of a healthy chronic carrier state has been detected, and no recurrence of hepatitis E has been reported. Association with hepatocellular carcinoma or persistent viremia are not features of HEV infection. Coinfection of young children with HEV and HAV may lead to severe forms of disease, including acute liver failure.

Diagnosis: Since cases of hepatitis E are not clinically distinguishable from other types of acute viral hepatitis, diagnosis is made by biochemical assessment of liver function (laboratory evaluation of: urine bilirubin and urobilinogen, total and direct serum bilirubin, ALT and AST, alkaline phosphatase, prothrombin time, total protein, albumin, IgG, IgA, IgM, complete blood count). Acute hepatitis E is diagnosed when the presence of IgM anti-HEV is detected. Storage of serum samples is acceptable for several days at 4^0C, although anti-HEV will be preserved at -20^0C, and a temperature of -70^0C should be preferred when viremia is suspected. Hepatitis E should be suspected in outbreaks of waterborne hepatitis occurring in developing countries, especially if the disease is more severe in pregnant women, or if hepatitis A has been excluded. If laboratory tests are not available, epidemiologic evidence can help in establishing a diagnosis. HEV RNA can be detected in acute phase faeces by PCR in approximately 50% of cases. Immune electron microscopy is positive in only about 10% of cases. The viral proteins pORF2 and pORF3 have been expressed in various recombinant systems and form the basis for diagnostic tests and vaccine studies. To confirm the results of EIA or ELISA tests, Western blot assays to detect IgM and IgG anti-HEV in serum can be used, along with polymerase chain reaction (PCR) tests for the detection of HEV RNA in serum and stool, immunofluorescent antibody blocking assays to detect antibody to HEV antigen in serum and liver, and immune electron microscopy to visualize viral particles in faeces. Currently ICT based (RDTs) formats are also available that can also be used at field settings too.

Host immune response: Viremia in bile and serum and shedding of HEV in faeces reach their peak during the incubation period and keep constant levels in the acute phase of the disease. At the same time, HEV antigens can be detected in the liver. The period of infectivity after acute infection has not been determined, but virus excretion in faeces has been demonstrated up to 14 days after onset of jaundice. Viremia precedes the major peak in ALT activity. Virus excretion in stools continues for up to 14 days after onset of illness, then disappears during the recovery phase. Antibodies to HEV (IgM and IgG) develop at the time symptoms occur, usually before the development of jaundice. IgM anti-HEV precedes the IgG anti-HEV. Viremia may persist after appearance of serum antibodies. Since IgM anti-HEV is usually present for 6-7 weeks but no longer than 3 months after infection its presence is approved of recent infection. Anti-HEV IgM is detectable only in about 80% of the HEV infections whose diagnosis was based on anti-HEV seroconversion. Therefore in a patient with acute hepatitis the absence of anti-HEV IgM does not rule out with certainty underlying acute hepatitis. IgG anti-HEV have been shown to persist for long periods of time (>14 yrs) and provide protection against subsequent infections. Monkeys infected with human HEV are protected against new challenge with homologous or heterologous strains from Asian and African countries. However, the immunity is incomplete since only the clinical disease seems to be prevented, while the virus is still excreted in stools.



Summary of clinical, biochemical, and serologic findings in acute hepatitis E.

Surveillance and Control: Surveillance and control procedures should include: Provision of safe drinking water and proper disposal of sanitary waste. Monitoring disease incidence. Determination of source of infection and mode of transmission by epidemiologic investigation. Detection of outbreaks. Spread containment.

Endemicity: Data on the endemicity of HEV infection have predominantly been collected in areas where outbreaks have been reported. As an exception, seroprevalence studies carried out in Egypt, where outbreaks of HEV have not been noted, showed rates of up to 60%, suggesting that most infections occurred early in life and were asymptomatic or mild. Outbreaks have been reported from Algeria, Bangladesh, Borneo, China, Egypt, Ethiopia, Greece, India, Indonesia, Iran, Jordan, Libya, Mexico, Myanmar, Nepal, Nigeria, Pakistan, southern Russia, Somalia, eastern Sudan, and The Gambia. Most outbreaks have occurred following monsoon rains, heavy flooding, contamination of well water, or massive uptake of untreated sewage into city water treatment plants.

Immune prophylaxis: There is no available immunoglobulin (IG) prophylaxis at present. IG prepared from donors in non-HEV-endemic countries does not prevent infection. The efficacy of IG prepared from donors in HEV-endemic areas is unclear, although convalescent human sera have given promising preliminary results for passive protection. 10 Experimental immune prophylaxis against HEV based on recombinant antigens appears to confer short-term protection and may be useful for pregnant women in endemic areas and travellers coming into these regions.

Vaccines: At present, no commercially available vaccines exist for the prevention of hepatitis E. However, several studies for the development of an effective vaccine against hepatitis E are in progress. Recombinant vaccines: A 55 kDa recombinant HEV-derived ORF2 protein has been used to vaccinate rhesus monkeys against different strains of hepatitis E. Although primates could still be infected, the vaccine protected them from the symptoms of disease. Subunit HEV vaccines: The direct intramuscular injection of purified plasmid DNA containing the full-length ORF2 of HEV has induced a prolonged humoral immune response (>12 months) to the expressed structural protein ORF2 in 80% and 100% of two separate groups of challenged mice, respectively. Because swine HEV is immunologically cross-reactive with human HEV and their capsid genes are very conserved, swine HEV may prove useful as an attenuated vaccine for immunization against human hepatitis E through the "Jennerian" approach.

Prevention: As almost all HEV infections are spread by the faecal - oral route, good personal hygiene, high quality standards for public water supplies and proper disposal of sanitary waste have resulted in a low prevalence of HEV infections in many well developed societies. For travellers to high endemic areas, the usual elementary food hygiene precautions are recommended. These include avoiding drinking water and/or ice of unknown purity and eating uncooked shellfish, uncooked fruits or vegetables that are not peeled or prepared by the traveller. Vaccines or specific IG preparations are currently under development.

Treatment: As no specific therapy is capable of altering the course of acute hepatitis E infection, prevention is the most effective approach against the disease. As with hepatitis A, hepatitis E patients generally do not require hospitalization. Admission is required for fulminant hepatitis and should be considered for infected pregnant women.

Guidelines for epidemic measures: Determination of mode of transmission. Identification of the population exposed to increased risk of infection. Elimination of common source of infection. Improvement of sanitary and hygienic practices to eliminate faecal contamination of food and water.

Future considerations: The development of more sensitive and more specific serologic tests for IgM and total anti-HEV antibodies will provide insight into the epidemiology of the disease. The manufacture of hyperimmune E globulin and the production of a vaccine are essential for the control of the disease. There is a need for determining the durability of anti-HEV neutralizing antibody after natural infection or vaccination. The development of differential diagnostic tests to distinguish between infections with swine HEV and human HEV is necessary. The pathogenesis of the disease, especially in infected pregnant women, needs to be elucidated. International measures should be established.

INTERPRETATION

Immunoglobulin E

Immunoglobulin E (IgE) is a class of antibody (or immunoglobulin "isotype") that has only been found in mammals. It plays an important role in allergy, and is especially associated with type 1 hypersensitivity. IgE has also been implicated in immune system responses to most parasitic worms like *Schistosoma mansoni, Trichinella spiralis*, and *Fasciola hepatica*, and may be important during immune defense against certain protozoan parasites such as *Plasmodium falciparum*. Although IgE is typically the least abundant isotype - blood serum IgE levels in a normal ("non-atopic") individual are ~75 ng/ml compared to 10 mg/ml for the IgGs (the isotypes responsible for most of the classical adaptive immune response) - it is capable of triggering the most powerful immune reactions. IgE was discovered in 1966 by the Japanese scientist Kimishige Ishizaka.

Receptors: IgE elicits an immune response by binding to Fc receptors found on the surface of mast cells and basophils, and are also found on eosinophils, monocytes, macrophages and platelets in humans. It has two main receptors:

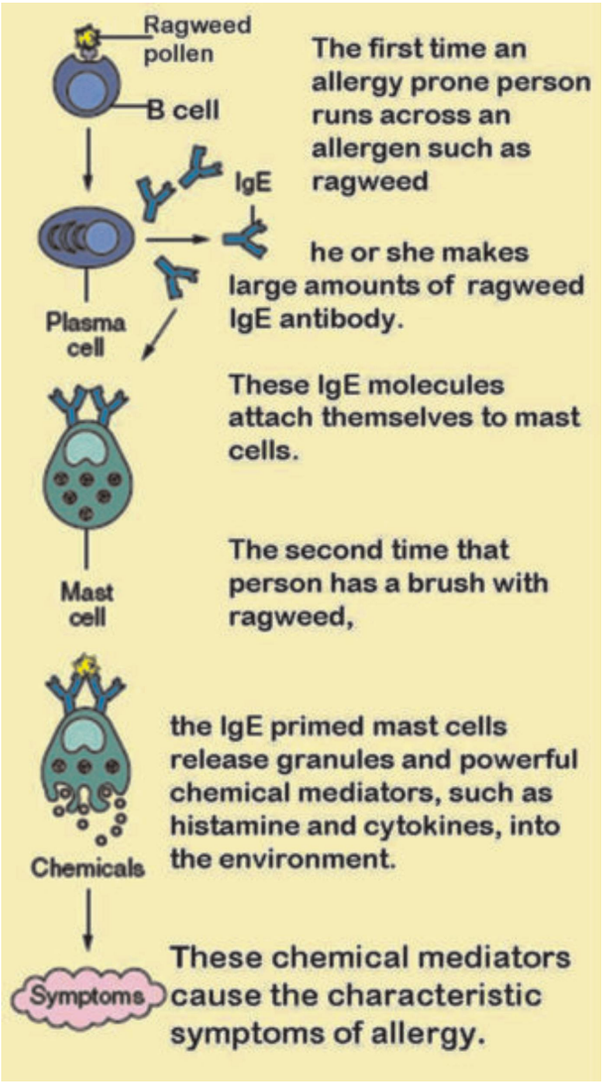
- Fc RI, the high-affinity IgE receptor
- Fc RII, also known as CD23, is the low-affinity IgE receptor

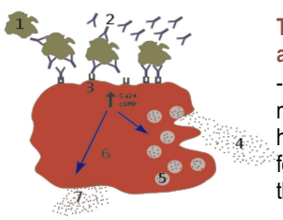
IgE can upregulate the expression of both Fc receptors. Fc RI is expressed only on mast cells and/or basophils in both mice and humans. Aggregation of antigens and binding of IgE to the Fc RI on mast cells causes degranulation and the release of mediators from the cells, while basophils cross-linked with IgE release type 2 cytokines like interleukin-4 (IL-4) and interleukin-13 (IL-13) and other inflammatory mediators. The low affinity receptor (Fc RII) is always expressed on B cells, but its expression can be induced on the surfaces of macrophages, eosinophils, platelets and some T cells by IL-4.

Physiology: There is much speculation into what physiological benefits IgE contributes, and so far, circumstantial evidence in animal models and statistical population trends have hinted that IgE may be beneficial in fighting gut parasites such as Schistosoma mansoni, but this has not been conclusively proven in humans. Although it is not yet well understood, IgE may play an important role in the immune system recognition of cancer in which the stimulation of a strong cytotoxic response against cells displaying only small amounts of early cancer markers would be beneficial. Of course, if this were the case, anti-IgE treatments such as omalizumab might have some undesirable side effects.

Role in disease: Atopic individuals can have up to 10 times the normal level of IgE in their blood (as do sufferers of hyper-IgE syndrome). However, this may not be a requirement for symptoms to occur as has been seen in asthmatics with normal IgE levels in their blood - recent research has shown that IgE production can occur locally in the nasal mucosa, without the involvement of lymphoid tissue. IgE that can specifically recognise an "allergen" (typically this is a protein, such as dust mite DerP1, cat FelD1, grass or ragweed pollen, etc.) has a unique long-lived interaction with its high affinity receptor, Fc RI, so that basophils and mast cells, capable of mediating inflammatory reactions, become "primed", ready to release chemicals like histamine, leukotrienes and certain interleukins, which cause many of the symptoms we associate with allergy, such as airway constriction in asthma, local inflammation in eczema, increased mucus secretion in allergic rhinitis and increased vascular permeability, ostensibly to allow other immune cells to gain access to tissues, but which can lead to a potentially fatal drop in blood pressure as in anaphylaxis. Although the mechanisms of each response are fairly well understood, why some allergics develop such drastic sensitivities when others merely get a runny nose is still one of science's hot topics. Regulation of IgE levels through control of B cell differentiation to antibody-secreting plasma cells is thought to involve the "low affinity" receptor, Fc RII or CD23. CD23 may also allow facilitated antigen presentation, an IgE-dependent mechanism whereby B cells expressing CD23 are able to present allergen to (and stimulate) specific T helper cells, causing the perpetuation of a Th2 response, one of the hallmarks of which is the production of more antibodies.

Pharmacology: IgE may be an important target in treatments for allergy and asthma. Currently, severe allergy and asthma is usually treated with drugs (like anti-histamines) that damp down the late stages of inflammation and relax airway smooth muscle. Unfortunately, these treatments are fairly broad in their action, and so many have unpleasant side effects; they may also inhibit important protective responses. In 2002, researchers at The Randall Division of Cell and Molecular Biophysics determined the structure of IgE. Understanding of this structure (which is atypical of other isotypes in that it is highly bent and asymmetric), and of the interaction of IgE with receptor Fc RI will enable development of a new generation of allergy drugs that seek to interfere with the IgE-receptor interaction. A new treatment, omalizumab, a monoclonal antibody, recognises IgE not bound to its receptor and is used to neutralise or mop-up existing IgE and prevent it from binding to cells. It may be possible to design treatments cheaper than monoclonal antibodies (for instance, small molecule drugs) that use a similar approach to inhibit IgE binding to its receptor. In 1975 Robert N. Hamburger, M.D. published "Peptide Inhibition of the P-K Reaction" based on blocking up to 89% of the IgE receptors on mast cells by the pentapeptide representing amino acids 320 to 324 on the epsilon chain of IgE.





The role of mast cells in the development of allergy. Degranulation processes 1 - antigen; 2 - IgE antibody; 3 - Fc RI receptor; 4 - preformed mediators (histamine, proteases, chemokines, heparine); 5 - granules; 6 - mast cell; 7 - newly formed mediators (prostaglandins, leukotrienes, thromboxanes, PAF).

TROUBLESHOOTING

Helicobacter pylori Tests

Helicobacter pylori tests are used to detect a *Helicobacter pylori* (*H. pylori*) infection in the stomach and upper part of the small intestine (duodenum). *H. pylori* can cause peptic ulcers; however, most people with *H. pylori* in their digestive systems do not develop ulcers. Four tests are used to detect *H. pylori*: Blood antibody test: A blood test to ascertain if the patient has antibodies to *H. pylori* bacteria. If found to be positive it implies that the patient is infected or has been infected in the past. Urea breath test: A urea breath test checks to find if the patient has *H. pylori* bacteria in his stomach. The breath test is not always available. Stool antigen test: A stool antigen test checks to see if substances that trigger the immune system to fight an *H. pylori* infection (*H. pylori* antigens) are present in the feces. Stool antigen testing may be done to help support a diagnosis of *H. pylori* infection or to determine whether treatment for an *H. pylori* infection has been successful. Stomach biopsy: A small sample (biopsy) is taken from the mucosa of the stomach and small intestine during an endoscopy. Several different tests may be done on the biopsy sample. Elisa/rapid immunochromatographic formats are also available.

Why It Is Done

A *Helicobacter pylori* (*H. pylori*) test is done to: Determine whether an infection with *H. pylori* bacteria may be causing an ulcer or gastritis. Determine whether treatment for an *H. pylori* infection has been successful.

How To Prepare

Blood antibody test or stool antigen test: No special preparation is required.

Stomach biopsy or urea breath test: The patient should not eat or drink for at least 6 hours before a breath test or a stomach biopsy. Many medicines may change the results of this test. The clinician must be informed about all the prescription and nonprescription medicines one takes. He may recommend that the patient should stop taking some of your medicines for up to 1 week before having this test. The patient should not take antibiotics, proton pump inhibitors (such as omeprazole or pantoprazole), or medicines containing bismuth (such as Pepto-Bismol) for 1 to 2 weeks before the test. The patient should not take H_2 blockers, such as Pepcid AC, Zantac, Axid, or Tagamet for 24 hours before the test.

How It Is Done

Blood antibody test: By venepuincture withdraw adequate amount of blood.

Urea breath test: The breath sample is collected when the patient blows into a balloon or blow bubbles into a bottle of liquid. The health professional taking a sample of your breath will: Collect a sample of your breath before the test starts. Give the patient a capsule or some water to swallow that contains tagged or radioactive material. Collect samples of your breath at different times. The breath samples will be tested to see whether they contain material formed when *H. pylori* comes into contact with the radioactive material. The urea breath test usually takes about 1.5 hours.

Stool antigen test: The stool sample for this test may be collected at home. If the patient is in the hospital, a health professional will help collect the sample. To collect the sample, one needs to: Pass stool into a dry container. Either solid or liquid stools can be collected. Be careful not to get urine or toilet tissue in with the stool sample. Replace the container cap and label the container with the patients name, referring doctor's name, and the date the sample is collected. The clinical attendent/ laboratarian should wash his/ her hands well after collecting the sample to avoid spreading bacteria. Deliver the sealed container as soon as possible to the laboratory. Sometimes the clinician may also use a cotton swab inserted into the patients rectum to collect a stool sample during an exam.

Stomach biopsy: Endoscopy is used to collect samples of tissue from the stomach and duodenum. The gastroenterologist may collect up to ten tissue samples. The tissue samples are tested in the lab to see if they contain *H. pylori*. In rare cases, a biopsy sample may be placed in a container that promotes the growth of *H. pylori* bacteria. A culture and sensitivity test may be conducted if felt appropriate.

How It Feels

Blood antibody test: Venepuncture usually doesn't cause any problem.

Urea breath test: A urea breath test does not normally cause discomfort.

Stool antigen test: Collecting a stool sample normally does not cause any discomfort. If the clinician collects the sample during a rectal exam, one may feel some pressure or discomfort as the cotton swab is inserted into the rectum.

Stomach biopsy: The local anesthetic sprayed into the throat usually tastes slightly bitter and will make the tongue and throat feel numb and swollen. Some people report that they feel as if they cannot breathe at times because of the tube in their throat, but this is a false sensation caused by the anesthetic. There is always plenty of breathing space around the tube in the mouth and throat. One should try to relax and take slow, deep breaths. One may experience some gagging, nausea, bloating, or mild abdominal cramping as the tube is moved. Even though the patient won't be able to talk during the procedure because he has a tube in his throat, he can still communicate. If the discomfort is severe, alert the doctor with an agreed-upon signal or a tap on the arm. The IV medications may make one feel sleepy. Other side effects-such as heavy eyelids, difficulty speaking, a dry mouth, or blurred vision-may last for several hours after the test. The medications may also cause one not to remember much of what happens during the test.

Risks

Blood antibody test: There is very little chance of a problem from having a blood sample taken from a vein. One may get a small bruise at the site. The patient can lower the chance of bruising by keeping pressure on the site for several minutes. In rare cases, the vein may become swollen after the blood sample is taken. This problem is called phlebitis. A warm compress can be used several times a day to treat this. Ongoing bleeding can be a problem for people with bleeding disorders. Aspirin, warfarin (Coumadin), and other medicines that thin blood can make bleeding more likely. If the patient has bleeding or clotting problems, or if on anti-coagulnts, the patient should inform the phlebotomist before the blood sample is taken.

Urea breath test: There are no known risks or complications with a urea breath test. If radioactive carbon is used, the amount of radioactivity exposure is extremely small-less than one normally gets from being outside during the day.

Stool antigen test: There are no risks or complications with a stool sample. However, if you do not wash your hands well after collecting the sample, you may spread germs.

Stomach biopsy: There is a slight risk (1 in 10,000) of puncturing the wall of the esophagus, stomach, or duodenum during an endoscopy to collect stomach biopsy samples. The biopsy may also cause some bleeding at the site where the samples are collected. However, the bleeding usually stops without treatment..

After the test

After the test, one may belch and feel bloated for a while. He also may have a tickling, dry throat; slight hoarseness; or a mild sore throat. These symptoms may last several days. Appropriate lozenges and warm saltwater gargles can help relieve the throat symptoms. One should not drink alcohol after the test. After the test, the patient should contact his clinician immediately if he: Vomit blood or notice black or bloody stools. Has trouble swallowing or talking. Is short of breath or have a fast heartbeat. Has increasing chest or abdominal pain. Has neck or shoulder pain. Has a fever.

Results

Helicobacter pylori tests are used to detect a *Helicobacter pylori* (*H. pylori*) infection in the stomach and duoenum. Results from the urea breath test or a stool antigen test are generally available within a few hours. Results from a blood antibody test are usually available within 24 hours. Results from biopsy samples obtained by endoscopy are usually available within 48 hours. Results from a biopsy sample that is cultured can take up to 10 days.

Blood antibody test: Normal: The blood sample does not contain *H. pylori* antibodies. Abnormal: The blood sample contains *H. pylori* antibodies.

Urea breath test: Normal: The breath sample does not contain the tagged hydrocarbon. Abnormal: The breath sample contains the tagged hydrocarbon.

Stool antigen test: Normal: The stool sample does not contain *H. pylori* antigens. However, a negative stool antigen test does not always mean that the patient does not have an *H. pylori* infection. Abnormal: The stool sample contains *H. pylori* antigens.

Stomach biopsy:
Normal: The biopsy sample does not contain *H. pylori* bacteria. *H. pylori* bacteria does not grow in a culture of the tissue biopsy samples.
Abnormal: The biopsy sample contains *H. pylori* bacteria. *H. pylori* bacteria grows in a culture of the tissue biopsy samples.

What Affects the Test

Reasons one may not be able to have the test or why the results may not be helpful include the following: The radioactive urea breath test for *H. pylori* is not usually done during pregnancy or while the patient is breast-feeding, because the radiation could harm the child. Use of antibiotics may affect the results of the urea breath test, the stool antigen test, and stomach biopsy by reducing the number of *H. pylori* bacteria in the stomach and duodenum. The use of lansoprazole, rabeprazole, sucralfate, omeprazole, famotidine, ranitidine, nizatidine, cimetidine, or medicines containing bismuth (such as Pepto-Bismol) can also interfere with the results of the urea breath test and stomach biopsy. A stomach biopsy may not detect an *H. pylori* infection that is present if the biopsy samples are taken from areas that are not infected by the *H. pylori* bacteria. Rough handling, contamination, or inadequate refrigeration of the blood sample can cause inaccurate blood antibody test results. When a blood antibody test is done early in an *H. pylori* infection, the results may be falsely negative because the level of antibodies is too low to measure. The likelihood of infection with *H. pylori* increases with age; older adults are more likely to have detectable amounts of the bacteria in their body.

Important Considerations

The radioactive urea breath test is not recommended for children or for pregnant or breast-feeding women because of exposure to a small amount of radioactivity. The stool antigen test is the newest and least expensive of the four tests for *Helicobacter pylori*, but it may not be as accurate as the other tests. The stomach biopsy is very accurate, but it is the most expensive and most risky of the four tests. A negative stool antigen test does not always mean that an *H. pylori* infection is not present. Although many people are infected with *H. pylori* bacteria, only a few of them will develop peptic ulcer disease. For this reason, other factors (such as a person's symptoms) should be considered when interpreting the results of an *H. pylori* test. Blood tests for *H. pylori* may be positive for several years after the infection; therefore, the urea breath test or a biopsy may be used to determine if treatment has been effective. If one's symptoms persist, an endoscopy may be needed. Having an infection with *H. pylori* increases one's chances of having cancer of the stomach; but the risk is very low.

BOUQUET

In Lighter Vein

A blonde is driving home and she gets caught in a really bad hailstorm. The hail is as big as tennis balls, and she ends up with her car covered with large dents. So the next day she takes her car to the repair shop.

The shop owner, seeing she is blonde, decides to have a little fun. He tells her just to go home and blow into the tail pipe, really hard, and all the dents will just pop out.

The blonde drives home, gets out of the car, gets down on her hands and knees and starts blowing into the tail pipe.

Nothing happened. So she blew a little harder, and still nothing happens. Meanwhile, her roommate, also a blonde, comes home and asks,"What in the world are you doing?"

The blonde car owner tells her how the repairman had instructed her to blow into the tailpipe in order to get all the hail dents to pop out.

Her blonde roommate rolls her eyes and says, "Hell-OOOO! Don't you think you should roll up the windows first?

Two friends, a blonde and a redhead, are walking down the street and pass a flower shop where the redhead happens to see her boyfriend buying flowers. She sighs and says, 'Oh, crap, my boyfriend is buying me flowers again.' The blonde looks quizzically at her and says, 'You don't like getting flowers?' The redhead says, 'I love getting flowers, but he always has expectations after giving me flowers, and I just don't feel like spending the next three days on my back with my legs in the air.'

The blonde says, "Don't you have a vase?"

Wisdom Whispers

- "To be a fool at the right time is also an art."
- "He who awaits much receives little."
- "One's shadow grows larger than life when admired by the light of the moon."
- "Women when injured are generally not easily appeased."
- "It is difficult to tie an unborn horse to the manger."
- "Grief pent up will burst the heart."
- "Haste makes waste and waste makes want."
- "No need to teach an eagle to fly."
- "Small men think they are small; great men never know they are great."

Brain Teasers

CHOOSE THE MOST APPROPRIATE/ CORRECT ANSWER.

1. Pelger Huet Anomaly
   A. Bilobed neutrophils B. Indented nuclei of lymphocytes
   C. Notched monocytic nuclei D. Hypersegmented neutophilic nuclei
2. For obtaining 1 ml of serum one should draw about … ml of blood
   A. 1.5 B. 2.5 C. 3.5 D. 4.5
3. Increased osmotic fragility is a feature of
   A. Iron deficiency anaemia B. Spherocytes
   C. Thalassaemia D. Reticulocytes
4. Schumm's test is done to estimate …. of plasma
   A. Hemopexin B. Haptoglobin C. Hemoglobin D. Methaemalbumin
5. The mean platelet life span is about… days
   A. 4-6 B. 8-10 C. 12-16 D. 21-28
6. The optimum diamteter for an ESR tube/ pipette is … mm.
   A. 1.5 B. 2.5 C. 3.5 D. 4.5

Answers: 1. A., 2. B., 3. B., 4. D., 5. A., 6. B.

Tulip Group Unfolds The Insight Range Gradually

Water-borne, food borne viral hepatitis may not be due to HAV alone, HEV may also be the causative organism. Detect and differentiate with...

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TULIP NEWS

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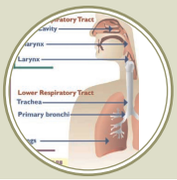
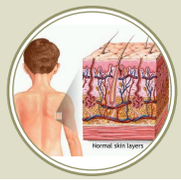


Reactive Airway Disease

Worm infestation



Skin allergies

Atopic dermatitis

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