Chapter

Hepatitis E: Disease in Humans

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Abstract

Hepatitis E virus (HEV) is one of the 7 viruses with mainly hepatic tropism. HEV determines 20 million new infections worldwide every year, 3.4 million acute hepatitis E and 44,000 deaths in 2015 (3.3% of the mortality due to viral hepatitis). Transmitted by the digestive tract mainly (fecal- orally, particularly by water infected with feces), the virus reaches the liver where it does not have a direct cytolytic effect, but immunological phenomena, especially cellular, activated by the replication of the virus in the hepatocytes. Clinically, over 95% of cases of HEV infection are asymptomatic and sel- limiting; in immunocompetent patients in tropics HEV can cause acute hepatitis with clinical features. On rare situations the infection can result in a severe, fulminant hepatitis with acute liver failure. In immunocompromised patients (organ transplant recipients, hematologic malignancies, HIV-infected) HEV may determine chronic hepatitis. In pregnant women or the elderly people or people with underlying liver disease HEV can cause fulminant forms which can become fatal (E.g.: 30% deaths among pregnant women in some parts of the world). Acute and chronic E hepatitis may be accompanied by extrahepatic manifestations: neurological, kidney, pancreatic, hematological diseases, autoimmune diseases with a pathogenesis not fully elucidated.

Keywords: hepatitis E virus (HEV), acute hepatitis, acute liver failure (ALF), acute-on-chronic liver failure (ACLF), cellular immunity, pregnant women, extrahepatic manifestations

1. Introduction

Hepatitis E virus (HEV) infection is a global public health problem. The World Health Organization (WHO) estimates that there are about 20 million HEV infections worldwide per year with a 3.3 million symptomatic cases E and approximately 44,000 deaths in 2015 [1].

HEV infection is a disease transmitted by enterically mainly in worldwide, special in the tropical countries. The source for infection is represented by zoonotic HEV - pigs, wild boar, deer camels (Genotype 7- GT 7) [2]. Transmission can be done through: direct contact with HEV infected animals, through heat processed meat incorrectly or through water of lagoons, streams and rivers polluted with the feces of sick animals. As such, other marine filter animals can become infected and transmit the disease (E.g. mollusks and seafood) or fruits and vegetables irrigated with infected fecaloid water. Organ transplantation in industrialized countries and blood products represent other ways of contamination for humans [2]. The virus can also be vertically transmitted from infected mother to fetus [3].

Hepatitis E occurs most commonly in adult men and with a lower prevalence in children [4].

In terms of clinical manifestations most infections caused by HEV in immunocompetent persons are asymptomatic (over 95%) and self-limiting; acute liver failure is rare [5]. In immunocompromised patients (solid organ transplant recipients, the patients with pre-existent chronic liver diseases, HIV infected patients, hematologycal diseases) HEV can cause chronic hepatitis, which may have an unfavorable evolution to acute fulminant hepatitis with to acute liver failure (ALF) or to cirrhosis [6]. In pregnant women, acute hepatitis can be benign or sever [7]; sever forms may occur during the third trimester with severe damage to the mother and fetus and their death (around 30% in India) [7, 8].

Extra-hepatic manifestations can also occur in patients with acute or chronic HEV infection such as neurological abnormalities (Guillain–Barre syndrome- GBS, neuralgic amyotrophy- NA, encephalitis and myelitis), acute pancreatitis, hematological disorders, kidney failure [9].

The pathogenesis of HEV infection is very complex and still unexplained. It involves the intervention of two categories of factors: the host organism and HEV. The host organism tries to stop the infection caused by HEV, and HEV tries to overcome the opposite barriers by the human body. This fight results in various clinical pictures of HEV infection.

There is a genetic predisposition of the human body to HEV infection, an increased susceptibility to this virus, to which is added the innate immunity and the adaptive response of the human body [7]. However, HEV uses different means to escape the defense of the human body, especially the genetic variation that leads to the appearance of genotypes, subgenotypes and quasi-species of HEV or a recombinant variants HEV-host cell with a different pathogenicity [2, 7], in general severe pathogenicity. HEV is not cytopathic in the liver, but it activates immune means, especially cellular immunity in determining liver damage.

One particular aspect is related to the pathogenicity of HEV in pregnancy when HEV infection can become fatal. In this situation there is a constellation of factors (immune, hormonal, viral, fetal) that can lead to severe clinical forms of hepatitis E with the death of the mother and fetus [10].

2. Clinical features- the main clinical manifestations of HEV infection

2.1 *A*cute hepatitis E in immunocompetent people

In developing countries most infections caused by HEV in immunocompetent persons are asymptomatic (over 95%) [6] and lead to spontaneous clearance of the virus [11] and acute liver failure is rare [5].

Clinically, manifest forms are found in only 5% of cases, especially in men aged 15–30 years as E acute hepatitis. These forms have an incubation period of 2–8 weeks (median 30 days) [12].

Clinical onset is characterized by non-specific symptoms: fever, nausea, vomiting, abdominal pain, anorexia, malaise and hepatomegaly; jaundice occurs only in 60% of cases accompanied by itching and light-colored stool and darkened urine [12].

The laboratory shows: increase in ALT (alanine aminotransferase), AST (aspartate aminotransferase) values (ALT is greater than AST), frequently accompanied by altered bilirubin, alkaline phosphatase and gamma-glutamyl transferase (GGT) [10].

E Acute Hepatitis is self-limiting illness, with full resolution of symptoms within weeks (usually) to months (less commonly) of onset. Viremia usually peaks during the early symptomatic phase and becomes undetectable about two weeks thereafter; excretion of the virus in the feces remains 2–3 weeks longer [10]. Progression of acute HEV infection to fulminant liver failure (FLF) remains rare and in the

literature there are only 2 examples of HEV induced acute fulminant failure requiring emergency transplantation [13].

This clinical manifestations are especially present in the Tropics where HEV-infections are endemic or epidemic (e.g. Asia and Africa-Western Africa, Latin America- as Mexico) and contaminated water and reduced hygienic conditions represents the source for fecal- orally transmission.

In developed countries, patients infected with HEV are usually middle-aged or elderly men (>55 years). In developed countries, in immunocompetent persons HEV infections is less severe. Severe HEV infections were not described in pregnant women. Severe forms can be found in these countries, in rare situations, in immunocompromised patients such as the elderly people or patients with chronic liver disease of other etiology, in whom HEV can cause acute liver failure [11].

2.2 Acute hepatitis E in chronic liver diseases patients

Acute hepatitis E is a concern in patients with underlying chronic liver disease. This is a particular problem in elderly patients where acute hepatitis may take a more severe course [2]. In patients with chronic liver disease, acute HEV infection causes a rapid deterioration of liver function (acute-on-chronic liver failure-ACLF) with the appearance of complications such as: ascites, hepatic encephalopathy and/or hepatic coagulopathy, which can lead to death (up to 70% of cases) [13].

2.3 Acute hepatitis E in pregnancy

In pregnant women, acute hepatitis with HEV etiology may have various clinical aspects. The clinical features of E acute hepatitis depend on several factors.

One of these factors is the geographical area in which the pregnant woman lives. So, HEV infection in pregnant women may be present with a higher or lower incidence and may be benign or severe, accompanied by an increased mortality rate or not. Example: in Northern India where HEV infection is endemic, HEV infection in pregnant women has a high incidence (representing 60% of viral hepatitis) and clinically is severe, evolving in 55% of cases with fulminant liver failure (FLF) that can leads to a maternal mortality of 41% [14]; in Egypt, another country where HEV infection is endemic, the incidence of HEV infection in pregnant women is lower and severe forms are rarely, although Egypt is part of the category of HEV endemic countries, compared to Northern India [14].

Another factor that influences the incidence and clinical appearance of HEV infection in pregnant women is the level of sanitation in that area, so there is a difference in the incidence and clinical feature of the disease in developed countries compared to the tropical region where the level of hygiene it is reduced and favors the fecal-oral transmission (especially water contaminated with feces) of HEV to pregnant women [14].

Other factors that may influence the clinical manifestation in pregnant women are:

- exposure to HEV infection, especially in early childhood, which leads to a protective immune fund, producing long-lasting immunity and/or modify subsequent responses to exposure to the virus; so the incidence of HEV infection may be lower and the severity of the disease rare [3, 15];

- different virulence of infectious HEV strains. E.g. HEV genotype(s) in Egypt could be less virulent than those in Asia [14];

- the pregnancy status, that means hormonal factors specially [8]

Conclusion: the clinical features of HEV infection of pregnant women can be benign or severe, with fulminant hepatic insufficiency that can lead to death, depend on a constellation of factors related to: the host (hormonal factors, immune status, nutritional, genetic status, infectious history), the infectious viral strain and external factors (e.g.: the hygienic-sanitary level, the prevalence of HEV infection in the respective area/country).

The mortality rate in pregnant women can be as high as 30% and usually occurs in the 3rd trimester [3] by obstetric complications such as hemorrhage or eclampsia, fulminant liver failure, premature delivery, low-birth-weight neonates and still-births, as well as the vertical transmission to infants, which leads to increased neonatal morbidity and mortality [10, 16]. HEV infection during pregnancy is also associated with more frequent miscarriages, preterm deliveries and perinatal mortality [7].

2.4 Acute and chronic HEV infectious in immunocompromised hosts

In immunocompromised patients, including solid organ transplant (SOT) [17] and those coinfected with the human immunodeficiency virus (HIV) with a T CD4+ count <200/mm^3 [7], patients with hematological disease receiving chemotherapy, those given stem cell transplants or patients with rheumatic disorders with heavy immunosuppression secondary the immunotherapy [7], HEV can determines acute or chronic hepatitis.

Acute HEV in immunocompromised patients generally presents asymptomatically [17]; in the case of clinical manifestation the symptoms are non-specific as well: jaundice, fatigue, diarrhea and myalgia [11].

Chronic hepatitis HEV infection in immunocompromised patients defined as HEV replication that persists for more than 3 months [18], can progress rapidly to cirrhosis in 10% of the chronically infected patients [7, 19]. Some of these patients may die from decompensated cirrhosis 2–3 years after 1the diagnosis [7]. HEV-infected transplant recipients did not develop fulminant forms [20].

The incubation period for the virus in the context of immunosuppression is longer than seen in immunocompetent hosts at 60 days, with chronicity itself being defined by viral persistence after the acute phase for either 3 or 6 months [21]. Chronic HEV infection in immunocompromised patients is almost exclusively secondary to HEV G3 infection; one case of chronic HEV G4 infection has been noted but none due to HEV G1 or G2 [21].

2.5 Extra-hepatic manifestations

Extra-hepatic manifestations can also occur in patients with acute or chronic HEV infection such as neurological (Guillain–Barré syndrome; GBS), neuralgic amyotrophy and meningoencephalitis, myositis, Bell's palsy, vestibular neuritis and peripheral neuropathy), acute pancreatitis, hematological disorders (aplastic anemia, hemolytic anemia, cryoglobulinemia, thrombocytopenia, monoclonal immunoglobulin), kidney failure and others (myocarditis, autoimmune thyroiditis, arthritis) [2].

3. Pathogenesis

The pathogenesis of hepatitis E is complex and still to be studied. Infection in humans with HEV is the result of 2 categories of factors: viral factors and factors related to the host organism.

3.1 Viral factors

HEV enters the human body through the digestive tract, especially fecal-oral. The intestine is the first site where HEV suffers the replication [22] or lymph nodes or colon [6] via the blood as a quasi-enveloped particle and reaches the liver for which it manifests a high tropism where it replicates, especially in the hepatocytes, without being directly cytotoxic, but with the initiation of immune phenomena, especially on the cell line by activating cytotoxic T lymphocytes and natural killer (NK) cells, that causes necro-inflammatory liver damages [23]. After replication in the hepatocyte cytoplasm, the virus is eliminated in the bile (at the apical membrane) [23, 24] and blood; most HEV particles are released at the apical membrane, then bile salts strip the lipids from the virus shed in the stool.

In the liver, the virus must enter the hepatocyte into which it will be replicated. In this process, the first stage of attaching the virus to the surface of the hepatocytes is extremely important in the viral development cycle replication, so as to HEV infection can be initiated or not. The attachment of the virus to the surface of the hepatocytes is achieved by fixing it at certain receptors from the surface of the hepatocytes resulting in viral inoculum.

3.1.1 The attachment of viral particles to the surface of the hepatocytes is different, depending on the morphology of the viral particles

HEV was discovered in 1983 by Dr. Mikhail Balayan who described the HEV particle as non-enveloped in the feces, with icosahedral symmetry, 27–30 nm, with spikes on the surface [6]. But in infected people, viral particles were also found in the blood. Under the electron microscope these particles appear enveloped, the capsid encased in limiting host-derived membranes; these particles have been called „quasienveloped" forms or „eHEV" [6, 7]. So, there are 2 categories of viral particles: enveloped and non-enveloped (naked). Each of them uses a specific means of attachment to the host cell. This is a very important step for HEV pathogenesis because the rate of attachment and penetration of the virus into hepatocytes influences the value of the viral inoculum, one of the viral pathogenic factors.

3.1.1.1 Attachment of non-enveloped particles

The receptor for HEV is unknown. However, the host cell provides a number of factors that can be used as receptors for the non-enveloped (naked) viral particles, so:

Heparan Sulfate Proteoglycans (HSGPs) - are glycans present on the cell surface that are involved in cell attachment of many nonenveloped and enveloped viruses. HSGPs, particularly syndecans, play a role in the binding of HEV VLPs to human hepatoma cells [25].

Glucose-Regulated Protein 78 (GRP78)- is a molecular chaperone in the ER, implicated in the attachment and entry of both enveloped and nonenveloped viruses [26].

Asialoglycoprotein Receptor (ASGPR)- is a protein receptor present on the basolateral membrane of hepatocytes that binds glycoproteins that lack sialic acid modifications. Experimentally, through different techniques, a direct interaction between the ectodomain of both ASGR1 and ASGR2 and HEV ORF2 was highlighted [27].

ATP Synthase Subunit 5β (ATP5B)- is largely a mitochondrial protein, but a small fraction is expressed on the cell surface and is implicated in other viral

infections [28]. Experimentally, the link between ATP5B and viral proteins p239 VLP has been demonstrated [28].

Integrin Alpha 3 (ITGA3)- a new HEV entry factor into the cell: overexpression of α3 integrin in nonpermissive cells made the cells permissive to HEV, while removal of α3 integrin in permitted cells abrogated permissiveness [29].

Conclusion: for non-enveloped (naked) viral particles, the range of receptors offered by the host cell is large, as such there is the possibility of making a high value viral inoculum that can cause even severe clinical manifestations.

3.1.1.2 Attachment of „quasienveloped" particles or "eHEV"

Quasi-coated HEV particles do not have viral proteins on the surface of their envelope, so, they must use different attachment factors and/or cellular receptors to initiate entry into the host cell. Thus, these qvasiparticles use for attachment to the host cell, as in the case of exosomes, the phosphatidylserine present at their outer membrane to bind at the TIM-1 receptor. However, this attachment to the surface of the host cell is less efficient than in the case of non-enveloped particles, which led to the theory that other unidentified substances present on the surface of non-enveloped particles participate in attaching of these particles to the host cell surface and make it inefficient [29]. On the other hand, this less efficient connection of this type of particles to the surface of the hepatocytes would explain their presence in the blood and could facilitate its penetration of immunologically privileged sites such as the central nervous system and other tissues and causes extrahepatic manifestations [30].

Similar to naked particles, eHEV enters hepatocytes mainly through the clathrin- and dynamin-dependent pathway [30, 31] or use a particular pathway that involves in degradation of the lipid membrane in the lysosome [30].

Conclusion: non-enveloped viral particles bind much more efficiently to the surface of hepatocytes, compared with eHEV; this aspect influences the clinical manifestations of the disease: the non-enveloped particles are located mainly in the liver and usually cause liver damages, while the eHEV cause especially extrahepatic manifestations.

3.1.2 Viral genetic variability- another pathogenic factor

So far, 8 HEV genotypes are known [8] with different hosts, including humans, and a certain geographical spread. Genotypes 1–4 and 7 are present in humans; genotype 1 (HEV-1) is predominant in Asia and Africa, HEV-2 in Mexico and parts of Africa, HEV-3 circulates among human, swine, rabbit and deer and has a world-wide distribution, HEV-4, mostly present in Southeast Asia, circulates between human and swine; HEV-5 and HEV-6, phylogenetically close to HEV-4, were identified in Japanese wild boars [31]; HEV-7 and the last, HEV-8 were identified as camel genotypes in the Middle East [32]; HEV-7 was implicated in chronic HEV infection in a liver transplant recipient consuming camel milk and meat [31], which suggests the possible transmission of this genotype to human and the possibility to affecting human health [23].

3.1.2.1 The correlation clinical features - viral genotypes

There is a close correlation between the clinical features caused by HEV and the viral genotypes, which demonstrates a different pathogenicity of HEV. So:

In case of acute hepatitis in immunocompetent hosts - genotypes G1 and G2 from tropical countries and endemic areas determine more aggressive forms of acute hepatitis [12] with clinical manifestations and changes in biochemical

parameters [2], compared to genotypes G3 and G4 [12]. The evolution of acute HEV infection to fulminant liver failure remains rare and there are only two examples in the literature of HEV acute fulminant liver failure that required emergency transplantation [11, 33].

In case of acute hepatitis in pregnancy: in pregnant women, particularly in the third trimester, HEV infection is associated with devastating maternal and fetal outcomes [21]. In this context, acute hepatitis with HEV is associated with the G1 genotype [11].

In case of immunocompromised hosts (SOT patients, HIV-infected patients or patients with chronic granulomatous diseases or connective tissue disorders like SLE or patients with hematological diseases receiving chemotherapy, those given stem cell transplants or patients with rheumatic disorders on heavy immunosuppression immunotherapy [7, 20, 34]: the incubation period for the virus in the context of immunosuppression is longer than seen in immunocompetent hosts at 60 days, with chronicity itself being defined by viral persistence after the acute phase for either 3 or 6 months [21]; they are not able to spontaneously clear the virus and as early as 12 months after HEV infection can involve significant hepatic fibrosis [35]. The genotype associated with these pathological conditions is HEV G3 [21].

In patients with pre-existing chronic liver diseases: HEV infection may exacerbate chronic liver failure (ACFL). The HEV genotype involved is G3 in Europe and G1 and G2 in China and India where the mortality rate can reach 67%, with an average of 34% [13].

In case of extrahepatic manifestations: acute pancreatitis is associated with HEV G1; kidney injury (membranous glomerulonephritis, membranoproliferative glomerulonephritis and even relapses of IgA nephropathy) is associated with HEV G3 [36]. A possible mechanism for these renal dysfunction in HEV infected patients is through the development of cryoglobulinaemia [36].

3.1.2.2 HEV quasispecies and pathogenicity

HEV may also present numerous quasispecies with different pathogenicity, in general more aggressive. These quasi-HEV species can be the consequence of:

- a high heterogeneity ORF1 and ORF 2 during the acute phase of the infection; these HEV quasi-species are associated with HEV persistence [6], so with a predisposition to chronicity;

- a high heterogeneity of the M domain at the viral capsid, a domain that contains epitopes for T cells, expressed by a low value of the Ka/Ks ratio (an indirect indicator of the selection pressure on a quasispecies) in patients with chronic HEV infection who are not able to achieve spontaneous HEV clearance [6] which means viral persistence and a tendency to chronicity;

- the heterogeneity of the P domain at the viral capsid that determines the progression to liver fibrosis in patients with chronic hepatitis E. Nearly 10% of SOT patients with HEV develop cirrhosis within 3–5 years following the primary infection [6].

3.1.2.3 The recombinant HEV-host variants

The recombinant HEV-host variants with replicative advantage in vitro [6, 37] in chronically infected patients. These HEV variants presented fragments of human genes (ribosomal genes S17 or S19, inter alpha trypsin inhibitor) in the PPR regions and duplications and insertions of the HEV genome [6].

3.2 The host organism

The human host can influence the pathogenicity of HEV and secondarily the clinical manifestations [6] by:

3.2.1 The presence of Apolipoprotein E (ApoE)

The presence of Apolipoprotein E (ApoE) considered a protective factor of the human body against HEV pathogenicity. ApoE is a plasma lipid transporter, but can also be found associated with lipids in the structure of cell membranes. In HEV infection, its intense activity was highlighted, suggesting its intervention in the pathogenesis of HEV infection [6]. An argument in this regard: the protection against HEV by ApoE highlighted in American non-Hispanic blacks by certain isoforms of ApoE (ApoE ε3 and ε4) [38]. The protection by ApoE against HEV action would be achieved by: blocking the attachment of HEV to the surface of the host cell by competition with the heparan-sulfate receptor, modulation of the entry of HEV particles in the host cell, given its presence in the membrane associated with eHEV particles in the blood, modulation of the anti-HEV immune response by regulating T lymphocytes activation and proliferation [6].

3.2.2 Genetic polymorphism in the promoter regions

Genetic polymorphism in the promoter regions for tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) in HEV infection *that stimulates their synthesis,* leading to severe clinical manifestations [39]. E.g.: a (G/A) polymorphism in 308 position of the promotor region of TNF-α will increase TNF production 7 times; a single nucleotide polymorphisms in the promotor IFN-γ (IFN-γ +874 T/A) will associate with a higher IFN-γ production and with symptomatic cases [39].

3.2.3 Innate immune response of the host

The synthesis of different types of IFN (interferon alpha, IFN beta) can influence the pathogenicity of HEV. HEV is more susceptible to IFN action, but has developed means of resistance to its action. There are experimental studies in animals (chimpanzees) that have demonstrated the role of interferon alpha (IFN-α) in the pathogenesis of hepatitis E, HEV being more susceptible than HCV to the innate immunity induced by IFN-α [40]. Studies in human cell cultures (human lung epithelial cells A549 [40] and Huh7 hepatocellular carcinoma cells [41]) have shown that HEV through the ORF3 region can inhibit IFN-induced phosphorylation of signal transducer and activator of transcription STAT1; the result is decreased synthesis of 2 key antiviral proteins: dsRNA-activated protein kinase and 2′,5′-oligoadenylate synthetase. Other authors [41] using other cell lines have shown that HEV by ORF3 protein enhanced type I IFN production by interacting directly with the pattern recognition receptor (PRR) retinoic acid-inducible gene I (RIG-I) [41]. In the same cell line was demonstrated the intervention of another HEV protein, namely ORF1 with inhibitory effect on the signaling and secretory pathway for IFN beta (IFN-β) by de-ubiquitination of RIG-I and tank binding kinase (TBK) [42]. So, gene suppression of key component of the Janus kinase (JAK)-STAT cascade of the IFN signaling, including JAK1, STAT1, and interferon regulatory factor (IRF) 9 stimulates replication of HEV [41].

An increased production of proinflammatory cytokines such as IL-6, IL-8, TNF-α and RANTES (regulated on activation, normal T cell expressed and secreted), as well as the activation of both nuclear factor kappa-light-chain-enhancer of B cells (NF-κB) and IFN regulatory factor 3 (IRF3), two transcription

factors activated in innate immune signaling pathways [42] *in vitro*, using HEV-infected A549 cell line.

NK and natural killer T (NKT) cells could also play a major role in the innate immune response to HEV [6]. Natural killer (NK) and natural killer T (NKT) cells constitute a major fractions of the lymphocytes in the liver, where they are important for the pathogenesis of viral hepatitis. In the peripheral blood of acute infected patients is present a higher proportion of $CD4^+$ cells than in uninfected controls [43]. This increase in CD4+ cells is not associated with an expansion of HEV ORF2-specific CD4+ CD69+ cells producing helper T cell type 1 (IFN-γ and TNF-α) T cytokines or helper T cell type 2 (IL-4) cytokines [43]. The expansion of $CD4^+$ cells could reflect an increase in NKT cells, which can be either $CD3^+$ $CD4^+$ or $CD3^+$ $CD4^-$ $CD8^-$ [43]. In acute hepatitis E the proportion and activation status of NK and NKT cells among PBMCs varies reversibly; there is generally a low proportion of NK ($CD3^-/CD56^+$) and NKT cells ($CD3^+/CD56^+$) in the periphery, but an excessive accumulation of them in the liver [43]. This aspect was supported by immunohistochemical liver biopsies obtained from patients infected with HEV with acute hepatic failure [44].

3.2.4 Adaptive response of the host

Humoral Immune Response

HEV elicits the appearance of IgM and IgG antibodies,. IgM anti- HEV appear in the early stages of the disease and may persist for several months (usually no longer than three to four months). IgG anti- HEV appear shortly after the appearance of IgM and may persist for several years with increasing antibody avidity over time [2]. Anti-HEV IgG antibodies are of the neutralizing type, directed against the neutralizing epitopes of the HEV capsid protein and are protective [45]. These antibodies can also occur after vaccination and confers protection against hepatitis E infection for up to 4.5 years [45] special in the China, although the minimum protective concentration of antibodies has not been determined. WHO suggests that an antibody concentration of 2.5 units of the WHO/ml postvacciunation is protective [45]. But in solid organ transplant recipients, HEV reinfection has been described at an IgG concentration below 7 WHO units/mL [46].

Cellular Immune Response

In acute HEV infection effector T cells are activated with CD8+ increased especially in the liver [44] and high proportions of PBMCs producing IFN-γ (after stimulation with recombinant ORF2 or ORF3 HEV proteins) [44].

An increased expression of CD11a integrin in naïve CD45RA+ T cells, as well as overexpression of CCR5 and CCR9, two chemokine receptors that play important roles in cell trafficking and homing, was also reported in peripheral blood of acutely infected patients. The expanded CD45RA+ CD11a high subpopulations present during the early phase of acute infection suggests the recruitment of these cells from the periphery to the liver, thus contributing to the pathogenesis of the infection [7]. This suggests that the immunosuppressive immune response is involved in the acute phase of the infection, but its exact role remains to be clarified.

General conclusion: the human body can modulate HEV infection using different resources (**Table 1**).

3.3 Specific/particular aspects of pathogenicity in HEV infection

3.3.1 Pathogenesis of fulminant hepatitis E

Fulminant hepatitis with HEV etiology may be present in patients with hepatic chronic diseases and in pregnant women.

	Pathogenic	Mechanisms	Changes present in HEV infection
HEV	Attachment of viral particles to the surface of the host cell	Naked- HEV particles Quasienveloped HEV particles („eHEV")	Inoculum value: the increased inoculum correlates with the severity of the infection
	Viral genetic variability:		
	• Genotypes	GT1–8 (HEV1–8)	• In immunocompetent hosts - genotypes G1 and G2 • In pregnancy: G1 genotype • In immunocompromised hosts: G3 genotype • In patients with pre-existing chronic liver diseases: G3 in Europe and G1 and G2 in China and India • Acute pancreatitis is associated with HEV G1 kidney injury: G3 genotype
	• Subtypes	—	—
	• Quasispecies	—	• A high heterogeneity ORF1 and ORF 2 • A high heterogeneity of the M domain at the viral capsid, • The weaker heterogeneity of the P domain at the viral capsid
	The recombinant HEV-host variants	—	The replicative advantage
	The rhythm of viral replication	—	Increased viral replication
HOST	Genetic susceptibility factors	Apolipoprotein E	Intense activity
		Genetic polymorphism in the promoter regions for tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ)	• G/A polymorphism in 308 position of the promotor region of TNF-α • the polymorphism in the promotor IFN-γ (IFN-γ +874 T/A)
		Surface receivers provided for attachment of HEV	HSGPs, GRP78, ASGPR, ATP5B, ITGA3 for naked- HEV
	Innate Immune Response of the Host	The synthesis of different types of IFN(IFN alpha, IFN beta)	HEV through ORF3 region can inhibit IFN-induced phosphorylation of signal transducer and activator of transcription STAT1
		The proinflammatory cytokines: IL-6, IL-8, TNF-α and RANTES	• An increased production of proinflammatory cytokines: IL-6, IL-8, TNF-α and RANTES • activation of both nuclear factor kappa-light-chain-enhancer of B cells (NF-κB) and IFN regulatory factor 3 (IRF3)
		NK and natural killer T (NKT) cells	Low proportion of NK ($CD3^-$/$CD56^+$) and NKT cells ($CD3^+$/$CD56^+$) in the periphery, but an excessive accumulation of them in the liver

	Pathogenic	Mechanisms	Changes present in HEV infection
	Adaptive Response of the Host.	Humoral Immune Response (IgM and IgG antibodies anti- HEV)	I- gM anti- HEV appear in the early stages of the disease and may persist for several months (usually no longer than three to four months). • IgG anti- HEV appear shortly after the appearance of IgM and may persist for several years
		Cellular Immune Response: CD4+, CD8+, CD11a integrin in naïve CD45RA+ T cells, CCR5, CCR9	• CD8+ increased especially in the liver • High proportions of PBMCs producing IFN-γ • An increased expression of CD11a integrin in naïve CD45RA+ T cells, • Overexpression of CCR5 and CCR9

Table 1.
Pathogenesis of HEV infection.

3.3.1.1 Fulminant hepatitis E in the general population

The pathogenesis of the fulminant liver failure (FHF) with HEV is unclear, but there are a number of issues related to HEV and the host organism that can be correlated with FLF.

It is discussed about associating FLF with the presence of IgM and IgG anti-HEV antibodies and some researchers believe that the humoral immune response is dominant in this fulminant forms [47], associated with increased amounts of IFN-γ, TNF-α, IL-2, and IL-10 produced by PBMCs stimulated by ORF2 HEV. FLF is also associated with changes in the cellular immune response anti-HEV, lower cellular immune response, but with the very important humoral immune response anti-HEV [47]. There is a difference between the peripheral blood and liver in FHF in terms of cellular immunity, so, in the periphery, the cellular immunity was lower, but in the liver the proportion of CD4 + and CD8 + T lymphocytes was increased [48]. CD8 + cytotoxic lymphocytes may play an essential role in the pathogenesis of liver injury in FHF caused by HEV [20].

On the other hand, FHF could be linked to the *viral genotype and/or the subgenotype*, perhaps due to specific mutations in the polyprotein of HEV such as F179S, A317T, T735I, L1110F, V1120I and FG1439Y in the ORF1 E polyprotein [49] or H105R, D29N, V27A mutations in the methyltransferase region of the HEV genome [50] or the association of FLF with HEV-4 [51]. These mutations could correlate with increased pathogenicity of HEV strains and progression to FLF [7].

3.3.1.2 Pathogenesis of HEV infection in pregnancy

Hepatitis E has both a high incidence and severe course in pregnant women in some geographic regions of HEV endemic countries, such as Northern India, [8] while in other HEV endemic countries, such as Egypt, it has been shown to have a benign course with little or no morbidity [7].

During pregnancy, especially in the third trimester, the course of acute hepatitis caused by HEV can be towards acute fulminant hepatitis which can lead to acute liver failure (ALF) and death. The pathogenic aspects of this evolution in pregnant women are related to: immunity, hormonal factors and the peculiarities of the virus [7]. In the initial period of pregnancy up to 20 weeks gestational age, all immune, hormonal factors are oriented to the protection of the fetus during the implantation period [7].

Immune status in pregnancy is characterized by a constellation of factors that lead to decreased cellular immunity that can no longer act on the fetus seen as an allograft. The decrease of cellular immunity is achieved by changing the immune status of the pregnant woman from the Th1 dominant state to that of "Th2 bias", a change initiated by the increased progesterone during pregnancy. The progesterone stimulate the synthesis of progesterone-induced binding factor (PIBF) by lymphocytes [52]. High concentrations of PIBF promote differentiation of $CD4^+$ T cells into helper T cell type 2 (Th2) cells that secrete high concentrations of anti-inflammatory cytokines, including IL-4, IL-5, and IL-10 which causes a decrease in the inflammatory effect of Th1 (e.g., production of IFN-γ), both at the maternal-fetal interface and systemically in humans [53]. These cytokines influence the functionality of monocytes/macrophages. As such, the decrease in cellular immunity protects the fetus, but it also alters the immune response mounted against infections [54]. Th2 status has been demonstrated in pregnant women infected with HEV and this status is likely to favor HEV replication, but its implication for the severity of a hepatitis E infection is unknown [54].

Decreased cellular immunity can also be caused by the placenta through the structural outer layer - the trophoblast. The trophoblast can cause a decrease in cellular immunity through various mechanisms:

- the cells of trophoblast does not express on their surface the major histocompatibility complex (MHC) which mediate antigen presentation; as a result T lymphocytes cannot be activated and cannot act on the fetus [16]. But, the NK cells, another immune cell effector, do not require MHC proteins for their activation. As such, the trophoblast also acts on them by expressing on its surface a special Human Leukocyte Antigen (HLA) molecule called *HLA-G*, which binds to NK receptors CD 16, and CD 56 and inactivates them [54].

- the trophoblast secretes an enzyme indoldeamin 2,3-dioxigenase, the enzyme that breaks down tryptophan, an amino acid essential for the function of T lymphocytes; as such, cellular immunity at the placental interface is suppressed [55].

- the placenta and trophoblast secrete cytokines, especially TGF-β, IL-4, IL-10 which inhibits cell-mediated immunity with protective effect on the fetus. Experimental laboratory animal studies have shown low levels of IL-1β, IL-2, IL-6, IL-10, IL-12 (p40), IL-12 (p90), IL-17, TNF-α in early pregnancy and significantly increased in the latter part of pregnancy and postpartum [56].

Another mechanism that leads to a decrease in cellular immunity in pregnant women is a decrease of the total T lymphocytes and TCD4 + lymphocytes, namely in the first part of pregnancy and then an increase or normalization towards the end of pregnancy or postpartum. This decrease in T lymphocytes and cellular immunity in pregnancy in general, protects the fetus, but would favor viral infections generally, including HEV infection [16, 57].

To summarize, the immunological changes during pregnancy promote the maintenance of the antigenic fetus in the maternal environment by suppression of T cell mediated immunity. Whether this suppressed immune system translates into increased risk of infections during pregnancy is still not clear [16].

Hormonal factors in pregnancy

Pregnancy is characterized by a hormonal storm, namely by the increase of progesterone, estrogens and human chorionic gonadotropin (HCG) [16, 57]. Hormones contribute significantly to the outcome of immune-related diseases during

pregnancy by altering the functioning of immune cells. Hormones can have additional effects on the outcome of infection during pregnancy. Experimentally in animals it has been shown that estrogens and progesterone acts on the thymus. So, progesterone determines the involution of the thymus with disorders in the development of T cells with lyTh1 inhibition and promoting lyTh2 status; this involution of the thymus is related to the expression of progesterone receptors at the thymic level; it can also cause the inadequate, early transition of pro-T to pre-T ly in the process of differentiating these cells [58]. The result will be: decreased cellular immunity of the pregnant woman.

The suppression of cellular immunity by progesterone may be correlated with another phenomenon: the mutations in the progesterone gene (the PROGINS haplotype) that will cause decreased progesterone receptor expression and progesterone-induced blocking factor (PINF) with NK cell inhibition and suppression of cellular immunity with anti-abortive effect [58].

Estrogens causes thymus contraction with the depletion of CD4+ and CD8+ ly T, thus the suppression of cellular immunity [59, 60]. This aspect can be correlated with other studies that show an increased predisposition to viral infections in certain states with high estrogens [61], especially in the third trimester of pregnancy, with an intensification of viral HEV replication (elevated levels of HEV RNA) by inhibiting estrogen receptors and type I IFNs synthesis [61].

Increased progesterone and estrogens in pregnancy can affect also the B lymphocyte population in the bone marrow and decrease mainly pre-B and immature (fractions B–D) bone marrow B cells of pregnant mice [16].

HCG is a chemoattractant protein secreted by the blastocyst after fertilization [62] which mediates migration of regulatory T cells to the pregnant uterus. Regulatory T cells are hypothesized to orchestrate immune tolerance of the fetus during pregnancy in mammals [62].

Steroid hormones are other hormonal markers that can influence viral replication through a mechanism similar to Cytomegalovirus that causes increased replication in pregnancy [16].

To summarize: Progesterone, estrogen, steroid hormones, HCG cause decreased immunity, especially on the cell line, favoring the acquisition of viral infections, including HEV.

HEV genotype

Previous studies have shown the correlation of HEV3 - pregnancy as dominant in the case of poorer outcome of HEV infection [63]. Histopathological HEV3 determines increased apoptosis and necrosis at the maternal- fetal interface with alterations in the architecture of the placental barrier and changes in the cytokine microenvironment - triggers the production of pro-inflammatory cytokines like IL-6 and chemokines [63]. HEV3 also correlates with elevated levels of pro-inflammatory cytokines (TNF-α, IL-6, IFN-γ and TGF-β1) in peripheral blood with „poor outcome" of HEV infection [64]. Subsequent studies have shown that the dominant genotype is not HEV3, but HEV1. The genotype HEV1 replicates more efficiently than HEV3 *in vivo* in tissue explants of decidua basalis and fetal placenta, and also in stromal cells [63].

Other factors that may influence the evolution of hepatitis E in pregnancy to acute liver failure are: the nutritional status of the pregnant woman including micronutrient or folate deficiencies [65] and the differences in the expression of MHC, molecule that can influence the immune response in pregnant women [16].

In certain regions of the world, the pregnancy is associated with acute fulminant E hepatitis and ALF can have an unfavorable evolution, accompanied by a very high maternal mortality (30% - 41% in Northern India) [8, 15].

Pathogenic mechanisms involved in this **high maternal mortality** remains unclear, but the factors involved in this situation are the same as in a common form pregnancy, but in much higher quantities and with a strong effect and much more deeply cellular immunity which favors HEV replication at a very high rate. Thus:

- Th2 "bias status", considered a major cause of death in pregnant women with fulminant HEV hepatitis (e.g., in Asia) [6, 16];
- higher CD8+ count and lower CD4+ count (Ly CD8 + seems to play an important role in the pathogenesis of fulminant hepatitis in pregnancy, being highlighted in large numbers in the liver of patients with fulminant hepatitis E) [59];
- MHC variations of the host pregnancy that mediate antigen presentation may explain the geographical variations in mortality in pregnant women infected with HEV [16];
- high concentrations of cytokines (TNF-α, IL-6, IFN-γ and TGF-β1) may also be associated with an adverse pregnancy outcome [64];
- elevated levels of estrogens, progesterone and beta -HCG can also contribute to a poor outcome in HEV-positive pregnant women who develop FHF [57];
- a reduced expression of toll-like receptor (TLR) 3/TLR7/TLR9 of the host body of the pregnant woman with acute liver failure, the key pattern recognition receptor in innate immunity. The consequence will be: an inadequate innate immune response with decreased phagocytosis capacity of macrophages/ monocytes and the possibility of appearance the severe acute liver failure in pregnancy [66];
- genetic factors in the host- an aspect more discussed in the case of pregnant women of Asian origin. The human genetic factor is not considered relevant in this case. The opposite argument: if the priority intervention of this factor is accepted in the evolution of HEV infection in pregnant women, the mortality rate should be very high in pregnant women with HEV infection in the endemic regions, an aspect that is not found in medical practice [67].

To these factors is added:

- **steroid hormones** present in increased amounts in pregnancy with unfavorable evolution in the presence of HEV infection. These steroid hormones may promote viral replication [16] and also has a direct inhibition on hepatic cells, which may predispose to hepatic dysfunction/failure when exposed to infectious pathogens [16]. Steroid hormones promote viral replication through the immunosuppressive effect [16] and mediate lymphocyte apoptosis by NF-kB factor [16]. NF-κB is a eukaryotic dimeric transcription factor which has a multiple cellular effects, including liver development and regeneration and its implications on the immune response [16]. NF-κB, physiologically down regulated during pregnancy, also plays an important role in sustaining the fetus during pregnancy [68]. An important role in the development and regeneration of the liver is the p65 protein, a component of the NF-kB factor. Experimentally, proteins p65 and p50 - another component of NF-kB - were studied in pregnant women with severe

hepatitis E infection. The absence of p65 protein was associated with a minimal or even absence of HEV in mononuclear cells in peripheral blood and in liver biopsy samples obtained post-mortem from pregnant women with fatal evolution of HEV infection. So, the absence of p65 in the NF-kB complex produced fulminant liver damage [68].

Conclusion: the NF-kB factor is a very important protective factor in the pregnant woman influencing her immune status; the modification of its structure determines the severe immune deficiency and favors high replication of HEV.

- **viral genotype.** Numerous studies have highlighted the role of HEV genotype or its subtypes in producing the severity of HEV infection in pregnant women, leading to a high percentage of deaths in some geographical areas [16, 67]. HEV1 in developing countries is associated as the cause of elevated maternal mortality (30%, with most deaths occurring in the third trimester) of pregnant women [16, 67]. Genotype 1 has been further classified into 4 subtypes and most of them have been grouped to genotype 1A. Sub-genotype shift, may have been responsible for the different geographic morbidity in pregnant women in Southern India and Egypt [16].

- **fetal infection with HEV**- may be responsible for the increased severity of the disease in the mother [69].

- In certain geographical areas **the use of herbal medicines by pregnant women** may influence the severity of HEV infection. This would explain the difference in mortality in certain geographical regions and could be used as a prognostic factor in the evolution of a fulminant liver failure due to other etiologies [70].

Conclusion: Severe pregnancy lesions due to HEV infection are caused by viral factors, host-related factors, immunological factors and hormonal factors and environmental factors.

3.3.1.3 Pathogenesis of chronic infection in immunocompromised patients

In immunocompromised patients HEV infection is present as chronic liver disease. HEV infection is present in a proportion of 0.9–3.5% in patients with **SOT** and the rates of chronicity ranging from 21% - 50% [7]. Chronic infection with HEV is characterizes by persistence of HEV in the organisms that are not able to realize the clearance of the virus. HEV persistence may be related to **the immunosuppressive condition of the patient** characterized by:

- a significantly lower of CD2+, CD3+ and CD4+ T cells comparative with the patients who spontaneously eliminate the virus [7].

- a lower concentration of IL-Ra and IL-2R, to which are added to higher concentrations of chemokines in the acute phase [37]

- refractory response of infected HEV cells to the action of interferon due to increased expression of interferon stimulated gene (ISG) [7]; these cells are not able to realize the clearance of the virus; in patients with kidney transplantation has been described this aspect and also *in vitro* in human hepatoma cell cultures and primary human hepatocytes despite the continuous

production of type III IFNs [71] and the persistent activation of the JAK/STAT signaling that confers to infected cells refractory to exogenous IFN [26].

- secondary immunosuppression due to HIV infection: in HIV-infected patients with low CD4+ T cell count <200/mm^3, HEV infection may become chronic [2].

- secondary immunosuppression due to the use of immunosuppressive drugs in the treatment of various diseases. In humans, HEV is more likely to be persistent in SOT patients treated with tacrolimus and cyclosporin or treatment used in rheumatoid arthritis. Cyclosporin and tacrolimus are both immunosuppressive drugs by inhibition the calcineurin phosphatase in lymphocytes (inhibition of cyclolines A and B); this immunosuppression promote viral replication [72].

Thrombocytopenia by HEV is another change associated with HEV infection in this category of patients: the decrease in platelet count is associated with the persistence of HEV infection and the reduction in the number of antiviral cytotoxic ly T in the liver. HEV-infected patients had low platelet counts [73], but, how HEV induces thrombocytopenia is unknown. It could be immune-mediated as in other virus infections or be linked to the development of fibrosis with splenomegaly [73].

Viral factors meaning the different types, subtypes, quasispecies belonging to HEV can be involved in the chronic evolution of the infection. Arguments:

- chronic infection HEV was found to be rare in a large cohort of Japanese liver transplant recipients, suggesting that there are differences in HEV subtype, strains, or host genetic factors that influence HEV persistence [74].

- greater heterogeneity of quasispecies in ORF1 and ORF 2 during the acute phase of infection is associated with HEV persistence [6, 7].

- the value of Ka/Ks ratio, an indirect indicator of selection pressure on quasispecies in the M domain of HEV capsid protein; M domain of HEV capsid contains epitopes for Ly T. This ratio is lower in patients with chronic HEV infection hence the importance of the cellular immune response in HEV clearance [7].

- the weak diversity of the P capsid domain of HEV, another component of the viral capsid, that can undergo to the evolution to liver fibrosis by the selection of aggressive variants of virus. Nearly 10% of SOT patients with HEV develop cirrhosis within 3 to 5 years after the primary infection [7].

- chronic HEV infection is associated with recombinant host-HEV variants, These recombinant variants have *in vitro* a replicative advantage. The PPR regions of these HEV variants contain fragments of different genes of human origin (e.g. ribosomal genes S17, S19, inter alpha trypsin inhibitor) [7].

Conclusion: HEV infection in immunocompromised patients is characterized by chronic hepatitis due to viral persistence. Viral persistence can be caused by multiple factors related to the immunosuppression of the host organism, but also to HEV.

3.3.1.4 Pathogenesis of extrahepatic manifestations in HEV infection

Neurological disorders have been reported in patients with acute or chronic HEV infections, namely: Guillain–Barré syndrome (GBS), neuralgic amyotrophy

(NA), encephalitis/meningoencephalitis, myositis, Bell's palsy, and polyradiculopathy [16].

Clinically, the evolution of these diseases is more severe in the presence of HEV infection. E.g.: cases of NA that clinically showed bilateral damage, with clamping of the brachial plexus, the phrenic nerve [22].

The pathophysiology of HEV-associated neurological injury remains uncertain. Some of these neurological conditions such as GBS and NA are immune-mediated, due to molecular mimicry [22], secondary to the immune response triggered by the virus. This immune response that cross-reacts with axolemmal or Schwann cell antigens damages peripheral nerves [22]. In case of NA direct infection of the brachial plexus cannot be excluded because HEV RNA was demonstrated in all HEV-associated patients at the start of their illness [19]. Another arguments for direct virus neurotropism:

- HEV variants found in the cerebrospinal fluid were different from those found in the serum of the same patient, which would lead to the theory that HEV may have variants with neurological tropism and replication in the central nervous system. [75]. Neurological disorders can be associated with special genotypes as HEV-1 in infected Asians and HEV-3 in infected Europeans [75, 76].

- the presence of HEV ORF 2 in the cytoplasm or nucleus of cells in brain and spinal cord tissues of the HEV RNA positive rabbits, such as glial cells, microglial cells, choroid epithelium cells, and neural cells, especially in cells located in perivascular areas. These aspects suggest that perivascular cells and neural cells are targets for HEV present in cerebrospinal fluid (CNS) [77] described in HEV infected gerbils the thickening of the basement membrane of blood vessels even reduplicated in brain and spinal cord tissues as a compensatory response to blood–brain barrier (BBB) disruption permeability. In conclusion: HEV can cross the BBB directly into the central nervous tissue. [77]

In summary, HEV entry to the brain. The BBB of brain is a potential target of HEV invasion into the CNS in experimentally infected rabbits. Components of the BBB include tight junction (TJ) and adhesion junction (AJ) between endothelial cells (EC), pericytes (PC), astrocytes endfoot (EF), as well as basement membrane (BM) surrounded ECs and PCs; HEV causes a decrease in TJ proteins, including ZO-1, Occludin, and Claudin5 and an increase in AJ protein VE-cadherin expression; the result will be in breaking the junctional complexes integrity between capillary ECs, facilitating HEV invasion into the brain tissue. This is the key factor in HEV pathogenicity at the CNS level [78].

Kidney injuries and impaired renal function

Both acute and chronic HEV infections can lead to kidney injuries and impaired renal function [79]. Both HEV antigen and RNA have been detected in the urine of patients with acute or chronic HEV4 [80] or HEV3 infections [41]. Experimentally, immunohistochemistry also detected ORF3 protein in the kidneys of infected rabbits [81]. This suggests that the kidneys or the urinary tract could be an HEV reservoir. Kidney biopsies from patients with glomerular disease and HEV infection revealed histological features of membranoproliferative glomerulonephritis (MPGN), with or without cryoglobulinemia, membranous glomerulonephritis [79, 82] and a relapse of immunoglobulin A nephropathy [79].

The pathophysiology of HEV infection at these patients is linked to the deposition of immune complexes formed from the HEV antigen, anti-HEV IgG antibodies, and a rheumatoid factor in the glomerulus [33]. It is also possible that the HEV antigen with lower molecular weight by-products of ORF2s could be secreted into

the urine by cross the glomerular filtration barrier [83] Both HEV antigen and RNA were detected in the urine of patients chronically infected with HEV [80] Kidney biopsies showed interstitial inflammatory cell infiltrates at tubule-interstitial. [36, 80]. But, there is still no evidence neither about viral replication of HEV in human renal cells nor of the direct nephrotoxic effect [7].

Hematological manifestations in HEV infection

Anemia and severe thrombocytopenia may be associated with HEV infection. Anemia related to HEV infection can be: hemolytic anemia due to deficiency of glucose 6-phosphate dehydrogenase (G6PD) [84, 85] or aplastic anemia secondary to severe forms of HEV infection [85].

Thrombocytopenia may be associated with HEV infection [29]. 11% HEV-infected patients had thrombocytopenia [86]. The pathogenic mechanisms involved by HEV could be: immune-mediated or be linked to the development of fibrosis with splenomegaly [86].

Cryoglobulenemia: there were only a few cases of Hepatitis E-associated cryoglobulenemia reported in the medical literature,; all of these patients are chronic hepatitis patients, immunocompromised, all from western Europe, with genotype 3 confirmed in eight cases, with all MC type 2 or 3 [22].

HEV-induced acute pancreatitis have been reported.

2.1% of patients with acute pancreatitis in a study conducted in India had serological arguments about a recent HEV infection. Acute pancreatitis associated with hepatitis E usually has a good prognosis. The mechanism of pancreatitis in patients with acute viral hepatitis (nonfulminant) is unknown, and it may be multifactorial. One proposed pathogenesis of pancreatitis associated with hepatitis is the development of edema of the ampulla of Vater with obstruction to the outflow of pancreatic fluid. A more plausible mechanism for virus-associated acute pancreatitis is the direct inflammation and destruction of pancreatic acinar cells by the virus [22].

4. Laboratory diagnosis

The laboratory is essential for establishing the etiological diagnosis.

4.1.Serology is the main way to diagnose HEV infection. It consists of highlighting IgG and IgM antibodies anti- HEV [8]. IgM rises rapidly within a month of acquiring the infection, the peak corresponds to the onset of clinical symptoms and increased liver enzymes (ALT, AST) and disappear up to 32 weeks to 5months after the initial disease onset; IgG occurs after the disappearance of IgM and they persist for a long time after infection (not yet defined), occasionally disappearing before the one year mark [8]. In endemic regions in acute infections, IgM antibodies may coexist with IgG antibodies for a certain period of time.

Clinical significance of the 2 types of anti-HEV antibodies: the presence of IgM antibodies means a recent acute HEV infection; presence of anti-HEV IgG antibodies means past HEV infection or post-vaccination status (protective value over 2.5 U/mL according to WHO) [87].

4.2. HEV RNA detection in blood or in the stool by Real-Time Polymerase Chain Reaction (RT-PCR).

HEV RNA detection is a method used to diagnose the infection in early stage. HEV viremia disappears after 3 weeks, and from feces HEV disappears after another 2 weeks [2].

NOTE: HEV RNA measurement is required in immunocompromised patients in whom antibodies tests may be negative, 3 months after an HEV infection to determine whether it becomes chronic, for monitoring antiviral therapy, or in blood donors as a screening test [8].

4.3. HEV antigen (HEV Ag) detection is another method for early acute HEV infection, comparable to RT-PCR. Antigen detection can be a good cost-effective alternative to real-time PCR [88].

4.4. HEV isolation *in vitro*, from serum or feces, using certain cell cultures, such as PLC/PRF/5 (human hepatocarcinoma) and A549 cells (human lung adenocarcinoma) or in hepatocytes derived from pluripotent stem cells. In these cell lines HEV replicates, the replication being dependent on the value of the inoculum [89, 90].

4.5. HEV genotyping to identify types, subtypes, variants, quasispecies HEV that has been shown to be correlated with the severity of the disease, with certain epidemiological aspects, with the area of spread of HEV infection [8].

5. Conclusions

Hepatitis E is a liver infection not yet sufficiently investigated, with an unclear pathophysiology, in which the confluence of several viral factors, the host or environmental factors can lead to different clinical features. Although most infections are subclinical, there are cases with severe forms of the disease that can progress unfavorably either to fulminant forms with acute liver failure, cirrhosis and death, or to chronicity, and during pregnancy can take benign or extremely severe forms that can lead to death. Mother and fetus. As such, hepatitis E is a topic that should be investigated in future studies.

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