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IMPORTANCE OF OPEN READING FRAMES (ORFS) IN HEV – A REVIEW.

Bisen JS[1], Sharma S[2], Shukla R[3]

[1]Shri Rama Krishna College of Commerce and Science, Satna, Madhya Pradesh, India

[2]Department of Biotechnology, S.R. Group of Institute Utter Pradesh Lucknow

[3]Department of Biotechnology, R.R. Group of Institute Utter Pradesh Lucknow

***Corresponding Author: Dr. Jaya Singh Bisen,**

Email ID: singhjaya685@gmail.com

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ABSTRACT

Hepatitis E virus (HEV) is the primary pathogenic factor of hepatitis E and the main source of enterically transmitting hepatitis virus worldwide. Increasing levels of HEV infections and no effective anti-HEV medication available refer to the global safety burden of the pathogens. Compared to many hepatotropic viruses, it was famously challenging to spread the hepatitis E virus (HEV) in cell culture, restricting experiments to uncover its genetics. Big advancements have been made lately with the processing of primary HEV isolates and the discovery of variants that reproduce efficiently in carcinoma cells. This review summarizes the current knowledge of the varients of hepatitis , morphology of HEV, Importance of HVR for efficient RNA replication, role of ORF1,ORF2,ORF3, effects of mutation in HEV and ORFs to investigated pathogenesis.

Key word: Hepatitis E virus, Mutation,Pathogenesis, Flaviviruses,Picornavirus**,** mutation in HEV

INTRODUCTION

Viral hepatitis has arisen as a serious global safety concern that threatens 10 corers of people all over the world. Viral hepatitis is a major source of disease and death in human beings. Hepatocellular carcinoma (HCC), that is one of the globe's ten most prevalent tumors, is strongly linked with hepatitis B and hepatitis C virus at least in certain parts of the world. The viruses of hepatitis encompass a number of unknown and sometimes very rare human pathogens. HAV (Hepatitis A virus), categorized as hepatovirus, is a miniscule, noneveloped RNA virus that expresses many of the picornavirus family's characteristics and is the causative agent of contagious or epidemic hepatitis transported by the rectal-oral route. HAV (Hepatitis B virus), a variant of the hepadnavirus community, has double-stranded DNA viruses that multiply by reverse transcription, uniquely. In human population hepatitis B virus is prevalent, and hyperendemic for some parts of world. There were variety of members identified forthis virus. Many species, including woodchucks, beechy ground squirrels and ducks, often suffer from normal hepadna -virus infections. HCV (Hepatitis C virus), is an enveloped RNA (single strand RNA) virus that tends to be distantly linked (possibly evolving) to flaviviruses, while arthropod vectors do not spread hepatitis C. various genotypes were described. In several countries infection with the more recently established virus is normal. In certain nations, hepatitis C virus is related to chronic liver disease and even to primary liver cancer. HDV (Hepatitis D virus) is an uncommon, circular RNA (single-stranded) virus with a proportion of resemblances to some viral satellites and viroids in plants. This virus involves hepadna virus aid operations for hepatocyte propagation, and is a significant cause of acute and severe chronic liver harm in many areas of the world. The unenveloped, RNA (single-stranded) virus, which shares several biophysical and biochemical characteristics with caliciviruses, is the HEV, (hepatitis E virus) the source of enterically transmitted non-A, non-B hepatitis. A plant virus, beet necrotic yellow vein virus, and similarities to rubella virus exist in the functional domains, is the most similar genome to HEV. Full taxonomic grouping also needs to be accepted. GB Viruses with Hepatitis (GBV-A, GBV-B and GBV-C).

Recently, the GB hepatitis viruses were cloned, and tentative genomic analysis indicates that they are linked to certain RNA(positive-stranded) viruses with sequence similarity local regions of distinct flaviviruses. Phylogenetic genomic sequence analysis demonstrated that such viruses are not genotypes of the hepatitis C virus. **[1]**

Table 1: Summary of Hepatitis Virus variants, their genetic makeup and family members.

HV	Envelop	Genome	Similar family
HAV	Non enveloped	RNA	Picornavirus
HBV		ds DNA	Hepadnavirus
HCV	Enveloped	ss RNA	Flaviviruses
HDV	-	single-stranded, circular RNA	Plant viral satellites and viroids
HEV	non-enveloped	s sRNA	Caliciviruses
GBV-A	-	positive-stranded RNA	Flaviviruses
GBV-B	-	positive-stranded RNA	Flaviviruses
GBV-C	-	positive-stranded RNA	Flaviviruses
Reference[40]			

Morphology and genome of Hepatitis E

Hepatitis E is an acute liver disorder that exists in many developed countries, and is a popular type of acute viral hepatitis.**[2, 3].**Hepatitis E virus (HEV), a small RNA virus of the genus hepevirus, Hepeviridae family, is the source of the disease. **[2].**In 1991, the Hepatitis E virus was cloned, and the whole sequence of 7.5 kb is identified. The genome composition is different from the Picornaviridae, the non-structured and structured polypeptides are located at the 5', and 3' ends respectively. Hepatitis E virus (HEV) is an Nonenveloped virus with an icosahedral structure and a diameter of approximately 32 and 34 nm.

The buoyant density of HEV in CsCl is between 1.39 and 1.40 g / cm^3 and its rate of sedimentation is 183S **[4]**. HEV genome Consist of positive RNA single-stranded molecule with three open reading frames (ORFs**) [3].** The ORF1 is the biggest reading frame at the genome's 5'-end, with 5 kb (5079 bp). ORF2 is 2 kb long (nucleotide 5147 to 7127), present at the 3' end of the genome. Three regions of glycosylation were established in ORF2, however their role is unclear. ORF3 partly interferes with ORF1 and ORF2 and calculates 369 bpnt (5106-5475) [**5**]. These ORFs are activated while viral infections as antibodies to these domains are present in spontaneously infected humans and in scientifically exposed monkeys**. [6].** Furthermore, the genome of 68 and 26 nucleotides has two Untranslated Regions (UTRs) in 3 'and 5' terminal sections, respectively.

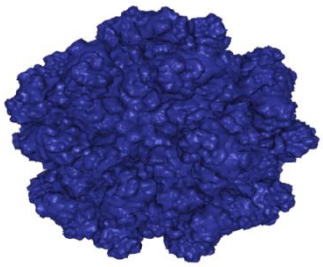

Figure 1: Hepatitis E virus ORF2 (doi:10.1093/bioinformatics/bty419)

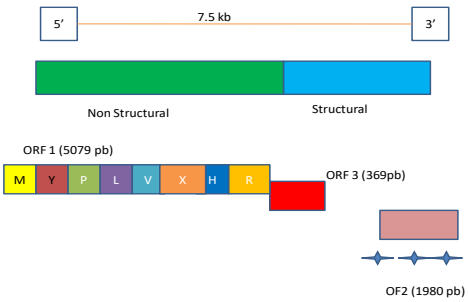

Figure 2: Hepatitis E Virus genetic structure. methyl transferase :; MY, : domain Y; P: papainlikecystein protease; L: hypervariable region; V: prolin "hinge"; X: domain X; H: helicase; R: RNA polymerase RNA- dependent; ; glicosilation site**. [7].**

Biology of HEV

Hepatitis E virus (HEV) genome comprises a remarkable hyper variable R region (HVR) with several tactical changes between isolates of the same genotype virus **[8]**. There are seven HEV genotype that are identified within the orthohepevirus**[9].** HEV1 to HEV4 are well identified as human pathogen while HEV5 and HEV 6 are acknowledged in wild boars **[10].**Camelid HEV-7 has recently been documented to infect humans and induce chronic hepatitis E, as seen in a patient transplanted to the liver **[11].**

The HEV 7.2-kb RNA genome comprises three open reading frames (ORFs), namely ORF3, ORF2 and ORF1, flanked by un-translated regions of 5'- and 3' **[12],** protease, Methyltransferase, RNA-dependent polymerase (RdRp) and helicase, sites are the presumed active sites in the ORF1 protein [**12],** protease . ORF2 encrypts the main protein capsid while ORF3 encrypts a tiny multipurpose protein [**13**]. The methyltransferase and guanylyltransferase performs viral RNA capping, **[15],**RdRp works in viral RNA replication **[14],** the helicase involve in 5'-triphosphatase activity **[16]**,and NTPase and RNA duplex-unwinding behaviors were both confirmed for HEV **[17]**.Recently an innovative ORF4 of 158 amino acids has been identified for HEV-1 inside ORF1. ORF4 associates with various viral proteins (helicase, RdRp, and X) and host factors such as eEF1α1 (eukaryotic elongation factor 1 isoform-1) and β-tubulin in HEV replication **[18].**HEV encrypts factors of replication with maintained ORF1 protein domains identical to rubella virus and alphavirus**[16]**The conserved X domain in the HEV ORF1 protein, flanked by the papain-like protease domain, is followed by a proline-rich hinge area that can shape a versatile character between the X domain and the upstream domains **[16]**The ORF1 protein hypervariable region (HVR) in HEV overlaps the proline-rich sequence situated between the X domain's N terminus and the C-terminal region of the presumed papain-like protease domain.**[19].**The HVR differs between various HEV types, in size and sequence. We have recently shown that HEV infection can withstand minor deletions in the HVR and that residues of amino acids in this vicinity are replaceable for virus infectivity **[20].**ORF2 encodes to the protein of capsid . ORF3 partly clashes with ORF2 and interprets a multifunctional phosphoprotein which can synchronize cell signaling and is consistent with particle secretion **[21].**

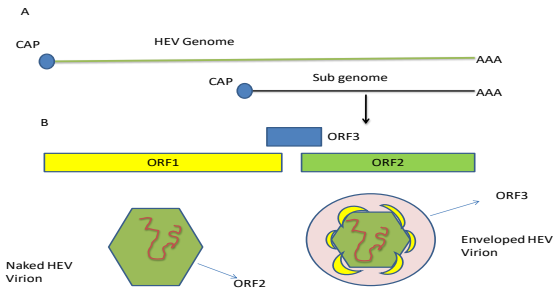

Figure 3: The hepatitis E virus (HEV) genome, its encoded proteins (a) and two types of virions (b).

Importance of HVR for efficient HEV RNA replication

The value of HVR for effective HEV RNA replication was analyzed by inserting overlapping deletions of different lengths into genotype 1 human HEV luciferase replicon (pSK-REP).The results revealed that minor deletions in region of the N-terminal and the central HVR region were correlated with notable reductions in the efficiency of HEV replication, as shown by significantly reduced luciferase operation.Higher deletions mutation in these locations lead to more dramatic decreases in the performance of viral RNA replication.Contrary to the comparatively steady reduction in replication rates of mutants with N-terminal and central area HVR deletions, the size of deletions in the C-terminal zone of HVR has evidently had a significantly smaller impact on the efficiency of viral RNA replication than that of deletions in the N-terminal and central regions **[22].**

Mutations in HEV

RNA viruses develop strong genetic diversity owing to gradual evolution, with an average rate of mutation varying from 10^{-6} to 10^{-4} substitutions per nucleotide per copying strand **[23].** During proliferation and sequential passages for adaptation to cell culture, mutations may occur frequently across the complete HEV genome [**24**]. The transcription mechanism is responsible for the strong heterogeneity and regular collection of mutations in the HEV genome. The viral RdRp, which lacks the capacity to proof-read DNA polymerases, possibly raises the differences in the HEV genome. In the other side, the selection pressure from antiviral medicines and host immune responses may also lead to increased HEV heterogeneity. **[25].**Even though life-cycle of the HEV is linked to more ssRNA viruses, further study is needed (Fig. 4). HEV sticks cells by ORF2 association with association receptors like HSPGs (heparan sulfate proteoglycans) and HSC70 (heat shock cognate protein 70) and reaches the cells via clathrin, dynamin-2, , membrane cholesterol and actin-dependent endocytosis **[26]**. Following entrance, the virionuncoats and delivers the RNA of virus into the cytoplasm . The virus translates the polyproteins of ORF1,by utilizing enzymes, of the host translation machinery. Replication accomplished of The viral genomes by viral RNA helicase and RdRp, while the proteins ORF2 and ORF3 are both derived through the translation of viral subgenomicRNA **[27].** The HEV replication complex is typically situated in the intermediate ER-Golgi pocket, where the positive single-stranded RNA and viral proteins may be found **[27].**

The aggregation of RNA and ORF2 protein shapes the particles of viral progeny , which are then emitted from the host cells via ESCRT(the transport machinery) necessary endosomal sorting complexes. The association between the preserved PSAP motifs in ORF3 protein of the virousand TSG101 (the tumor susceptibility gene 101) (a part of the ESCRT machinery) is probably necessary for the maturation and egress of HEV **[28].**

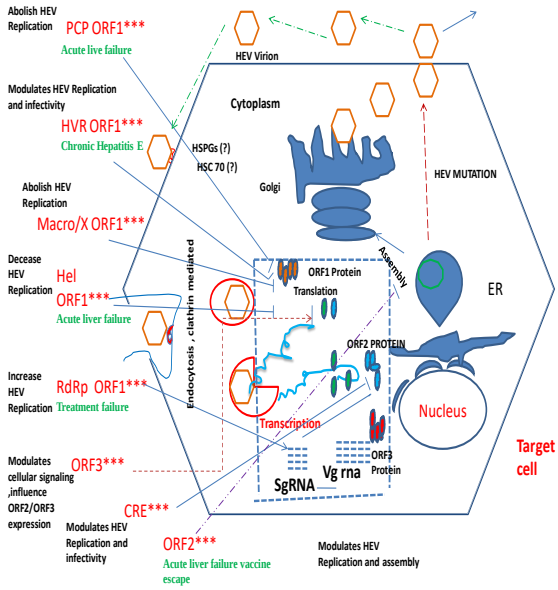

Figure 4 : Impact of mutations on clinical importance and on process of HEV replication.

Schematic description of the HEV replication process and impact of HEV genome mutations (red color; domain and area are indicated) on the HEV transcription / translation machinery (blue dotted box). Asterisks (* * *) display mutations as mentioned in the document.

The potential consequence of mutations on HEV replication is denoted at the hand, defined green color as the clinical outcome.

ORF3 is a Transmembrane Protein

Our initial bioinformatical review found that HEV ORF3 formed a putative transmembrane domain **[29].** This prediction was verified by HEV ORF3 expression in HepG2C3A cells, a widely used human hepatoma cell line permissive for HEV infection. **Qiang Ding et.al.,2016** detected ORF3 colocation with the calnexin(ER-associated protein) in certain cells suggested interaction of ORF3 with intracellular membranes probably acquired from ER. FLAG-tagged ORF3 immunoprecipitated with anti-HA in lysatesresulting from FLAG- and HA-tagged ORF3 cells of HepG2C3A. By comparison, co-expression of HA-tagged STING, an excellently-characterized ER-resident protein **[30],** didn't pull off ORF3, suggesting that the interactions between various ORF3 molecules are unique. Furthermore, even under denaturation circumstances, greater protein clusters were found in western blots of HepG2C3A lysates, indicating multimerisation.

These results jointly suggest that HEV ORF3 is a membrane protein that is located at ER membranes **[31]** and forms multimeric complexes, possibly by homophilic interactions. Researchers noted that HEV ORF3 resemblance to recognized viroporins, i.e. protein clusters virally encrypted acting as functional ion channels. ORF3 is, like many viroporins, a weak hydrophobic protein that appears to oligomerise in membranes isolated from ER. Analogous to class IA viroporins such as IAV M2 **[29**], HIV-1 Vpu**[29]**, or coronavirus E protein **[29],** ORF3 has a short tail at the N terminus which resides at the ER lumen and a lengthy cytosolic tail at the C terminus, which is vulnerable to phosphorylation at the serine at location 70 **[32].**

Diagnostic methods

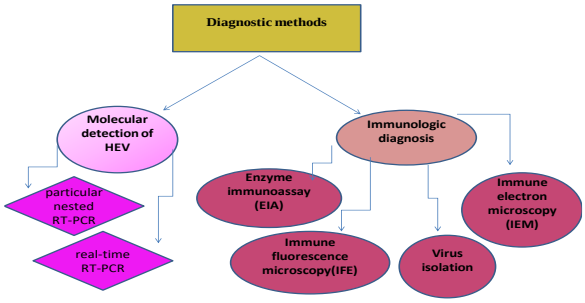

Figure 5: Flow diagram of HEV diagnostic tests

Molecular detection of HEV

HEV genome sequencing has permitted the creation of a variety of different diagnostic tests. For eg, HEV RNA was identified in stool samples collected during a recent epidemic in Kanpur (North India), using the polymerase chain reaction (PCR). Nucleic acid-based strategies, in particular nested RT-PCR and real-time RT-PCR, soon emerged as the first-choice approach for responsive and accurate identification of RNA viruses. This approach is quite effective in analysis to classify different HEV strains whose serological reactions have not been observed by some tests, particularly in non-endemic countries **[33]**.RT-PCR is a standard approach for the identification of viral RNA, not just in serums and feces during severe human disease period**[34].**

Immunologic diagnosis

Enzyme immunoassay (EIA)

EIA is a realistic, highly reactive, and economical way of diagnosing antibodies to HEV. Antigenic region is present in all ORFs proteins of the HEV**[35]**:

(I) 12 ORF1 antigenic domains (particularly within the alleged RNA-dependent RNA polymerase domain)

(II) Six antigenic ORF2 Protein domains.

(III) three antigenic domains within the ORF3 protein

Transgenic proteins are used to identify IgG and IgM anti-HEV, arising from the ORF2 and ORF3 C-end domains or from a wider portion of ORF2 and full ORF3.

A broader variety of antigens produced by a greater portion of ORF2 or "capsid-like" molecules are more successful in detecting antibodies at the convalescent stage of the disease than uncommon ORF2 and 3 C-end antigens or ORF3 as a whole **[36].**

Immune fluorescence microscopy(IFE)

Several advanced laboratories choose this method for antibody identification. IFE semiquantitatively identifies antibodies that respond to the HEV antigen.

Anti-HEV antibodies prevent the attachment of anti-HEV IgG-conjugated fluorescein to HEV antigen in frozen liver tissue. Semiquantitatively, the production of anti-HEV antibodies is measured **[37].** This approach is tedious and costly, and is therefore not suitable for regular diagnosis. **[3, 7].**

Virus isolation

Establishing a functional cell culture method for the dissemination of HEV in vitro is important for virological characterization but also for the detection and avoidance of HEV infection. A few in vitro culture models have been recorded for HEV reproduction, such as human heart, kidney or liver (2BS, A549, Hep-G2), and macaque hepatocytes.However, most of these cannot have credible HEV particles or strong VLP titers and are poorly reproducible.**[3,7]**

Immune electron microscopy (IEM)

IEM recognizes VLPs in clinical studies **[38].** The HEV proteins are precipitated with the active- or convalescent-phase sera-derived native HEV antibody. Levels of antibodies against HEV may be semiquantitatively calculated by evaluating the antibody coating. Although IEM is a greater sensitivity tool, the assay's sensitivity is inadequate for routine analysis.IEM is challenging to conduct because most clinical samples don't have adequate VLPs to identify **[39,40].**

Summary and Outlook

The outbreak of HEV has risen dramatically in recent years, and HEV has become the primary agents in acute viral hepatitis. The combination of cell culture and infectious clone has given significant success to HEV researchers in recent years.

Mutations that influence the efficacy of RdRp activity but the exact function of such identified mutations remains unknown. Except for drug-resistance-related HEV mutations in the RdRp domain, most mutations observed in clinical isolates in certain regions do not confirm with findings from artificial mutations in functional experiments, reflecting the existence of mutational sophistication. More research may further explain the future role of HEV variants in the metabolism, pathogenesis and clinical importance of HEVs. Several concerns regarding HEV remain to be explored further, in particular the restricting factors of the host population, the cellular receptor of HEV, the cause for high fatal levels in pregnant women, etc.

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