Journal of Clinical Virology 91 (2017) 1–4

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Short communication

Report of novel H105R, D29N, V27A mutations in the methyltransferase region of the HEV genome in patients with acute liver failure

Jayanta Borkakoti[a,b], Giasuddin Ahmed[b], Arvind Rai[c], Premashis Kar[a,*]

[a] PCR Hepatitis Laboratory, Department of Medicine, Maulana Azad Medical College, University of Delhi, New Delhi, India
[b] Department of Biotechnology, Gauhati University, Assam, India
[c] Department of Biochemistry, National Centre for Disease Control, New Delhi, India

ARTICLE INFO

Article history:
Received 22 December 2016
Received in revised form 17 March 2017
Accepted 18 March 2017

Keywords:
HEV
Methyltransferase
Acute liver failure
D29N
V27A
H105R

ABSTRACT

Background: The Hepatitis E virus (HEV) has been responsible for major outbreaks in the developing countries affecting millions of people and acute sporadic hepatitis worldwide. The HEV methyltransferase is important for capping the 5′-end of the viral pregenomic RNA which is critical for viral infection.
Objectives: We aimed to assess the substitutional profile in the HEV methyltransferase region in patients with acute liver failure (ALF) and acute viral hepatitis (AVH) from North Indian population and associate the substitutions with the poor outcome of the disease.
Study design: HEV RNA was detected and partial region encoding the Methyltransferase domain in the HEV genome was amplified by Reverse Transcriptase(RT-PCR). Viral load of HEV was quantified utilizing Real time PCR.32 representative samples consisting of 16 AVH and 16 ALF were directly sequenced and amino acid changes were compared using Fischer's exact (two-tailed) test.
Results: Novel mutations Valine27Alanine (V27A), Aspartate29Asparagine (D29N) and Histidine105Arginine (H105R) mutation corresponding to 107T > C, 115G > A and 341 A > G substitutions respectively were significantly ($p < 0.0001$) obtained in 16/16(100%) ALF patients compared to none (0/16) of the AVH patients. HEV viral load and disease severity parameters corresponding to the samples with D29N and V27A mutations were significantly higher compared to the isolates lacking these mutations while the H105R mutation was associated with decreased viremia.
Conclusion: The D29N and V27A mutations had significant association with the poor outcome in ALF patients suggesting key role in enhancing HEV replication while the association of H105R mutation with decreased viremia creates interest on its antiviral aspects.

1. Background

The Hepatitis E virus (HEV) has been responsible for major outbreaks in the developing countries affecting millions of people and acute sporadic hepatitis worldwide. The HEV Methyltransferase is responsible for capping the 5′ end of the genomic RNA [1]. HEV belongs to the family Hepeviridae which is divided into 2 genera *Orthohepevirus*, further subdivided into species *Orthohepevirus* A-D and *Piscihepevirus* [2]. The function of Methyltransferase can be possibly blocked by small molecule inhibitors [2]. Mutations in the methyltransferase region of the alphavirus have been reported to affect the infectivity and methyltransferase activity of Brome Mosaic Virus, Semliki Forest Virus and Sindbis virus [3–5]. Due to the worldwide incidence and its association with acute liver failure (ALF), effective antiviral therapeutics against HEV infections are warranted.

2. Objectives

The present study was aimed to identify the involvement of nucleotide or amino acid substitutions, if any, in the Methyltransferase region of the HEV genome in ALF patients from North India and associate them with the disease severity.

* Corresponding author at: Department of Medicine, Maulana Azad Medical College and associated Lok Nayak Jai Prakash Hospital, University of Delhi, New Delhi.
E-mail address: borkakoti.jayanta@gmail.com (P. Kar).

http://dx.doi.org/10.1016/j.jcv.2017.03.017
1386-6532/

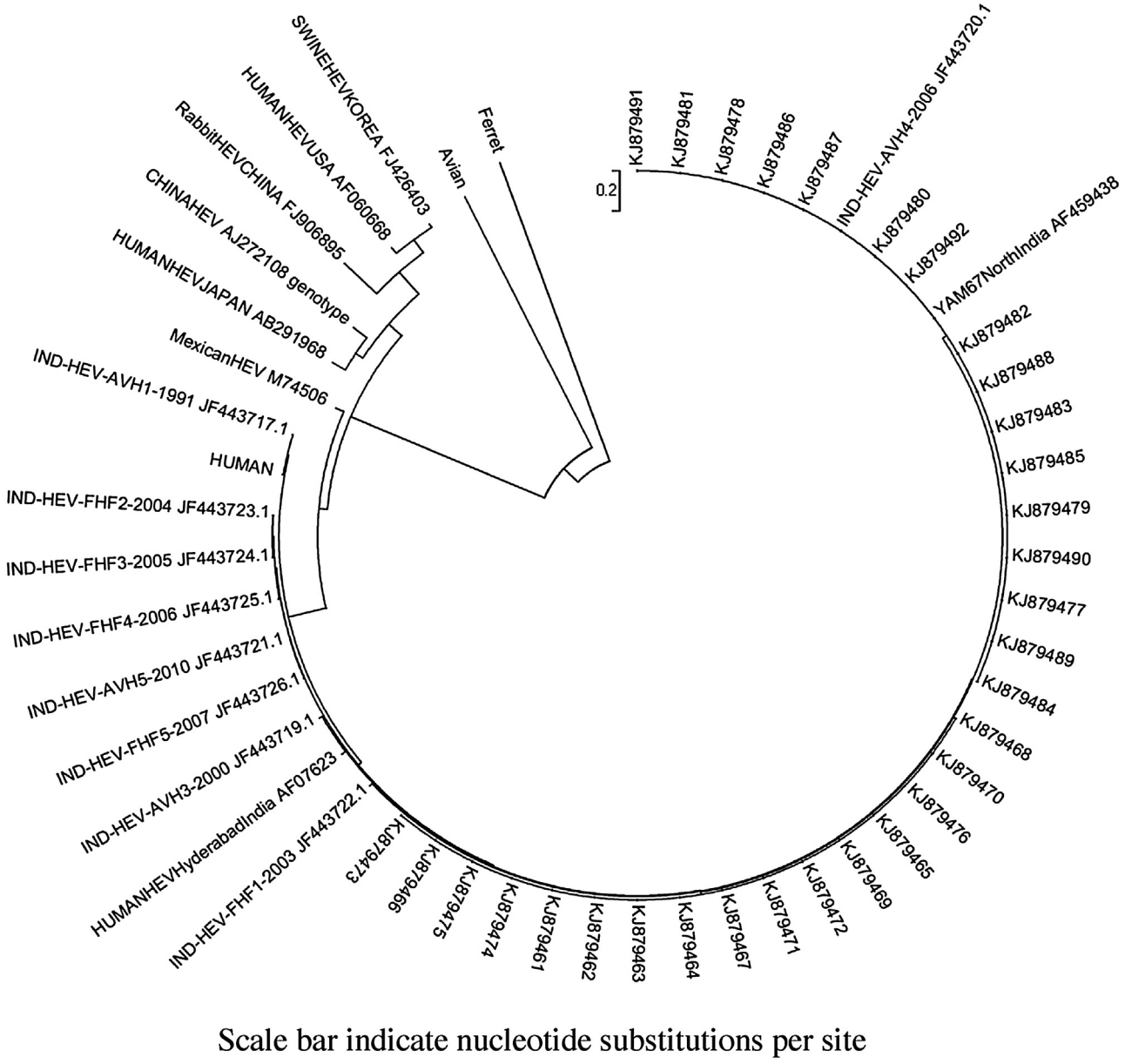

Fig. 1. Phylogenetic tree of the Methyltransferase region in the ORF 1 of the Hepatitis E virus genome. Clustal X was used for sequence alignment along with the MEGA4.0 software package (http://www.megasoftware.net), and the trees were constructed using the neighbor-joining method with p-distance (gap/missing data treatment; pairwise deletion) and 1000 bootstrap replicates.

3. Study design

The study was conducted in the LNJP Hospital, New Delhi during January 2013 to September 2015 and approved by the Institutional Ethics Committee, Maulana Azad Medical College, New Delhi. ALF was diagnosed as any evidence of coagulation abnormality, usually an International Normalized Ratio (INR) > 1.5 and any degree of mental alteration (encephalopathy) without preexisting cirrhosis and with an illness of <26weeks' duration [6] whereas acute viral hepatitis (AVH) was evaluated as acute illness with 1) discrete onset of symptoms (e.g., nausea, anorexia, fever, malaise, or abdominal pain) and 2) jaundice or elevated serum aminotransferase levels [7]. The study included 126 patients positive for HEV IgM consisting of 23 ALF and 103 AVH. A total of 42 patients were pregnant of which 25 had AVH and 18 had ALF. Viral RNA was extracted by the Viral RNA extraction kit (Qiagen, Hiedelberg, Germany). cDNA synthesis was done by the cDNA synthesis kit (Massachusets, USA) using reverse transcriptase PCR (RT PCR). The representative 32 sequences from AVH and ALF patients were directly sequenced.

The primers for the partial Open Reading Frame (ORF 1) of the HEV genome encoding the Methyltransferase region having a length of 505 bp were designed using manual instructions (Primer 3 software) and were named as JB F15(Forward primer) and JB R15(Reverse Primer). The primers were:

Forward JB F15:5′ GGAGGCCCATCAGTTTATCA 3′,

Reverse JB F15: 5′CATATCATGGAGGGAGTAGAGG-3′.

The PCR was performed using 2 μl c-DNA as template, 10 mM TrisHCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 400 μM dNTPs, 0.4 μM of each primer and 1.2 U of Taq Polymerase in a total of 25 μl reaction mixture. The PCR was carried out with the following temperature profile as follows- initial denaturation at 94 °C for 3 min followed by 35 cycles of DNA denaturation carried out at 94 °C for 3 min, annealing at 50.2 °C for 45 s, nucleotide extension at 72 °C for 45 s respectively followed by a final elongation at 72 °C for 7 min.