

Viral Etiology and Assessment of HBV Genotypes in Fulminant Hepatic Failure: The Present Scenario

Sami Hiba^{1*}, Rizvi Meher², Azam Mohd², Ajmal MR³, Shukla Indu², Khan Haris M²

¹Department of Microbiology, Shri Ram Murti Smarak Institute of Medical Sciences, Bhojipura, Bareilly, U P, India

²Department of Microbiology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh

³Department of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh

Keywords: Fulminant hepatic failure, Genotype D, HAV, HCV, HEV.

ABSTRACT

Background: Fulminant hepatic failure (FHF) is defined as the rapid development of acute liver injury with severe impairment of synthetic functions and hepatic encephalopathy in patients without obvious previous liver disease. The present study was carried out to evaluate the etiology of FHF on one hand and to assess the role of HBV genotypes in acute liver failure in India on the other hand.

Methods: The patients who developed fulminant hepatic failure (FHF) were screened for HAV, HBV, HCV, HEV and HIV and Hepatitis B Core IgM, HBeAg, anti-HBs and anti-HBe were assessed. HBV genotyping was performed using Kirschberg's type specific primers (TSP-PCR), heminested PCR, and Naito's monoplex PCR. SEN and TTV viruses were detected in Non-A to Non-E hepatitis (NANEH).

Results: Majority of FHF were detected in HBV 13 (38.2%) followed by 12 (35.3%) HEV. There were 8(23.5%) cases of NANEH. 3(0.88%) and 1(0.29%) of the NANEH cases were positive for SEN and TTV viruses respectively. Genotype D was the most prevalent HBV genotype 7(55.55%) followed by genotype F 2(15.38%) and genotype A (7.69%).

Conclusion: FHF continues to be a major challenge for the clinician because of its high mortality rate and the requirement for a multidisciplinary approach.

***Corresponding author:**

Dr. Hiba Sami, M.B.B.S., MD (Microbiology) Assistant Professor, SRMSIMS, Bareilly UP, India
Phone: +91 7895239786



Introduction

Fulminant hepatic failure (FHF) or acute liver failure (ALF) is defined as the rapid development of acute liver injury with severe impairment of synthetic functions and hepatic encephalopathy in patients without obvious previous liver disease. The aetiology of ALF has a wide geographic variation.^[1] In the West, acetaminophen overdose, non-A non-E hepatitis (NANEH) and idiosyncratic drug reactions constitute the bulk of cases.^[2] In developing countries, on the other hand, hepatotropic viruses- hepatitis A or hepatitis B in the Far East^[3] and hepatitis E in India and Bangladesh^[4] seems to be the dominant cause. The most important aetiological cause of ALF in the Indian subcontinent is hepatitis E virus (HEV).^[5] HEV has a high predilection for pregnant women.^[6] The pathogenesis leading to the development of fulminant hepatitis B (FHB) is still being investigated. Although enhanced replication of the hepatitis virus^[7] and an exuberant immune response by the host^[8] are considered to be the main cause, various issues are not fully understood.

Approximately half of all acute HBV infections are subclinical. At the opposite range of the scale, the fulminant cases constitute less than 1% of acute hepatitis B (AHB) infections. Genetic analysis of HBV has shown there to be six different genotypes, A to F, based on an intergroup divergence in nucleotide sequence of 8% or more.^[9] Following reports of outbreaks of fulminant hepatitis B (FHB), it has been suggested that HBV genotypes may be associated with the development of fulminant disease.^[10] The present study was carried out to evaluate the etiology of FHF on one hand and to assess the role of HBV genotypes in acute liver failure in India on the other hand.

Material and Methods

The study was conducted over a period of one and a half years from January 2011 to November 2012 in the Department of Microbiology, J.N. Medical College, A.M.U., Aligarh. During this period 225 cases of acute hepatitis presented to our facility amongst which 34 cases developed fulminant hepatic failure (FHF) and were recruited. Detailed history was elicited and rigorous physical examination was conducted on all the patients. The study was conducted after obtaining permission from institutional ethics committee of J.N. Medical College and the procedure followed in the study was in accordance with institutional guidelines and informed consent was obtained from all the patients before including them in the study.

Patients positive for HBV were re-grouped as follows:

a Acute hepatitis B was defined as a condition associated with prodromal symptoms preceding

the onset of jaundice by 1-2 weeks (e.g. anorexia, nausea, vomiting, and fatigue), fever, an onset of clinical jaundice, diminished constitutional prodromal symptoms, hepatomegaly, jaundice, hyperbilirubinemia (>17 mol/l), serum amino alanine transaminase (ALT), and aspartate amino transferase (AST) at least fivefold greater than normal, HBsAg (+), anti-HBc IgM (+), anti-HBc IgG (-), anti-HBs (-).

- b) Fulminant Hepatic failure/Hepatic encephalopathy:** The diagnosis of FHF/ALF was made if encephalopathy developed within 8 weeks of the onset of symptoms of liver disease, in a patient with no prior known liver disease.^[11] The diagnosis and grading of complications of FHF were made on standard criteria.^[12] All variables included in determining the prognosis were those recorded at presentation. For complications namely cerebral oedema, renal failure and gastrointestinal bleeding, findings recorded within 48 h of hospitalization were included for analysis.
- c) Non-A to Non-E hepatitis (NANEH):** It was diagnosed in a patient with: (I) clinical, biochemical and histological features of acute hepatitis, (II) absence of acute viral markers of known hepatitis viruses (A–E), (III) no exposure to drugs, hepatotoxins, systemic infections, biliary obstruction/infection and metabolic liver diseases.

Exclusion criteria: Patients with autoimmune hepatitis, alcoholic hepatitis, drug induced hepatitis, human immunodeficiency virus co-infection, patients giving history of recent infection, surgery, trauma within the preceding two months, renal insufficiency or with other acute or chronic inflammatory diseases were excluded from this study. None of the participants had received any antiviral or immunosuppressive therapy before or during the course of this study.

Collection of specimen: For all serological assays, venous blood samples were obtained after taking an informed consent. After centrifugation the serum was stored at -40 °C until used for study.

Routine Investigations: Liver function tests (LFT) like serum amino alanine transaminase (ALT), serum aspartate amino transferase (AST) and alkaline phosphatase (ALP), bilirubin (direct & indirect) total bilirubin, albumin, globulin, creatinine and international normalized ratio for prothrombin time were performed. Specific investigations like ultrasonographic examination of liver, upper GI endoscopy and liver biopsy were performed wherever feasible.

Serological investigations: All patients with hepatitis were screened for HAV (Hepatitis A virus), HBV (Hepatitis B virus), HCV (Hepatitis C virus), HEV (Hepatitis E virus) and HIV by commercially available ELISA kits: HBsAg, third generation anti-HCV, fourth generation anti-HIV (J. Mitra & Co. Pvt. Ltd., India), anti-HAV IgM and anti HEV IgM (DRG International, Inc., USA). HBc IgM antibodies were tested in HBsAg positive samples using DRG Anti- Hepatitis B Core IgM Antigen ELISA kit, (DRG International Inc., USA). The tests were performed according to the manufacturer's instructions.

Genotyping of HBV: HBV positive cases were genotyped. DNA was extracted from 100 µL serum by phenol chloroform extraction method. A 125 base pair sequence of the surface gene of HBV was amplified using a thermal cycler (Labnics, USA). A genotyping system based on PCR using type-specific primers was used in this study for the determination of genotypes D of hepatitis B virus according to previously described methods by Kirschberg et al. [13]

Heminested PCR was performed on all the sera which could not be genotyped by the above method. Samples which could not be identified by heminested PCR were subjected to monoplex PCR using the method of Naito et al. [14] In brief, 10 ml of extracted DNA was subjected to 40 cycles of first round PCR using primers 5'-TCA CCA TAT TCT TGG GAACAA GA-3' (nt 2823-2845, universal, sense) and 5'-CGA ACC ACT GAA CAA ATG GC-3' (nt 685-704, universal, antisense) amplifying a 1063 bp region of S gene [14]. TSP-PCR was performed in two separate mixes A and B utilizing 1 ml of 1st round PCR product and subjected to two rounds PCR cycles (20 cycles each) as described by Naito et al. [14]

In mix A, primers specific for genotype A (5'-CTC GCG GAG ATT GAC GAG ATG T-3' nt 113-134, type A specific, antisense), genotype B (5'-CAG GTT GGT GAG TGA CTG GAG A-3' nt 324-345, type B specific, antisense), genotype C (5'-GGT CCT AGG AAT CCT GAT GTT G-3' nt 165-186, type C specific, antisense) and a common universal sense primer (5'-GGC TCA AGT TCA GGA ACA GT-3' nt 67-86, types A to C specific, sense) were used.

In the mix B a common antisense primer (5'-GGA GGC GGA TCT GCT GGC AA-3' nt 3078-3097, specific for types D to F, antisense) along with genotype specific primer D (5'-GCC AAC AAG GTAGGA GCT-3' nt 2979-2996, type D specific, sense), E (5'-CAC CAG AAA TCC AGA TTG GGA CCA-3' nt 2955-2978, type E specific, sense), and F (52-GTT ACG GTC CAG GGT TCA CA-3 nt 3032-3051, type F specific, sense) were used. Mix A

allowed for the specific detection of PCR products for types A (68 bp), B (281 bp), and C (122 bp), and mix B allowed for detection of types D (119 bp), E (167 bp), and F (97 bp).

SEN and TTV viruses were looked for in all Non-A to Non-E hepatitis (NANEH) by the following method:

Detection of SEN virus: SEN virus DNA (349 bp) was detected using nested-PCR procedures standardized for the detection of specific sequences for SEN virus and its genotypes SENV-D (193bp) and SENV-H (118bp). [15] The primer sequence are as follows: SENV common primers: SP15' TWCYCMAACGACCAGCTAGACCT3', SP2 5' G T T T G T G G T G A G C A G A A C G G A 3'; SENV-D primers: SP35' CTAAGCAGCCCTAACACTCATCCAG-3', SP45' GCAGTTGACCGCAAAGTTACAAGAG3'; SENV-H primers: SP55' TTTGGCTGCACCTTCTGGTT3', SP6- 5' AGAAATGATGGGTGAGTGTTAGGG3', where W=A/T, Y=C/T, and M=A/C.

The outer PCR for SENV was carried out with a reaction mixture consisting of 12.5 µl of 2x PCR master mix (MBI Fermentas, USA) containing 0.05 units/µl Taq DNA polymerase in reaction buffer; PCR buffer consisting of 4 mM MgCl₂, 0.4 mM dATP, 0.4 mM dCTP, 0.4 mM dGTP and 0.4 mM dTTP, 5 pmol concentration of SP1 and SP2 primers and 5 µl DNA sample. The reactions consisted of preheating at 94°C for 4 min, 40 cycles of denaturation at 94 °C for 15 sec, annealing at 55°C for 50 sec, extension at 72°C for 50 sec, and a final extension at 72°C for 7 min. The first nested PCR for SENV-D was carried out with the same reaction mixture with SP3 and SP4 primers and 5 µl of the outer PCR product. The second nested PCR for SENV-H was carried out with the same reaction mixture used as above with SP5 and SP6 primers and 5 µl of the outer PCR product. Cycling conditions for both SENV-D and SENV-H PCR were the same as for SEN-V.

TTV DNA detection: 16S rRNA genes were targeted for amplification of TTV specific nucleic acid by nested PCR. Primers were synthesized from Operon, Germany (Genetix). The first set of primers were NS1 5'-GGGTGCCGAAGGTGAGTTTAC-3', NS2- 5' GCGGGGCACGAAGCACAGAAG-3' while the second set of primers were: NS3- 5'-AGTTTACACACCGAAGTCAAG-3' and NS4- 5'-AGCACAGAAGCAAGATGATTA-3' as described by Biagini et al. [16] The first round PCR was carried out for 40 cycles with preheating at 96°C for 2 min, followed by each cycle consisting of denaturation at 94°C for 15 seconds, primer annealing at 55°C for 45 seconds and extension at

72°C for 45 seconds, followed by an additional extension at 72°C for 7 minutes in a solution containing primer NS1 (5 pmol), primer NS2 (5 pmol), DNA template (5 µl), nuclease free water (3 µl), and 2x PCR master mix (10 µl). The second round PCR was carried out for 40 cycles, each cycle consisting of denaturation at 94°C for 15 seconds, primer annealing at 50°C for 45 seconds and extension at 72°C for 45 seconds, followed by an additional extension at 72°C for 7 minutes in a solution containing primer NS3 (5 pmol), NS4 (5 pmol), PCR product of round one (2 µl), nuclease free water (6 µl), 2x PCR master mix (10 µl). The 2x PCR master mix contained the reagents as described previously. The amplified PCR products (5 µl each) were subjected to electrophoresis and visualized under a gel documentation system (Biorad, USA).

Statistical analysis: Statistical analysis was performed with the IBM SPSS Statistics 19. Results were expressed as means ± standard deviation or as percentages. Means were compared between groups by using the t –test, ANOVA (one way analysis of variance) and frequency distributions were compared by using the chi- square test.

Results

The study group comprised of 34 FHF patients. In the two year period, majority of FHF were detected in HBV 13 (38.2%) followed by 12 (35.3%) HEV. Only one case was attributed to HCV. HAV was not associated with FHF. There were 8(23.5%) cases of NANEH (Figure 1). 3(0.88%) and 1(0.29%) of the NANEH cases were positive for SEN and TTV viruses respectively. Half of pregnant females with HEV had a fulminant outcome. The mortality rate was 30.8% in HBV and a higher 41.7% in HEV. Patients with HEV had higher grades of encephalopathy, Sepsis, renal failure and gastrointestinal bleeding was more often observed in HEV than in the non-HEV group.

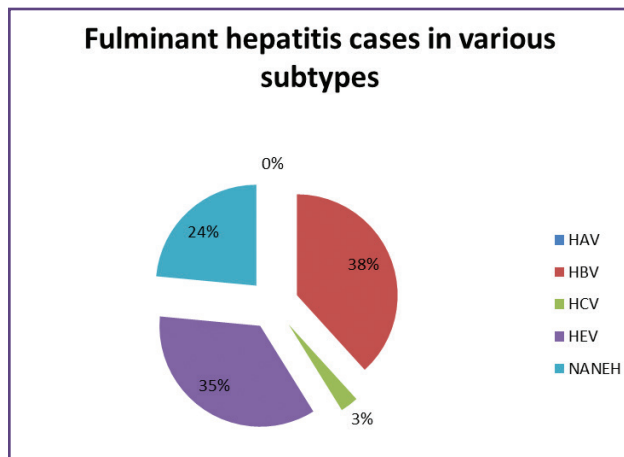


Fig. 1

Characteristics of the Fulminant Hepatitis B Patients: The clinical characteristics of the 13 FHB (Fulminant Hepatitis B) patients are shown in Table 1. The mean age was 36.0 years (range, 29–71), and the ratio of male to female was 1.6 :1. The majority (61.5%) of patients were in the third decades of life. The mean peak total bilirubin was 18.5, the mean peak alanine aminotransferase was 200 IU/ml, and the mean lowest prothrombin time was 1.3.

Determination of HBV Genotype: In our study, genotype D was the most prevalent genotype, 7 (55.55%) in FHB patients (figure 2), followed by genotype F 2(15.38%) and genotype A (7.69%). 3(23.07%) of the FHB samples were not typable. Although liver enzymes were highly elevated in all patients with FHF, genotype D had highest elevations in LFT followed by genotype A and F.

In FHB patients, majority of patients who were HBeAg positive were older. Of the 7 genotype D cases, majority (71.42%) were HBeAg positive as against genotype F where 100% were HBeAg negative. This may indicate that apart from human immune response, viral factors of genotype D may also play a role in FHF.

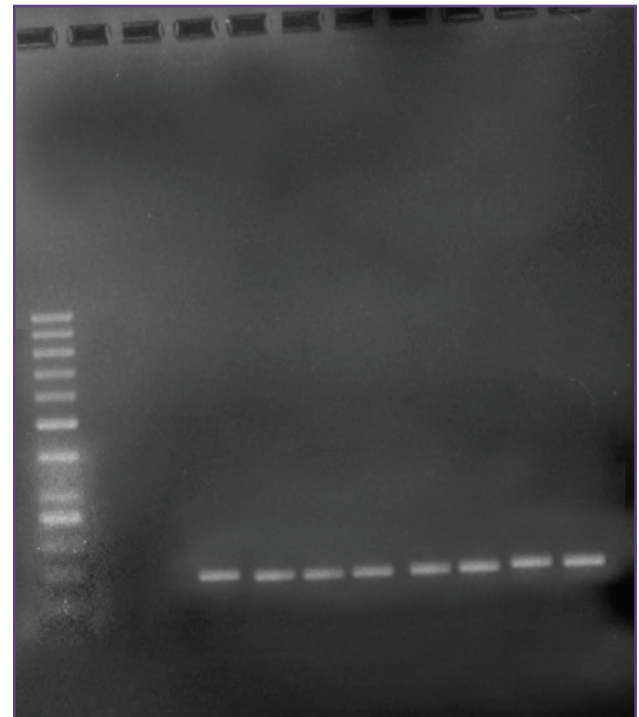


Fig. 2: Agarose gel electrophoresis of HBV genotype D (using heminested PCR). PCR product of 147 bp. Lane M: 50 bp DNA ladder; lane NC: Negative Control; lane 11: positive control; lanes 3-10 samples; lanes 4,5,6,7,8,9,10: genotype D

Table 1: Demographic profile, hepatitis B e antigen (HBeAg) status, and Biochemical profile of patients with Fulminant Hepatic Failure comparing different genotypes (n=13)

Variable	Genotype D(n=7)	Genotype F(n=2)	Genotype A(n=1)	Nontypable (n=3)
Age, mean years \pm SD	34.00 \pm 17.5	50	40	30.5 \pm 27.57
Ratio of male to female subjects	5:2	1:1	1:0	1:2
Complications				
Encephalopathy	3 (42.8%)	1 (50%)	0	0
ARF	0	0	1 (100%)	0
Bleeding tendencies	2 (28.6%)	0	1 (100%)	0
HBeAg positive, no(%)	5(71.4%)	0	1(100)	0
HBeAg negative, no(%)	2(28.5%)	2(100)	0	3(100)
ALT (IU/L) (Normal 2-15)	88.2 \pm 81.99	12	60	110.5 \pm 48.7
AST (IU/L) (Normal 2-20)	82.8 \pm 71.72	21	90	97 \pm 45.25
PT-INR (Normal 0.9-1.3)	3.3 \pm 2.03	2.1	4.3	2.91 \pm .83
MELD	26.4 \pm 17.7	24	27	31.00 \pm 2.82

Discussion:

The specific physiopathology of fulminant hepatic failure induced by viruses has not yet been well delineated, but some studies have suggested that the disease severity induced by these viruses may be related to genetic variability.^[17] In this study, we determined the prevalence of different hepatitis viruses in patients of FHF.

Hepatitis A was not associated with FHF in the present study. HAV is a ubiquitous agent in developing countries, is highly pathogenic and spreads through person-to-person transmission. The entire childhood population in such countries is exposed to HAV in the first 10 years of age and all adults are protected from HAV infection.^[18] In fact, hepatitis A is a rare cause of acute viral hepatitis in adults in developing countries and the majority of HAV infections occur in children, with mild or anicteric disease.^[19]

The prevalence of HBV was found to be 38.2% in FHF and the mortality rate was 30.8%. The prevalence of HBV infection in previous reports of FHF has reportedly ranged from 10%-65%.^[20] The mortality rate from FHF resulting from HBV has been reported to be as high as 61%-77%.^[21]

HCV infection was determined in 2.94% of our patients. Hepatitis C is a rare cause of FHF in United States and Europe but a number of studies from Japan and India have found evidence of hepatitis C in the non-A, non-B group of patients.^[22, 23]

In our study, a high prevalence of HEV was observed (35.3%) in FHF cases which is similar to those found in other studies who reported 23-62% prevalence of HEV in the fulminant hepatic failure (FHF).^[24, 25, 26]

Eight (23.5%) patients in the present study lacked acute markers of known hepatitis viruses and were classified as NANEH. SENV and TTV were found in 0.88% and 0.29% of NANEH patients. Previous studies from India have reported the existence of TTV other than A-G hepatitis viruses, causing liver diseases.^[27,28] SENV has been recently identified as a candidate agent of non A-E hepatitis virus.^[29] However, the exact role of this virus in the pathogenesis of chronic liver diseases, including chronic hepatitis and cirrhosis, and the development of hepatocellular carcinoma (HCC) remains to be verified.^[30]

FHB patients were further genotyped and assessed for their influence on disease severity. We found genotype D to be the most prevalent genotype followed by genotype F and genotype A. In our patients of FHB, mean LFTs in the patients with genotype D were raised five to six folds compared to normal whereas PT-INR and MELD were extremely high. HBeAg positivity was observed in 66.7 % of patients suggesting active replication in FHF patients. Mean ALT and AST were higher in HBeAg negative patients than HBeAg positive patients.

There is a paucity of data on the clinical course of patients with genotypes other than B and C. One study from Spain reported that HBeAg seroconversion rates were similar in patients with genotypes A and D, but sustained biochemical and virological remission was more common in patients with genotype A who had HBeAg seroconversion.^[30] Patients with genotype A also had a higher rate of HBsAg clearance. However, the need for liver transplantation and the deaths related to liver disease were comparable between patients with genotypes A and D. In this study,

patients with genotype D were more likely to die from liver disease than those with genotype F.

The role of HBV genotype in FHB is controversial.^[32] In a study from Japan, HBV genotype B was found more frequently in patients with acute forms of liver disease than in those with chronic liver disease, and more frequently in patients with fulminant hepatitis than in those with acute hepatitis.^[33] These results suggested that HBV genotype B may induce more severe liver damage than other genotypes. A study from USA showed that genotype D was more frequently found in ALF patients than in those with chronic HBV infection.^[34] Based on these findings, it seems that the development of fulminant hepatitis is not clearly linked to a particular HBV genotype, and may merely reflect the predominance of certain genotypes in different populations as genotype D is the most predominant genotype in this part of North India.^[35,36] However, further studies are needed to ascertain the effect of genotypes on fulminant outcome of HBV.

Conclusions

FHF continues to be a major challenge for the clinician because of its high mortality rate and the requirement for a multidisciplinary approach. HEV is a common cause of FHF in this region of North India HBV genotypes can be used to predict the course of fulminant hepatitis B.

Conflicts of interest

No conflicts of interest.

References

1. Lee W, Sorrell M. Developing a world view toward acute liver failure. *Hepatology*. 1996; 24: 270–1.
2. Schiodt F, Rochling F, Casey D, Lee W. Acetaminophen toxicity in an urban county hospital. *N Engl J Med*. 1997; 337: 1112–7.
3. Trey C, Davidson C. Inc: Popper H, Schaffner F, editors. The management of fulminant hepatic failure. *Progress in Liver Disease*. 1970; Volume 3. New York: Grune and Stratton: 282–98.
4. Vaquero J and Andres T. Etiology and management of fulminant hepatic failure. *Current Gastroenterology Reports*. 2003; 5:39–47.
5. Khuroo M. Acute liver failure in India. *Hepatology*. 1997; 26: 244–246.
6. Khuroo M, Teli M, Skidmore , Sofi M, Khuroo M. Incidence and severity of viral hepatitis in pregnancy. *Am J Med*. 1981; 70: 252–255.
7. Baumert T, Rogers S, Hasegawa K, Liang T. Two core promoter mutations identified in a hepatitis B virus strain associated with fulminant hepatitis result in enhanced viral replication. *J Clin Invest*. 1996; 98: 2268–76.
8. Rivero M, Crespo J, Fabrega E, et al. Apoptosis mediated by the Fas system in the fulminant hepatitis by hepatitis B virus. *J Viral Hepat*. 2002; 9:107–13.
9. Norder H, Courouce A, Magnius L. Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. *Virology*. 1994; 198:489–503.
10. Mahtab M, Rahman S, Khan M, Mamun A, Afroz S. Etiology of fulminant hepatic failure: Experience from a tertiary hospital in Bangladesh. *Hepatobiliary Pancreat Dis Int*. 2008; 7: 161–4.
11. Riordan S, Williams R. Treatment of hepatic encephalopathy. *N Eng J Med*. 1997; 337: 473–478.
12. Sherlock S, Dooley J. Diseases of the liver and biliary system, Oxford: Blackwell Scientific Publications; 1997: 9th edn.
13. O. Kirschberg, C. Schuttler, R. Repp, and S. Schaefer. A multiplex-PCR to identify hepatitis B virus—genotypes A–F. *Journal of Clinical Virology*. 2004; 29: 39–43.
14. Naito H, Hayashi S, Abe K. Rapid and specific genotyping system for hepatitis B virus corresponding to six major genotypes by PCR using type-specific primers. *J Clin Microbiol*. 2001; 39: 362–4.
15. Tang Z, Chen X, Yu Y, Zang G. Prevalence and clinical significance of SEN virus infection in patients with non A-E hepatitis and volunteer blood donors in Shanghai. *World J Gastroenterol*. 2008; 14:4204–8.
16. Biagini P, Gallian P, Attoui H, Cantaloube J, de Micco P, de Lamballerie. Determination and phylogenetic analysis of partial sequences from TT virus isolates. *J Gen Virol*. 1999; 80:419–24.
17. Fujiwara K., Yokosuka O, Ehata T, et al. Association between severity of type A hepatitis and nucleotide variations in the 5' non-translated region of hepatitis A virus RNA: strains from fulminant hepatitis have fewer nucleotide substitutions. *Gut*. 2002; 51: 82–8.
18. Khuroo M. Study of an epidemic of non-A, non-B hepatitis: possibility of another human hepatitis virus distinct from post-transfusion non-A, non-B type. *Am J Med*. 1980; 68: 818–823.
19. Khuroo M, Rustgi V, Dawson G et al. Spectrum of hepatitis E virus in India. *J Med Virol*. 1994; 43: 281–286.

20. Schiodt F, Atillasoy E, Shakil O, Schiff E, Caldwell C, Kowdley K, Stribling R, et al. 1999. Etiology and outcome for 295 patients with acute liver failure in the United States. *Liver Transpl Surg.* 1999; 5: 29–34.
21. O’Grady J, Alexander G, Hayllar K, Williams R. Early indicators of prognosis in fulminant hepatic failure. *Gastroenterology.* 1989; 97: 439–445.
22. Farci P, Alter H, Shimoda A et al. Hepatitis C virus-associated fulminant hepatic failure. *N Engl J Med.* 1997; 335: 631–634.
23. Takahashi Y, Shimizu M. Aetiology and prognosis of fulminant viral hepatitis in Japan: a multicentric study. *J Gastroenterol Hepatol.* 1991; 6: 159–164.
24. Nanda S, Yalchinkaya K, Panigrahi A, Acharya S, Jameel S, Panda S. Etiological role of hepatitis E virus in sporadic fulminant hepatitis. *J Med Virol.* 1994; 42: 133–137.
25. Nejdard S, Mitchell F, Biswas R. Simultaneous amplification and detection of specific hepatitis B virus and hepatitis C virus genomic sequences in serum samples. *J Med Virol.* 1994; 42: 212-6.
26. Tan D, IM S, Yao J, Ng M. Acute sporadic hepatitis E virus infection in Southern China. *J Hepatology.* 1995; 23: 239-45.
27. Irshad M, Chaudhuri P, Joshi Y. Superoxide dismutase and total anti-oxidant levels in various forms of liver diseases. *Hepatol Res.* 2002; 23: 178-184.
28. Rizvi Meher, Jahan Shafquat, Azam Mohd, Ajmal M.R, Shukla Indu, Malik Abida, et al. Prevalence and assessment of role of SEN virus in acute and chronic hepatitis in India. *Tropical Gastroenterology.* 2013; 34(4):227–234.
29. Umemura T, Yeo A, Sottini A, et al. SEN virus infection and its relation to transfusion-associated hepatitis. *Hepatology.* 2001; 33:1303–1311
30. Schroter M, Laufs R, Zollner B, Knodler B, Schafer P, Sterneck M, et al. Prevalence of SENV-H viraemia among healthy subjects and individuals at risk for parenterally transmitted diseases in Germany. *J Viral Hepatology.* 2002; 9: 455 – 459.
31. Sanchez-Tapias , Costa J, Mas A, Bruguera M, Rodes J. Influence of hepatitis B virus genotype on the long-term outcome of chronic hepatitis B in western patients. *Gastroenterology.* 2002; 123: 1848-1856.
32. Hou J, Lin Y, Waters J, et al. Detection and significance of a G1862T variant of hepatitis B virus in Chinese patients with fulminant hepatitis. *J Gen Virol.* 2002; 83:2291-8.
33. Imamura T, Yokosuka O, Kurihara T, et al. Distribution of hepatitis B viral genotypes and mutations in the core promoter and precore regions in acute forms of liver disease in patients from Chiba, Japan. *Gut.* 2003; 52:1630-7.
34. Wai C, Fontana R, Polson J, et al. The US Acute Liver Failure Study Group. Clinical outcome and virologic characteristics of hepatitis B-related acute liver failure in the United States. *J Viral Hepat.* 2005; 12:192-8.
35. Sami Hiba, Rizvi Meher, Azam Mohd, et al. Emergence of Hepatitis B Virus Genotype F in Aligarh Region of North India. *Advances in Virology.* 2013: Article ID 846849, 8 pages.
36. Chattopadhyay S, Das B, Kar P. Hepatitis B virus genotypes in chronic liver disease patients from New Delhi, India. *World J Gastroenterol.* 2006; 12:6702-6706.