ISSN Print: 2664-6536
ISSN Online: 2664-6544
Impact Factor: RJIF 5.4
IJBB 2025; 7(1): 98-105
www.biosciencejournal.net
Received: 06-01-2025
Accepted: 09-02-2025

Nahida Sultana
Department of Virology, Bangabandhu Sheikh Mujib Medical University, Shahabag, Dhaka, Bangladesh

Dr. Umme Shahera
Department of Virology, Bangabandhu Sheikh Mujib Medical University, Shahabag, Dhaka, Bangladesh

Mohammad Enayet Hossain
Virology Laboratory & Infectious Disease Division Emerging Infection, International Center for Diarrhoeal Research Laboratory, Dhaka, Bangladesh

Saif Ullah Munshi
Department of Virology, Bangabandhu Sheikh Mujib Medical University, Shahabag, Dhaka, Bangladesh

Shahina Tabassum
Department of Virology, Bangabandhu Sheikh Mujib Medical University, Shahabag, Dhaka, Bangladesh

Corresponding Author:
Dr. Umme Shahera
Department of Virology, Bangabandhu Sheikh Mujib Medical University, Shahabag, Dhaka, Bangladesh

Hepatitis C Virus molecular characterization in Bangladeshi patients

Nahida Sultana, Dr. Umme Shahera, Mohammad Enayet Hossain, Saif Ullah Munshi and Shahina Tabassum

DOI: https://www.doi.org/10.33545/26646536.2025.v7.i1b.103

Abstract
Background: Every year, over 3-4 million new cases of HCV infection are reported worldwide. Enhancing the effectiveness and tolerability of HCV treatment has been made possible by advances in our understanding of the genome and proteins of the hepatitis C virus (HCV). Because HCV genotype influences treatment and medication dosage, it is necessary to determine HCV genotype prior to beginning treatment. The purpose of this study was to determine which HCV genotypes and subtypes were prevalent among Bangladesh's HCV-infected population.
Methods: The study participants were chosen from among anti-HCV-positive blood donors at BSMMU's Department of Virology for HCV RNA measurement. Following the measurement of the HCV viral load, 36 randomly chosen specimens from 150 HCV RNA-positive samples were included in this cross-sectional study and subjected to One Step testing for NS5B gene amplification. The RT-PCR. After 36 readable partial sequences of the NS5B gene were discovered, the NCBI Genotyping tool was utilized to genotype the circulating HCV.
Findings: Of the study population, 31 (86.1%) had genotype 3 infection, whereas only 5 (13.9%) had genotype 1 infection. In the study population, subtype 3b was the most common (42%) and was followed by subtype 3a (39%), 1b (11%), 3g (5%), and 1a (3%). The circulating strains of Bangladeshi HCV clustered closely with strains from Pakistan, China, Thailand, Malaysia, Indonesia, Germany, the United States, and Canada, according to phylogenetic analysis of the virus's sequences. In addition to working abroad and sharing one or two drug trafficking routes, all of these nations share immigration-related migration.
Conclusion: The current study's findings, which will help clinicians better treat chronic HCV patients, showed that at least two genotypes and five distinct HCV subtypes are prevalent among Bangladesh's hepatitis C-infected population.

Keywords: Non-structural 5b, non-coding sequencing, hepatitis C virus, and HCV genome typing

Introduction

Overview

First discovered in the late 1980s, the hepatitis C virus (HCV) is a leading cause of liver disease worldwide [1]. It is a positive-sense single-stranded RNA virus that is a member of the Flaviviridae family's genus Hepacivirus. A single long polyprotein with the following gene order is encoded by the HCV genome, an RNA molecule with roughly 9600 nucleotides organized in a coding region that contains one large open reading frame (ORF): E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-C-E1-E2 [2]. The hepatitis C virus's genome is incredibly diverse. The non-structural (NS) 5b-encoded RNA-dependent RNA polymerase used for HCV replication is highly prone to errors and produces mutations at a rate of approximately 10±5 per replication per nucleotide [3]. Under the selective pressure of the host immune system during the course of infection, it produces quasispecies, which are made up of a collection of diverse RNA sequences centered on a dominant nucleotide sequence that varies [4]. According to phylogenetic analysis, average sequence divergence can be used to distinguish between HCV types, subtypes, and isolates. HCV isolates fall into seven main categories, each of which has 67 subtypes [5]. Globally, HCV genotypes exhibit varying geographic distributions [6]. For the clinical implementation of tailored treatment for chronic hepatitis C infection, HCV genotyping is required [7]. Direct-acting antiviral agents (DAAs) have advanced in the treatment of HCV, moving

from interferon-containing regimens in 2011 to interferon-free regimens, which are currently the norm in the majority of Western nations [8]. The available DAAs are genotype and/or subtype specific, and response guided therapy (RGT) with interferon (IFN)-based therapy is genotype specific for HCV. To improve management and lower treatment costs, the HCV genotype, including genotype 1 subtypes (1a or 1b), should be evaluated before starting treatment [9]. Investigating infection outbreaks and comprehending the epidemiology and virological characteristics of the virus are further benefits of genotyping.

Bangladesh has a chronic HCV carriage rate of 0.8% and a low endemic HCV rate. Between 0.8% and 2.4% of the general population and a number of high-risk groups, such as intravenous drug users, have chronic HCV [10, 11]. However, there is a dearth of information from Bangladesh on the prevalence of recombinant viruses, HCV genotypes and subgenotypes, and virulence-associated traits like drug resistance mutations. We conducted a prospective cross-sectional study to identify the dominant HCV genotypes and subgenotypes in order to address this lack of data. Consequently, HCV genotyping and precise subtype identification are regarded as a clinically significant metric for the possible duration and response of HCV treatment, as well as helpful for comprehending the HCV epidemiology and developing vaccines.

Materials and Methods

The study population consisted of anti-HCV-positive patients who gave blood for HCV RNA measurement at the Department of Virology, BSMMU. Only 36 HCV RNA patients were ultimately chosen based on selection criteria for NS5B gene PCR amplification out of 150 HCV RNA positive patients [HCV RNA > 600 IU/ml] following HCV viral load quantification. RNA levels below 600 IU/ml (Analytik Jena AG, 07745 Jena/Germany) were not genotyped in specimens that were kept at -80˚C.

RNA extraction and RT-PCR: In accordance with the manufacturer's instructions, 150 µl of human plasma was used to extract HCV-RNA using the Instant Virus RNA KIT (Analytik Jena AG, 07745 Jena, Germany). International units per milliliter are used to present the results. The RT-PCR process was performed using a 25 µl reaction volume that contained 5 µl of extracted RNA, 5X QIAGEN OneStep RT-PCR buffer, 400 µM of each of dATP, dCTP, dGTP, and dUTP, and 0.5 µM antisense and sense primers (Forward primer-DM100: 5'-TACCTVGTCATAGCCTCCGTGAA-3', Position (nt): 8616-8638 and more). In reverse Primers: DM101: 5'-TTCTCRTATGAYACCCGCTGYTTTGA-3', position (nt): 8250-8275; amplification size: 389 bp; 2.0 µl QIAGEN OneStep RT-PCR enzyme mix; 10 U RNAguard RNase inhibitor (Amersham Biosciences Inc.). Samples were incubated at 50°C for 30 min for reverse transcription and 95°C for 15 min for initial PCR activation. DNA amplification was performed for 40 cycles each consisting of 55°C for 30 s, 72°C for 60 s, and 94°C for 15 s in a GeneAmp PCR System 9600 or 9700 (Applied Biosystems, Foster City, CA). The last cycle was followed by a 10-min extension step at 72°C. The PCR products were run on 1.5% agarose gel, stained with EtBr and visualized under UV. Samples showing a band of the appropriate size were further analyzed by DNA sequencing.

DNA purification and sequencing: The ExoSAP-IT (Affymetrix, California, USA) method, a special one-step enzymatic cleanup of PCR products that removes un-incorporated primers and dNTPs so they do not obstruct downstream sequencing, was used to purify amplicons. The ABI Prism Big Dye Terminator cycle sequencing ready reaction kit v3.1 (Applied Biosystems, USA) was used to perform the sequencing reactions, and it was electrophoresed on an ABI Prism 3500 XL genetic analyzer (Applied Biosystems). For NS5B-derived PCR products, which were typically sequenced using antisense primer DM100 (Plus), amplicons were sequenced in a single direction using the antisense amplification primers.

Editing, assembling sequences, and calling bases

Chromas 2.3 (Technelysium, Australia) or BioEdit (version 5.0.9) were used to manually edit the sequence [12, 13]. Primers were eliminated and degenerate base codes from the International Union of Biochemistry were added as part of the sequence editing process.

Genotype determination: After comparing each sample's sequence to that of HCV prototypes found in GenBank, additional genetic analysis was conducted to ascertain each sample's genotype. Phylogenetic analysis was used to assign genotypes. Sequences were manually edited in Bioedit [12], version 7.1.3, after being aligned using Clustal W Multiple Alignment. The MEGA software package version 5.05 was used to perform molecular evolutionary and phylogenetic analyses [14]. To create neighbor-joining (NJ) trees, MEGA 5.05 software (Sinauer Associates, Inc.) was utilized. Using the Kimura 2-parameter method and bootstrap analysis [15] with 1000 replicates, the reliability of the NJ trees was statistically assessed. The Kimura 2-Parameter method was used to calculate genetic distances [16]. The neighbor-joining method was used to create the dendograms [17].

Analysis of statistics

The chi-square test and a Student's unpaired t test were used to verify the overall proportions of HCV subtypes identified by sequencing the NS5b region. P-values less than 0.05 were regarded as significant.

Findings

HCV RNA quantification tests were performed on 500 consecutively identified HCV-infected patients. Following the measurement of the viral load, NS5B region sequencing was performed on the 36 randomly chosen positive patients.

The demographic characteristics of the population being studied

The mean age of the 36 patients in this study was 43.53±13.03 years (range: 23-72 years), with 22 (44.9%) being male and 14 (28.9%) being female. Seven (14.3%) of these 36 patients had a past history of hepatitis, while 3 (6.1%) had a family history of hepatitis. Table 1 summarizes the demographic characteristics of every patient who was enrolled.

Table 1: Demographic features of the study population

Characteristics	Patients
Gender, N (%)	
Male	22 (44.9%)
Female	14 (28.6%)
Age years	
Median (range)	40 (23-72)
Mean±SD	43.53±13.03
Family History of hepatitis, n (%)	
Present	3 (6.1%)
Absent	33 (67.3%)
Past History of hepatitis, n (%)	
Present	7 (14.3%)
Absent	29 (59.2%)

Biochemical profile

Patients with genotypes 1 and 3 had an average HCV viral load of 14.11±6.77 [log (copies/ml)]. 12.80±2.05 [log (copies/ml)] and 13.53 to 14.98 [log (copies/ml)] are the ranges. Table I shows that the range is 7.17 to 15.82 [log (copies/ml)]. Patients with genotype 1 infection had an average ALT level of 51.2±34.4 U/L (range: 17 to 108 U/L), whereas patients with genotype 3 infection had an ALT level of 89.6±86.6 U/L (range: 18 to 446 U/L). Patients with genotype 1 and genotype 3 infections did not differ statistically significantly in serum ALT ($p>0.05$) or plasma viral load ($p = 0.174$).

Table 2: HCV viral load comparison [log (copies/ml)] and the ALT (U/L) level in patients with various HCV genotypes

Features	Genotype 1	Genotype 3	P value
Genotypes	5 (13.9%)	31(86.1%)	_
Log Mean HCV viral load±SD (Range)	(14.11±6.77) (13.53-14.98)	(12.80±2.05) (7.17-15.82)	0.174*
Mean Serum ALT level ± SD (Range)	(51.2±34.4) (17-108)	(89.6±86.6) (18-446)	>0.05*

*Independent Students t test.

$p \leq 0.05$ indicates statistical significant

HCV genotype and subtype distribution

The HCV genotype and subtype of each of the 36 patients were successfully identified through direct sequencing and phylogenetic analysis of the HCV NS5B gene. Of the 36 patients who tested positive for HCV RNA, 31 (86.1%) had genotype 3 infection, whereas only 5 (13.9%) had genotype 1 infection. Subtype 3b (42%) was the most common type, followed by subtype 3a (39%), subtype 1b (11%), subtype 3g (5%), and subtype 1a (3%), according to the subtype evaluation.

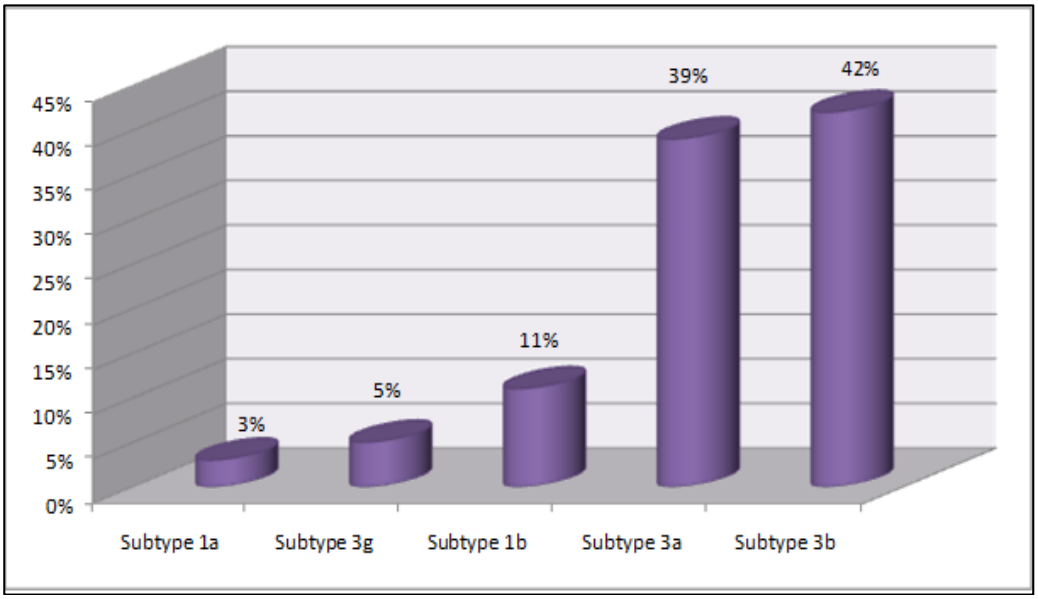

Fig 1: A column diagram displaying the study participants' percentage distribution of HCV subtypes.

Genotype 3 variants: Subtypes were evaluated, and the most common subtype was subtype 3b (42%) followed by subtype 3a (39%). 86.1% of genotype 3 isolates were subtypes 3a, 3b, and 3g. Additionally, variations from subtype 3g (5%) were found (Figure 1).

Genotype 3a: Out of the fourteen HCV 3a strains identified in this investigation, matrix analysis revealed that M0388 and M0618 had the highest nucleotide similarity (97.4%) in the partial sequence of the NS5B gene. These fourteen HCV 3a strains ranged in similarity from 89.5% to 97.4%. Compared

to the other 3a study strains, strain M0672 showed comparatively lower levels of similarity (89.5%-91%) strain M0539 showed 89.5% similarity, while strains M0210, M0283, and M0549 showed 91% similarity. The 3a strain M0210, however, had the highest (99.3%) similarity with a strain from Thailand (HQ229367/CUTH319) when compared to other globally circulated strains, while thirteen other strains from Bangladesh showed higher similarity (94.8%-95.9%) with other Asian strains: North American (USA>UFOMN0526/Canada>HEP-5074), South American (Brazil>0307HC), European (UK>71737927K7, Patients 275 and France>TL53). Nine of the HCV-3a study strains were grouped together in a common clade, according to the phylogenetic tree created to ascertain the evolutionary relatedness of the 3a strains of this study with other globally circulating strains (Figure 2-a).

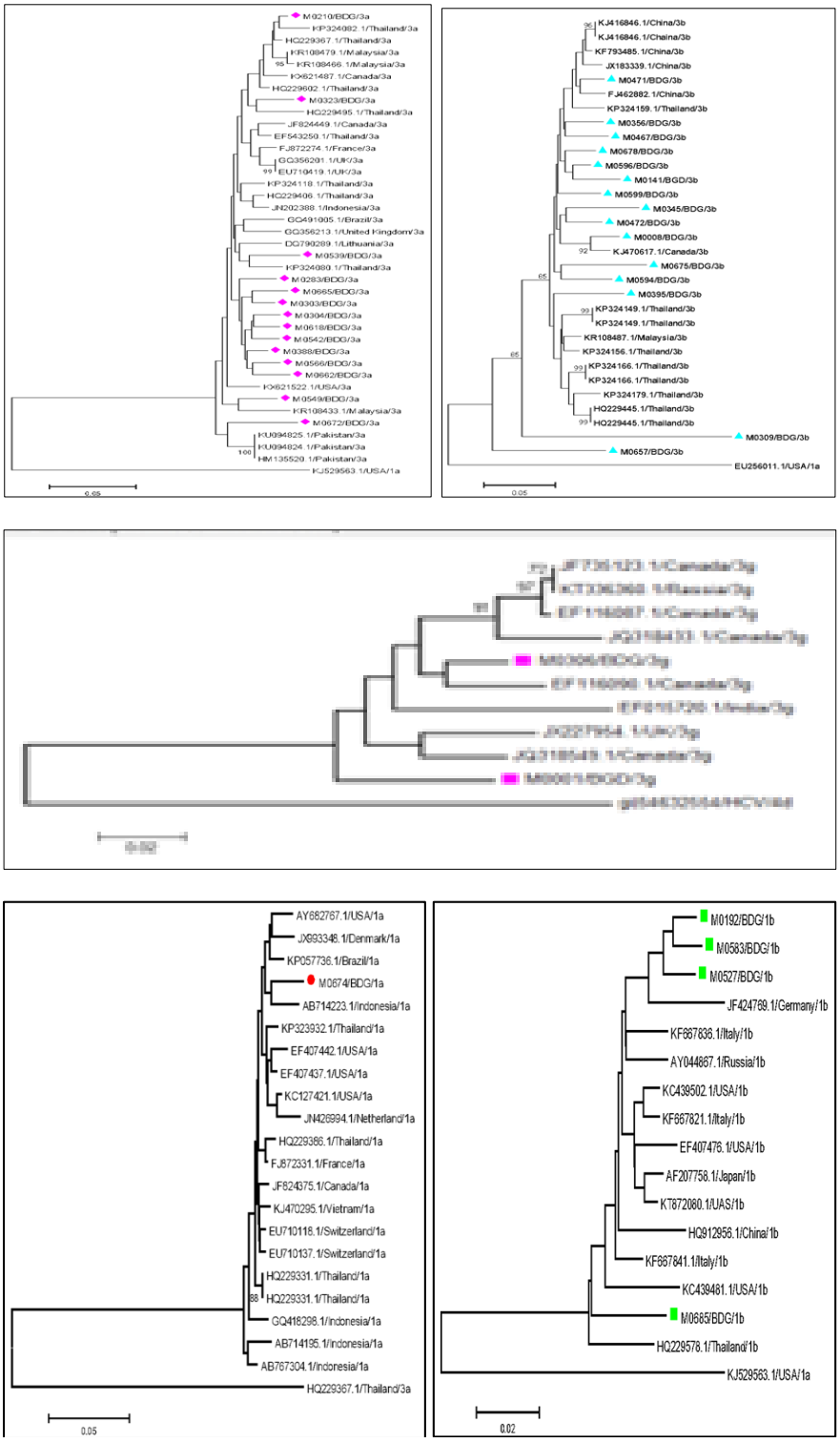

Fig 2: NS5B sequences (340 bp) with neighbor-joining trees (positions 8276 to 8615). Phylogenies are displayed for the sequences of genotypes 3a NS5B (a), 3b NS5B (b), 3g NS5B (c), 1a NS5B (d), and 1b NS5B (e). Genotype 1a sequence KJ529563 was used as the out-group in the phylogenetic tree for genotype 3a sequences, whereas genotype 1a (KJ529563), 1a (EU256011), 4d (gi/54632554), 3a (HQ229367), and 1a (KJ5295631) were used as the out-group in the phylogenetic trees for sequences of genotypes 3b, 3g, 1a, and 1b. The percentage bootstrap support for 1,000 replicates is shown by the numbers at the nodes. Only values greater than 70% are displayed. The genetic divergence is displayed by bars at the base of the trees

None of the nine strains from Bangladesh had any branches in common with the other strains under study or with any strains that were widely distributed. Rather, these nine research strains were positioned on a distinct node that was closely associated with the primary clusters. M0210, M0323, and M0539, on the other hand, clustered with strains from Thailand (KP324048/SK96_south_3a, HQ229495/CUTH216, and KP324080/SK90_south_3a), while M0594 appeared to be related to a strain from Malaysia (KR108433/10MYKJ022). Study strain M0672 shared the common branch with Pakistani strains (KU094825/KP10, KU094824/KP9 and HM135520/PK/FG5).

Genotype 3b: The 15 HCV 3b study strains, the range of similarity ranged from 74% to 96.9%. Nevertheless, two 3b strains, M0471 and M0008, had a higher (96.6%) similarity with the Chinese strain (FJ462882/DL310) and the Canadian strain (KJ470617/QC88) when nucleotide comparison with other globally circulating strains was performed. Furthermore, thirteen additional strains from Bangladesh exhibited high (80.5%-95.8%) similarity to strains from other Asian countries, including China, Malaysia, and Thailand.
The six HCV-3b strains used in this investigation were grouped together in two common clades, according to phylogenetic analysis (Figure 2-b). Six more strains from Bangladesh did not have a common branch with any of the other strains under study or with any strains that were in circulation worldwide. Three strains (M0471, M0356, and M0467) were linked to Canadian strains (KJ470617/QC88), while strains from Thailand (KP324159) and China (FJ462882/DL310) were clustered together. Several 3b strains from Thailand shared a common branch with study strain M0395. It is interesting to note that the dendrogram's locations for the other two study strains (M0309 and M0657) were in an odd spot on the tree. Neither this strain nor any strains that are widely distributed had any branches in common with the other study strains. Rather, it positioned itself on a node that was far from the primary clusters.

3g genotype
M0306 and M0001 had a high degree of similarity (92% at the nucleotide level) according to the similarity matrix of the two 3g genotypes used in this investigation. The strain M0001 displayed the greatest resemblance to strains JX227954/HCV-3g/GB/BID-G1234 from the UK (91.5%). The HCV 3g subtype's similarity matrix showed that it was 89.7%-96.2% similar to strains of HCV 3g that were circulating globally. Comparing nucleotide sequences revealed that M0306 and the Canadian strain EF116090/QC229 were largely (96.2%) similar. It was interesting to note that the other study strain (M0001) was positioned in an odd spot on the tree in the dendrogram. Neither this strain nor any strains that were in widespread circulation had any branches in common with the other study strains. Instead, it positioned itself on a node that was far from the primary clusters (Figure 2-c).

Genotype 1 variants: Subtypes were evaluated, and the most common subtype was subtype 1b (11%) followed by subtype 1a (3%). Thirteen percent of genotype 1 isolates were subtypes 1a and 1b. During this investigation, no additional genotype 1 variants were found (Figure 1).
Genotype 1a: The local strain (M0674) and global strains were highly similar, according to the similarity matrix of the 1a genotype. The study strain (M0674) showed high (96.4%) similarity to strains from Asia (Indonesia/ YOGHhcv120 & Thailand/CUTH127 & CUTH348 & CUTH263), Europe (France/CF4, Vietnam/VN185 & Denmark/TNCC), North America (USA/VO415 and Canada/SN104) and South America (Brazil/028HD) according to nucleotide sequence comparison. Additionally, the study strain exhibited 96% similarity to certain USA strains (EF407437/1030 & AY682767/01448).

M0674 clustered with the Indonesian strain
AB714223/YOGHhcv120 in a single branch with other European and American strains, according to the dendrogram that was created using the partial sequence of the NS5B regions of Bangladeshi 1a subtypes and reference strains from various geographic regions that were available in the GenBank database (Figure 2-d).

Genotype 1b: At the nucleotide level, all four of the subtype 1b study strains exhibited a high degree of similarity (95.4%-98.3%). Additionally, this subtype's three study strains (M0527, M0192, and M0583) shared 96.4% to 97% of their similarities with the German strain Pat21. In contrast to the other three strains, strain M0685 exhibited 95.7%-96.4% similarity. The strains of 1b, with the exception of M0685, were grouped with the German strain Pat-21 and thus belonged to the same clade, according to phylogenetic analysis (Figure 2-e). It is interesting to note that the dendrogram's placement of the other study strain, M0685, was in an odd spot on the tree. This strain positioned itself on a different node that was distantly related to the main clusters, but it did not share any branches with any globally circulating strains or other study strains.

Discussion
Hepatitis C is a known illness with worldwide significance. Since most HCV infections do not go away but instead cause chronicity [18], which is a contributing factor to about one-third of liver cancers worldwide [19], controlling HCV infection is a significant public health concern. Significant nucleotide sequence heterogeneity has been observed in HCV genome sequences from different parts of the world [20]. In addition to being helpful for managing patients, determining the HCV genotype is now a part of medical practice to help comprehend the virus's epidemiological characteristics [21]. DAA combination therapy is currently the gold standard for clinical management of HCV-related morbidity. In order to customize the dosage and length of treatment, the majority of treatment protocols require genotype and subtype information. Therefore, accurate knowledge of HCV's genetic diversity is crucial for patient care.
This study sought to determine the circulating HCV strains in order to address the need for accurate information on the genetic diversity of HCV and to look into the origin of circulating HCV strains. HCV genotypes in Bangladeshi patients with HCV infection. Sequencing the entire virus genome is the standard method for determining HCV genotypes and subtypes. However, this is impractical in a typical clinical laboratory, so surrogate HCV typing methods are employed. Five HCV subtypes 1a, 1b, 3a, 3b, and 3g as well as two distinct genotypes 1 and 3 were identified in this investigation. Among Bangladeshi HCV strains, subtype 3b (42%) was the most common type, followed by subtype 3a (39%), subtype 1b (11%), subtype 3g (5%), and subtype 1a

(3%). In South Asian nations, these are the most common genetic groups [22-25]. According to earlier research from Bangladesh, patients with chronic hepatitis had both HCV genotypes 3b and 3a, with 3b being more common than 3a [26, 25]. The distribution of HCV genotypes did not differ between this study and the earlier research. According to two other studies, this result demonstrated a similar pattern of HCV genotypes circulating among HCV-infected patients in Bangladesh. While genotypes 3b, 3a, 1a, and 1b were more common, genotypes 2a, 4a, 4c, and 2c were less frequently found [27, 28]. In this study population, the identified genotype 3a was also found to be a prevalent genetic group from India and Pakistan, two nearby countries [23, 22]. The other circulating genotypes in this study were 1a, 1b, and 3g. According to some research, genotype 1 is widespread throughout the world, and subtypes 1a and 1b are common in North America, Europe, and Australia, as well as in some regions of Asia [20, 29-31]. With the exception of China, where genotype 1b is more common than 1a, the distribution pattern of HCV genotypes 1a and 1b seen in this study differs from that of Pakistan, India, Australia, and Korea [25], [20]. Nonetheless, genotype 3g was found in this study among Bangladeshi chronic HCV patients, which is remarkable as this genotype is not common in South-East Asia. In addition to being identified in previous research from northern and western India, HCV-3g was only found in Indonesia [32, 33].

There are various ways to conceptualize the hepatitis C virus's (HCV) origin. Its dramatic spread throughout the twentieth century in both developing and Western nations may be considered its origins. New parenteral transmission pathways linked to medical treatment, vaccination, blood transfusion, and more recently, injecting drug use, fueled this blood-borne virus epidemic [34]. Using phylogenetic analysis, one of the study's objectives was to investigate the origin of the HCV strains. The current study's HCV 3a strains clustered with HCV 3a strains from South and Southeast Asia. They also displayed greater similarity to HCV genotype 3a from three South and Southeast Asian countries, including Pakistan, Thailand, and Malaysia (Figure 2-a), where 3a is the most common subtype [23, 35], [36], indicating that they shared a common ancestor. Since both Bangladesh and Pakistan are SAARC members, people frequently travel back and forth between them. Therefore, it is possible that Pakistan is where the HCV 3a strains that are currently circulating in Bangladesh came from. Bangladesh maintains significant trade relations with Malaysia and Thailand. Although the source of the infection and the mode of transmission are still unknown, increased trade and migration related to jobs with Southeast Asian nations raise the possibility that the HCV 3a strain could spread to Bangladesh. Figure 2-b showed that the HCV 3b strains were similar to those from China, Thailand, and Canada. The HCV strains were most likely brought to Bangladesh from China. One HCV 3g strain in the current study displayed sequence similarity with the HCV 3g Canadian strain, while three more HCV 3b study strains were clustered together with the Canadian strain (Figure 2-c). Given that all HCV 3b and 3g have Asian ancestry, it is hypothesized that population migration from south Asian nations may have also influenced the evolution of HCV origin in Canada [37, 38]. Thus, it is possible to infer from the current study that the evolution of HCV genotype 3 (3a, 3b, and 3g) in Bangladesh is probably from South and South-East Asian countries where genotype 3 is endemic. The current study's HCV genotype 1a strain clustered with Indonesian HCV 1a strains as well as other European (Brazil and Denmark) and American HCV 1a strains (Figure 2-d). The strains used in this 1b study exhibited a high degree of similarity to one another. Figure 2-e shows that three HCV 1b study strains clustered with the German HCV 1b strain, confirming their shared origin. In addition to HCV genotype 1, which is an epidemic globally, subtypes 1a and 1b are common in North America, Europe, and Australia, as well as in some regions of Asia [20, 29-31]. Given that Bangladesh's HCV genotypes 1a and 1b exhibited strong similarities to those of Indonesia, Europe, and other western nations, it is reasonable to assume that the strains of HCV genotypes 1a and 1b used in this investigation may have spread from these nations. Drug trafficking routes between nations may play a major role in the spread of HCV in Bangladesh from developed nations where HCV genotype 1 is epidemic in an era of widespread international travel and human migration. Given that HCV genotype 1 is common in developed nations, the evolution of HCV genotype 1 (1a and 1b) in Bangladesh may be a reflection of population migration history.

The current study provides a thorough scenario of HCV genotype distribution among HCV patients from Bangladesh, despite the study's relatively small sample size and the paucity of pertinent data on genotype and subtype distribution from Bangladesh. Additionally, this study shows that at least five distinct genotypes of HCV are circulating in Bangladesh, and it makes the hypothesis that the strains of the virus that are currently there may have migrated from both nearby and far-off countries. It is imperative to carry out a comprehensive molecular epidemiological study among HCV-infected individuals to gain a deeper comprehension of Bangladesh's HCV distribution pattern.

Conclusion

The current study concluded that among Bangladesh's hepatitis C-infected population, at least two genotypes and five distinct HCV subtypes are circulating. The results of the study highlight the significance of conducting a more thorough HCV molecular epidemiological study in Bangladesh. This study will help public health authorities create various intervention programs to stop the spread of HCV among the Bangladeshi population and will also help clinicians manage chronic HCV patients more effectively.

Recognition

The funding for this research project came from core donors BSMMU and ICDDR, B, who give the Virology department unrestricted support for its operations and research. We are appreciative of these donors' dedication to and support of the Department of Virology's research.

Conflicting interests

The authors have disclosed no financial or other conflicts of interest.

Statement of Ethics

The BSMMU, Dhaka, Bangladesh, Research Review Committee (RCC) and Ethical Review Committee (ERC) gave their approval to the study.

Reference

1. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. The Lancet Infectious Diseases. 2005;5(9):558-567.

2. Moradpour D, Penin F, Rice CM. Replication of hepatitis C virus. Nature Reviews Microbiology. 2007;5(6):453-463.
3. Bartenschlager R, Lohmann V. Replication of hepatitis C virus. Journal of General Virology. 2000;81(7):1631-48.
4. Bukh J, Miller RH, Purcell RH. Genetic heterogeneity of hepatitis C virus: quasispecies and genotypes. Seminars in Liver Disease. 1995;15(1):41-63.
5. Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, Simmonds P. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. Hepatology. 2014;59(1):318-327.
6. Simmonds P, Bukh J, Combet C, Deléage G, Enomoto N, Feinstone S, *et al*. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology. 2005;42(4):962-73.
7. Yu ML, Chuang WL. New treatments for HCV: perspective from Asia. Clinical Liver Disease. 2015;5(1):17-21.
8. AASLD/IDSA/IAS-USA. HCV testing and linkage to care. In: Recommendations for Testing, Managing and Treating Hepatitis C, 2014. Available at http://www.hcvguidelines.org/
9. European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C. Journal of Hepatology, 2016.
10. Rahman S. Hepatitis C: A New Future Everyday. Bangladesh Journal of Medicine. 2016 Aug 3;27(2):74-7.
11. Khan M, Hussain M, Yano M, Hashizume K, Yousuf M, Tanaka E, *et al*. Comparison of seroepidemiology of hepatitis C in blood donors between Bangladesh and Japan. Gastroenterology Japan. 1993;28(5):28-31.
12. Hall TA. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposia Series. 1999;41:95-98.
13. Staden R, Beal KF, Bonfield JK. The Staden package, 1998. Methods in Molecular Biology. 1998;132:115-130.
14. Tamura K, Dudley J, Nei M, Kumar S. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4. Molecular Biology and Evolution. 2007;24(8):1596-1599.
15. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution. 1985;39(4):783-791.
16. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution. 1980;16(2):111-120.
17. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution. 1987;4(4):406-25.
18. Hwang SJ, Lee SD, Lu RH, Chu CW, Wu JC, Lai ST, *et al*. Hepatitis C viral genotype influences the clinical outcome of patients with acute posttransfusion hepatitis C. Journal of Medical Virology. 2001;65(3):505-9.
19. Lavanchy D. Evolving epidemiology of hepatitis C virus. Clinical Microbiology and Infection. 2011;17(2):107-15.
20. Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, Barnes E. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology. 2015;61(1):77-87.
21. Murphy DG, Willems B, Deschênes M, Hilzenrat N, Mousseau R, Sabbah S. Use of sequence analysis of the NS5B region for routine genotyping of hepatitis C virus with reference to C/E1 and 5′ untranslated region sequences. Journal of Clinical Microbiology. 2007;45(4):1102-1112.
22. Singh S, Malhotra V, Sarin SK. Distribution of hepatitis C virus genotypes in patients with chronic hepatitis C infection in India. Indian Journal of Medical Research. 2004;119(4):145-148.
23. Idrees M, Riazuddin S. Frequency distribution of hepatitis C virus genotypes in different geographical regions of Pakistan and their possible routes of transmission. BMC Infectious Diseases. 2008;8(1):1.
24. Narahari S, Juwle A, Basak S, Saranath D. Prevalence and geographic distribution of Hepatitis C Virus genotypes in Indian patient cohort. Infection, Genetics and Evolution. 2009;9(4):643-645.
25. Sievert W, Altraif I, Razavi HA, Abdo A, Ahmed EA, AlOmair A, *et al*. A systematic review of hepatitis C virus epidemiology in Asia, Australia, and Egypt. Liver International. 2011;31(s2):61-80.
26. Mellor J, Holmes EC, Jarvis LM, Yap PL, Simmonds P. International HCV Collaborative Study Group. Investigation of the pattern of hepatitis C virus sequence diversity in different geographical regions: implications for virus classification. Journal of General Virology. 1995;76(10):2493-2507.
27. Mahtab MA, Rahman S, Karim MF, Khan M, Foster G, Solaiman S, *et al*. Epidemiology of hepatitis B virus in Bangladeshi general population. Hepatobiliary Pancreatic Disease International. 2008;7(6):595-600.
28. Islam MJ, Habib A, Jamiruddin R. Prevalence of hepatitis C virus genotypes in Bangladesh. Hepatitis Monthly. 2011;6:193-198.
29. Kaba S, Dutta U, Bythi K, Crewe EB, Khan MH, Coverdale SA, *et al*. Molecular epidemiology of hepatitis C in Australia. Journal of Gastroenterology and Hepatology. 1998;13(9):914-20.
30. Xie Y, Zhao H, Ou WN, Yan J, Du SC, Xu DZ. The difference in distribution of HCV genotypes between patients infected with HCV by transfusion and non-transfusion routes. Chinese Journal of Experimental and Clinical Virology. 2004;18(3):247-50.
31. Mousavi SF, Moosavy SH, Alavian SM, Eghbali H, Mahboobi H. Distribution of hepatitis C virus genotypes among patients with hepatitis C virus infection in Hormozgan, Iran. Hepatitis Monthly. 2013;13(12):e14324.
32. Hotta H, Kemapunmanus M, Apichartpiyakul C, Handajani R, Barzaga NG. Differential distribution of hepatitis C virus subtypes in Asia: Comparative study among Thailand, Indonesia, the Philippines and Japan. The Southeast Asian Journal of Tropical Medicine and Public Health. 1996;28:23-31.
33. Kumar A, Kumar B. Study of the distribution of hepatitis C virus genotypes in patients with chronic hepatitis C infection in Moradabad (UP) India. Global Journal for Research Analysis. 2016;5(7).
34. Simmonds P. The origin of hepatitis C virus. In: Hepatitis C Virus: Molecular Virology to Antiviral Therapy. Springer Berlin Heidelberg; 2013. p. 1-15.

35. Sunanchaikarn S, Theamboonlers A, Chongsrisawat V, Yoocharoen P, Tharmaphornpilas P, Warinsathien P, *et al*. Seroepidemiology and genotypes of hepatitis C virus in Thailand. Asian Pacific Journal of Allergy and Immunology. 2007;25(2-3):175.
36. Hanafiah MK, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: New estimates of age-specific antibody to HCV seroprevalence. Hepatology. 2013;57(4):1333-42.
37. Lu L, Nakano T, He Y, Fu Y, Hagedorn CH, Robertson BH. Hepatitis C virus genotype distribution in China: Predominance of closely related subtype 1b isolates and existence of new genotype 6 variants. Journal of Medical Virology. 2005;75(4):538-49.
38. Bernier L, Willems B, Delage G, Murphy DG. Identification of numerous hepatitis C virus genotypes in Montreal, Canada. Journal of Clinical Microbiology. 1996;34(11):2815-2818.

Supplementary

List of reference sequence

The following sequences were also used for constructing trees shown in Fig.1: HCV 1a- AB714223, EF407442, KP323932, EF407437, HQ229386, HQ229331, GQ418298, JF824375, FJ872331, EU710118, EU710137, HQ229331, KJ470295, AB714195, AB767304, KC127421, JN426994, AY682767, KP057736, JX993348, HQ229367; HCV 1b- KF667836, HQ229578, AF207758, KC439481, KF667841, EF407476, AY044867, KC439502, HQ912956, KT872080, JF424769, KF667821; HCV 3a- HQ229367, KR108479**,** KR108466, HQ229602, KP324082, GQ491005, JF824449, GQ356213, EF543250, KX621522, KP324118, KP324080, KX621487, KR108433, HQ229406, GQ356201, EU710419, KU094825, KU094824, HM135520, FJ872274, JN202388, DQ790289; HCV 3b- KF793485, KJ416846, KP324179, HQ229445, KP324149, KR108487, JX183339, HQ229445, KP324159, FJ462882, KP324166, KP324156, KJ416846, KP324149, KJ470617, KP324166; HCV 3g- JX227954, JQ318549, JF735123, KT336360, EF116090, JQ318433, EF116087, EF015720