Jagannath University Journal of Life and Earth Sciences, **8**(2) pISSN 2414-1402, eISSN 2791-1845

Research Article JnUJLES

PREVALENCE OF HEPATITIS CVIRUS GENOTYPE-3 PATIENTS IN DHAKA CITY, BANGLADESH

Md. Arifur Rahman[1,4], Md. Monirul Islam[2], Md. Eunus Ali[3,4], Mohammad Ariful Islam[1], Farhana Afroze[1], Mohammad Shamim Hossain[5] and Ahmed Abu Rus'd[1*]

[1]*Department of Microbiology, Jagannath University, Dhaka-1100, Bangladesh*

[2]*PCR & Molecular Diagnosis Section, Labaid Limited (Diagnostics), Dhaka-1205, Bangladesh*

[3]*Department of Microbiology and Immunology, BSMMU, Dhaka-1000, Bangladesh*

[4]*PCR Laboratory, Ibn Sina Diagnostics and Imaging Center, Dhaka-1209, Bangladesh*

[5]*Department of Biology, University of Copenhagen, Denmark*

Received: 19 July 2022, Accepted: 10 November 2022

ABSTRACT

The hepatitis C virus is a small, enveloped, positive-sense single-stranded RNA virus. This virus can cause liver cirrhosis. The identification of the infecting HCV virus genotype is crucial for the therapy of hepatitis C virus- affected human. This study aims to diagnose HCV genotypes from the HCV affected peoples to decide the correct treatment of hepatitis C sufferers in Bangladesh. Total 390 blood samples were collected from HCV positive patients. The nucleic acid was extracted from the samples and HCV genotype was detected by multiplex Real Time PCR system. HCV genotypes of 297 samples have been detected from 390 samples of patients tested. Out of the 390 study subjects, 200 were male (51.28%) and 190 (48.71%) were female of 5 to 78 years of age. and 166 detected HCV positive from 200 males and 131 were detected from 190 females. Out of 297 total positive samples, 213 (54.61%) samples identified as HCV Genotype-3 positive, 78 (20%) as Genotype 1 positive, 6 (1.53%) as genotype 6 positive and remaining 93 (23.85%) samples were unclassified due to low/undetected viral load. Here genotype-1a subtype was shown to be more prevalent in males, and genotype-3 was found to infect males and females approximately equally. The viral load was observed higher in male for genotype 3. In this study, we detected the highest percentage (30.89%) of HCV genotype 3 in the age group (51-60 years) among the patients tested. The results suggested that HCV genotype 3 is frequently present in Bangladesh and it is usually better responsive to interferon therapy. However, HCV genotype 1 and genotype 6 has also been found circulating in this country which demands longer treatments tenure as well as effective control measures.

Keywords: *Genotypes, Cirrhosis, Hepatocellular carcinoma, Reverse transcription, cDNA, Antiviral therapies, Viral load.*

Introduction

Hepatitis C is a disease that causes acute and chronic inflammation in the liver by viral infection. It is one of the public health issues around the world and approximately 1.4 million

[*] **Correspondence:** *roosho31@gmail.com.*

people die annually from viral hepatitis-associated cirrhosis and liver cancer (Jefferies *et al*., 2018 and Strader *et al.,* 2004). Every year about 170 million people are severely infected and 3-4 million humans get new infections by hepatitis c virus (Ashraf *et al.*, 2010). The superiority of HCV contamination varies throughout the global arena with considerable local and ethnic variations (Ashraf *et al.,* 2010). The incidence of HCV taint is notably higher in a few nations and regions, which include Europe with about 25 million instances, are HCV positive (Rockstroh *et al*., 2012). In Bangladesh, the actual pattern of the existing genotypes has no longer been properly studied but it was mentioned to be 0.6% within the rural population (Islam *et al*., 2015) and in the published literature the figure is about 0.2% to 1% within the general population (Mahtab *et al.*, 2016). In another study, it was shown that HCV prevalence could be very excessive among people who inject drugs (PWID) (Azim *et al.*, 2002 and Rahman *et al*., 2018), but, in 2011 it was recorded that the HCV occurrence became 95.7% in a north-western city and 39.6% in the capital Dhaka (IEDCR and Icddrb, 2011). Consequently, HCV stays an essential reason of morbidity and mortality in Bangladesh (Mahtab *et al.*, 2008). Even though a few threat factors for acquiring HCV infections are found in 50% of cases but no recognizable transmission aspect can be considered in the remaining 50% cases (Yee, *et al.*, 2001). However, the main chance issue for transmission of HCV in Bangladesh is from quacks, shaving, dialysis procedure and hair cut in barbershops, body piercing, dental procedure and intravenous injection, etc. (Lapa *et al.,* 2019). HCV shows an extraordinarily high degree of genetic diversity creating a major challenge for the development of both HCV vaccines and pan-genotypic drug treatment plans (Cuypers *et al.*, 2015 and Islam *et al.*,.2011). The updated geographic HCV genotype flow in the world is very complex. Curiously, it seems to be that, genotype 1, 2, 3 [especially 1a, 2b, 2a and 3a] are widely distributed around the globe, and other genotypes are confined in certain geographic locations (Petruzziello *et al*., 2016, Messina *et al.*, 2015 and Petruzziello *et al.*, 2019). It's far obtrusive that both HCV and HBV are major global health issues and they are swiftly spreading in developing countries due to lack of health education, poverty, illiteracy and vaccination, etc. (Jobayer, *et al*., 2017). Genotype records of HCV infection is important to make individual treatment and required to maximize the scope of successful treatment outcome for every patient and it is a useful tool to optimize treatment type, length and doses of medicine (Zein, 2000 and Navaneethan *et al.,* 2009). Genotype study for HCV infection in a large population of a country is quite difficult. In Bangladesh, some studies have been published of HCV genotype, prevalence, risk factors, etc. from which exact treatment strategies have not been practical. This study was designed to determine the active HCV RNA infection in addition to know about the HCV genotypes circulating within the study place. It will also help the physicians to prescribe more suitable treatment for the HCV infection and implements pointers to provide customized medicine.

Materials and Methods

Study population

A total of 390 suspected HCV-positive samples were collected for this study. The study period was from January 2019 to December 2021. The blood samples had been collected from different

branches of Ibn Sina Hospital and Diagnostic centers in Dhaka, Bangladesh. Plasma was separated from the whole blood within 6 hours of collection and was stored at -20°C until further testing. While collecting blood samples, a patient's history was collected for clinical information from all the individuals.

Extraction of Nucleic Acid

At first viral nucleic acid was extracted from blood plasma by Qiasymphony Sp Auto Extraction System (Qiagen, Germany). Isolation, purification, and concentration are the three main steps in the extraction of nucleic acids. In the laboratory, commercial extraction kits are frequently employed (Tang *et al.,* 2005). Extraction process was followed by the manufacturer's guidelines where the input volume of plasma/serum was 200 µl, and 60 µl purified RNA was eluted with RNAse free water.

HCV Genotype Specific Real Time-PCR

Genotyping with genotype-specific primers for the target region of the HCV genome was performed by Rotor-Gene Q MDX 5 plex HRM Real-Time PCR system and the selected reporter dye was green for tube 1, orange for tube 2 and yellow for tube 3. For PCR reaction profile, after an initial denaturation step at 94°C for 10 sec, the PCR protocol defines the thermal profile as follows: 40 cycles at 94°C for 15 sec; 58°C for 45 sec; and 72°C for 15 sec.

Data Interpretation

HCV Genotyping is a qualitative assay. In case of two genotype amplification, earlier CT value of genotype was considered the dominant genotype and for positive result, CT value must be <37.

Table1: Interpretation of genotype between reporter dye and PCR-tube: one sample loaded into 3 separate tubes. If one or multiple reporter dyes shows a signal curve, the sample indicates the following genotype specific HCV.

Table 1. Interpretation of genotype between reporter dye and samples

PCR Tube No	Reporter dye	Signal	HCV genotype
1	Green	Present	HCV positive
	Yellow	Present	5
	Orange	Present	1
2	Green	Present	1a
	Yellow	Present	4
	Orange	Present	IC
3	Green	Present	3
	Yellow	Present	2
	Orange	Present	6

Results and Discussion

Results

Real Time PCR was performed for a total of 390 samples for the detection and quantification of HCV load and to identify their genotypes (Table-2). Genotypes of 297 samples out of 390 were detected. Signal curves for the reporter dyes are illustrated by Figure 1. 4 curves for 4 standard and another 2 curves for unknown samples (A); Comparison between given and calculated concentrations (B); the standard curve for standard and samples (C).

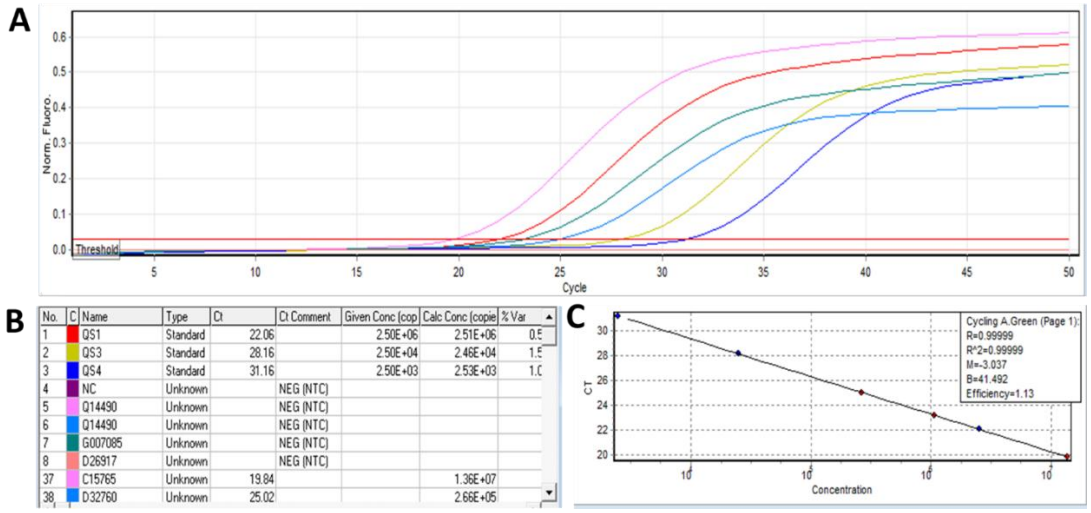


Fig. 1. HCV Viral load analysis in Rotor Gene Q Multiplex PCR.

HCV RNA Quantitative real time PCR data analysis was performed in Rotor Gene Q HRM PCR (Fig. 1).

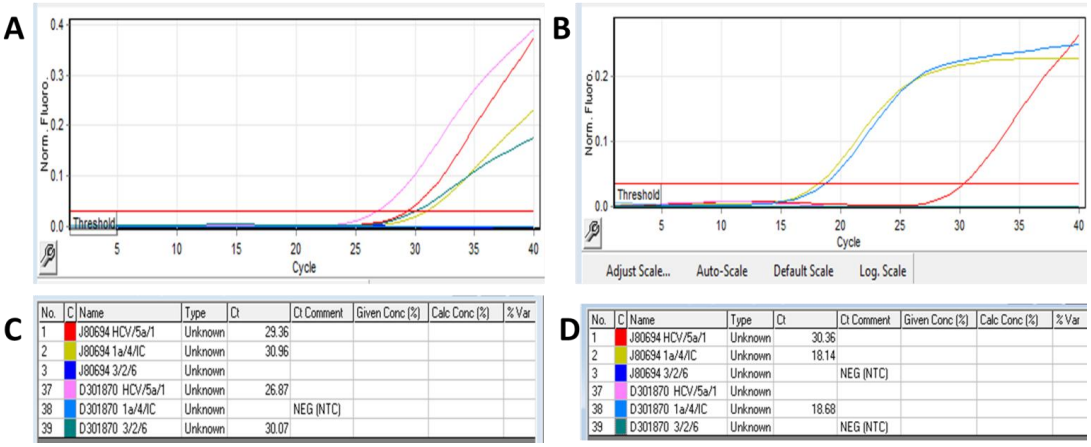


Fig. 2. HCV Genotyping analysis in Rotor gene Q HRM PCR.

Prevalence of HCV Genotypes in Studied Population

Table 2 represents the percentage of genotypes in the studied population. Out of 390 patients, more than 57% were male and about 42% were female. In 390 tested samples, classified PCR fragments were seen in 297 (76.15%) samples whereas unclassified genotypes were found for 93 (23.85%) samples.

Table 2. Presence of HCV genotype among the infected patients (n=390)

Genotype	Subtype	Male (%)	Female (%)	Total (%)
1		51(22.87%)	27(16.16%)	78(20%)
	1a	42(18.83%)	21(12.57%)	63(16.15%)
	1b	9(4.04%)	6(3.59%)	15(3.85%)
3		111(49.78%)	102(61.08%)	213(54.61)
6		4(1.79%)	2(1.98%)	6(1.53%)
Unclassified		57(25.56%)	36(21.56%)	93(23.85%)
Total		**223**	**167**	**390**

The distribution of classified genotypes is as follows: genotype-1a counted for 63 (16.15%), type-1b for 15 (3.85%), type-3 for 213 (54.61%) and genotype-6 for 6 (1.53%)**.** Predominant genotype of this study is '3' (54.61%) followed by genotypes '1' (20%), '1a' (16.15%), '1b' (3.85%) and '6' (1.53%). The predominant genotype-3 for males was 111 (49.78%), followed by genotype-1 (22.87%), genotype-6 (1.79%) and undetermined (25.56%). Similarly, the frequent genotype among the infected female patients was type-3 (61.08%), followed by genotype-1 (16.16%), genotype-6 (1.98%) and undetermined (21.56%). The HCV sub-genotype pattern in male was '1a' as identified in 42 (18.83%) and '1b' was identified in 9 (4.04%) HCV infected patients. Among the females, it was: subtype '1a' in 21 (12.57%) and '1b' was identified for 6 (3.59%) patients. Genotype-3 was found to infect male and female approximately equally whereas genotype-1a subtype was found higher for male.

Distribution of HCV Genotypes in Different Age Groups

The distribution of HCV genotype in different age group patients (390) was shown in Table 3. In this study it was found that type-3 was of highest percentage among the age group 51-60 and it was calculated as 30.98% of total patients. The second highest infection for type-3 was found in the age group 41-50 years. Genotype-1a was the predominant type in older patients (ages >60 years) which was 42.86% of total patients. Type-6 showed the lowest percentage of the patient in all age groups tested.

Table 3. Percentage of HCV genotypes in different age groups (of 390 Patients)

Genotypes	21-30 Y	31-40 Y	41-50 Y	51-60 Y	>60 Y
1a	3(4.76%)	3(4.76%)	24(38.09%)	6(9.52%)	27(42.86%)
1b	0	3(20%)	7(46.67%)	2(13.33%)	3(20%)
3	15(7.04%)	21(9.86%)	60(28.17%)	66(30.98%)	51(23.94%)
6	0	3(50%)	0	1(16.67%)	2(33.33%)
Unclassified	24(25.81%)	9(9.68%)	12(12.90%)	39(41.94%)	9(9.685%)
Total	42(10.77%)	39(10%)	103(26.41%)	114(29.23%)	92(23.59%)

HCV RNA Viral Loads in Male and Female Patients with Different Genotypes

The copies of HCV for type specific genotype in male and female patients were shown in Table 4. It is classified into three categories based on their viral load which include low (<600,000 IU/ml), intermediate (60, 0000-800,000 IU/ml) and high (>800,000 IU/ml) viral copies (one IU equal to 4 copies of HCV RNA).

Table 4. HCV RNA viral load categories in gender and genotype in studied population

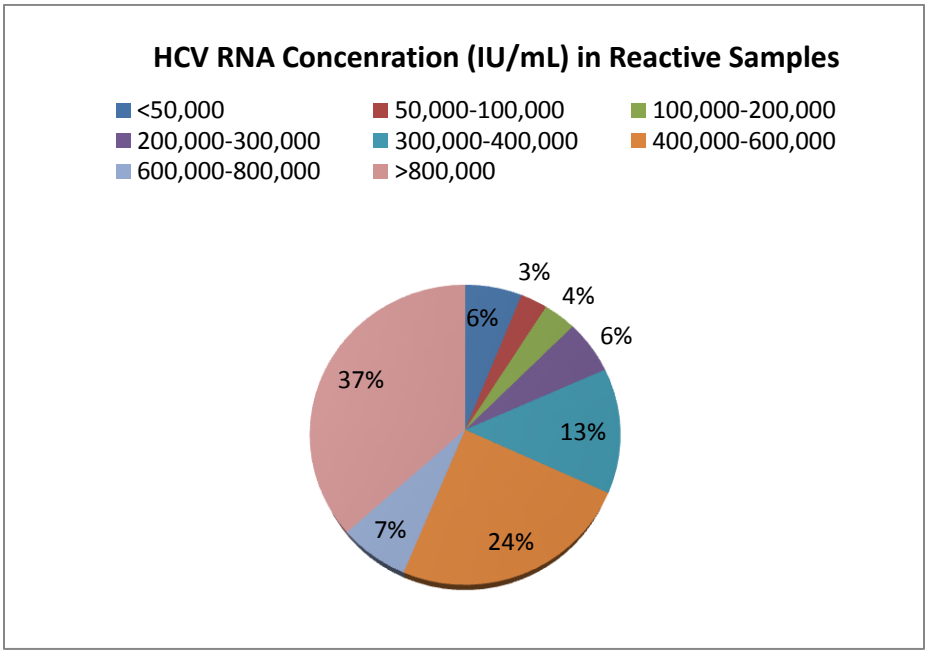
Genotype	Viral load (IU/mL)			P value
	<600,000	**600,000-800,000**	**>800,000**	
Genotype 3	104(48%)	15(7.04%)	94(44.13%)	p <0.07*
Other genotypes	57(67.85%)	7(8.33%)	20(23.80%0)	
Male	78(48.45%)	14(63.63%)	79(69.29%)	p < 0.03*
Female	83(51.55%)	8(36.36%)	35(30.70%)	

*Significant difference was calculated by student t-test.

Pre-treatment viral load was determined significantly high (p< 0.07) in patients infected with HCV genotype 3 as compared to other genotypes. A significant difference was observed in male and female for a high viral load of HCV-infected patients (p< 0.03).

Minimum Viral Load Among Tested Samples

HCV RNA genotype was detected in 297 individuals and viral load test adopted the IU/mL, so sensitivity was estimated based on this observation. The minimum viral loads were observed for 19 samples at <50,000 IU/mL, whereas the highest number of samples (114) reacted strongly at >800,000 IU/mL (Graph 1.).



Graph 1. Minimum viral load among the tested samples.

Discussion

The clinical consequences of different patients of hepatitis C virus (HCV) infection found to fluctuate starting from acute hepatitis to persistent liver diseases along with liver cirrhosis (Lee *et al.,* 2001). Documentation of HCV genotype is essential for the research of numerous features of HCV infection, which include epidemiology, pathogenesis and response to therapy (Forns *et al.*,1996 and Nagayama *et al*., 2000). An appropriate and trust worthy HCV Genotyping method is obvious for large-scale epidemiological and experimental studies (Sarrazin *et al*., 2010). Few numbers of laboratory procedures had been described for the detection of HCV genotypes. For genotyping, PCR has been used broadly which is based on the type-specific primer based upon the amplification of virus sequences in clinical specimens (Pawlotsky *et al*., 1997 and Frank *et al*., 2000). In comparison to other existing in-vitro assays, RT-PCR has extra perspective for its diagnosis as it offers an ultimate detection of HCV (Albadalejo *et al.*, 1998). Our data showed that genotype-3 (54.61%) followed by genotype 1a **(**16.15%) were detected in studied patients. In this study, genotype-3 was observed in the highest percentage for the age group 51-60 which is considered as 30.98% of total patients. Some studies, regarding the occurrence of HCV genotype showed that in Asia like Pakistan and Iran, genotype-3 is more prevalent (Ali *et al.*, 2014). The high prevalence and extreme diversity of type-3 and 6 along with their subtypes in Asia, and type-1, 2, and 4 along with their subtypes in Africa suggested that these types and the corresponding subtypes emerged and diversified in these regions, where they have probably been circulating for a very long time among local populations. HCV prevalence is variable in various regions of the world and in different groups of the community. For example, HCV genotype '1a' is most prevalent in the United States and Northern Europe, while genotype-1 is the more

common in the world about 83.4 million cases (42.6% of total HCV cases), of which about one third are present in East Asia (Ali *et al.*, 2011). Several other studies confirmed the high prevalence of genotype-1a and -1b in HCV patients from different parts of the world (Mohd.Hanafiah *et al.*, 2013 and Bell *et al.*, 1997). Our findings carried some important allegations for therapeutics deterrent that genotype-3 was the most collective genotype in Bangladesh. We found 15 (7.04%) patients infected with genotype-3, which was quantitatively detected in `intermediate viral load (<8000000 IU/mL). Research revealed that patients having high viral load (>80, 0000 IU/mL) with genotype-3 should be treated for 24 weeks, whereas patients with low RNA viral load (<6000000 IU/ml) might be treated for 16 weeks for those patients whose HCV RNA was undetectable by PCR at week 4 of treatment. Therefore, extensive information about HCV genotype and basal RNA viral load is essential when scheduling therapy approaches against HCV at the domestic level. The result of this study showed that the occurrences of HCV types-3 were frequent in Bangladesh that could better respond to interferon therapy. Type-1 and 4 are also circulating which need longer duration for remedy. Moreover appropriate preventive measures should be considered to control the spread of this dreadful disease.

Conclusions

In our study we emphasized to reveal the prevalence of HCV genotype in Bangladesh and HCV genotype 3 was observed as persistent and predominant. As genotype has a direct relation with the time duration of the treatment of severe Hepatitis C positive patients, this study will be helpful to get proper treatment. The national difference is also present in HCV genotypes. The majority of the infected patients found were of 51-60 years of age, and of which majorities were male. Baseline viral load was observed as significantly elevated in patients infected by HCV genotype-3 as compared to other genotypes such as '1' (subtype a and b) '6' and unclassified genotypes. The highest number of samples (114) were detected at >800,000 copies/mL, and minimum number of samples (19) were detected at < 50,000 copies/mL. Therefore, to evaluate the exact scenario of the national population besides widespread clinical data collection, a national survey of HCV genotyping might be the prime source for determining the etiology and/or present consequences of HCV genotype circulation for HCV control.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

Albadalejo, J., Alonso, R., Antinozzi, R., Bogard, M., Bourgult, A. M., Colucci, G., Fenner, T., Petersen, S., Sala, E., Vincelette, J., and Young, C. (1998). Multicenter evaluation of the COBAS AMPLICOR HCV assay, an integrated PCR system for rapid detection of hepatitis C virus RNA in the diagnostic laboratory. *Journal of Clinical Microbiology*, 36: 862–865.

Ali, A., Nisar, M., Ahmad, H. Saif, N., Idrees, M., and Bajwa, M. A. (2011). Determination of HCV genotypes and viral loads in chronic HCV infected patients of Hazara Pakistan. *Virology Journal*, 8: 466.

Ali, S., Ahmed, A., Khan, R. S., Khan, S., Hamayun, M. Khan, S. A., Iqbal, A., Khan, A. A., Wadood, A., Rahman, T. U. and Baig, A. H. (2014). Genotyping of HCV RNA reveals that 3a is the most prevalent genotype in Mardan, Pakistan. *Advances in Virology*, 2014: 606201.

Al-Mahtab, M. (2016). Past, Present, and Future of Viral Hepatitis in Bangladesh. *Euroasian Journal of Hepato-Gastroenterology*, 6:43–44.

Ashraf, H., Alam, N. H., Rothermundt, C., Brooks, A., Bardhan, P., Hossain, L., Salam M. A., Hassan, M. S., Beglinger, C. and Gyr, N. (2010). Prevalence and risk factors of hepatitis B and C virus infections in an impoverished urban community in Dhaka, Bangladesh. *BMC Infectious Diseases*, 10: 208.

Azim, T., Bogaerts, J., Yirrel, D. L., Banerjea, A. C., Saker, M. S., Ahmed, G., Amin, M. M., Rahman, A. S., and Hussain A. M. (2002). Injecting drug users in Bangladesh: Prevalence of syphilis, hepatitis, HIV and HIV subtypes. *AIDS*, 16:121–123.

Bell, H., Hellum, K., Harthug, S., Mealand, A., Ritaland, S., and Myrvang, B. (1997). Genotype, viral load and age as independent predictors of treatment outcome of interferon-α2a treatment in patients with chronic hepatitis C. *Scandinavian Journal of Infectious Diseases*, 29:17–22 .

Cuypers, L., Li, G., Libin, P., Piampongsant, S., Vandamme, A. M., and Theys, K. (2015). Genetic diversity and selective pressure in hepatitis C virus genotypes 1–6: Significance for direct-acting antiviral treatment and drug resistance. *Viruses*, 7: 5018–5039.

Forns, X., Maluenda, M. D., Ampurdanes, S., Olmedo, E., Costa, J., Simmonds, O., and Rodes, J. (1996). Comparative study of three methods for genotyping hepatitis C virus strains in samples from Spanish patients. *Journal of Clinical Microbiology*, 34: 2516–2521.

Frank, C., Mohamed, M. K., Strickland, G. T., Lavancy, D., Arthur, R. R., Magder, L. S., Anwar, W., and Sallam, I. (2000). The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. *Lancet*, 355:887–891.

IEDCR, and ICDDRB. (2011). National HIV serological surveillance 2011, Bangladesh- 9th round technical report, Dhaka, Bangladesh. National AIDS/STD Program (NASP), Directorate General of Health Services, Ministry of Health and Family Welfare, Government of the People's Republic of Bangladesh,

Islam, M. J., Habib, M. A., Jamiruddin, M. R., Ahmed, F., and Hoassin, A. (2011). Prevalence of Hepatitis C Virus Genotypes in Bangladesh. *Proceeding of 13th Annual Scientific Conference*, ICDDR, B. 14–17

Islam, M. S, Miah, M.R., Roy, P.K., Rahman, O., Siddique, A.B., Chowdhury, J., Ahmed, F., Rahman, S., and Khan, M.R. (2015). Genotypes of hepatitis C virus infection in Bangladeshi population. *Mymensingh Medical Journal*, 24: 143–151.

Jefferies, M., Rauff, B., Rashid, H., Lam, T., and Rafiq, S. (2018). Update on global epidemiology of viral hepatitis and preventive strategies. *World Journal of Clinical Cases*, 6: 589–599.

Jobayer, M., Afroz, Z., Rahman, M., Akter, N., Samsuzzaman, S. M., and Islam, K. M. S. (2017). Hepatitis: Knowledge and awareness among the infected population. *Bangladesh Medical Research Council Bulletin*, 43: 126–130.

Lapa, D., Garbuglia, A., Capobianchi, M., and Del Porto, P. (2019). Hepatitis C Virus Genetic Variability, Human Immune Response, and Genome Polymorphisms: Which Is the Interplay? *Cells*, 8:305.

Lee, Y. S., Yoon, S. K., Chung, E. S., Bae, S. H., Choi, J. Y., Han, J. Y., Chung, K. W., Sun, S. H., Kim, B. S., and Kim, B. K. (2001). The relationship of histologic activity to serum ALT, HCV genotype and HCV RNA titers in chronic hepatitis C. *Journal of Korean Medical Science*, 16: 585–591.

Mahtab, M. A., Rahman, S., Karim, M. F., Khan, M., Foster, G., Solaiman, S., and Afroz, S. (2008). Epidemiology of hepatitis B virus in Bangladeshi general population. *Hepatobiliary and Pancreatic Diseases International*, 7: 595–600.

Messina, J. P., Humphreys, I., Flaxman, A., Brown, A., Cooke, G. S., Pybus, O. G., and Barnes, E. (2015). Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology*, 61:77–87.

Mohd Hanafiah, K., Groeger, J., Flaxman, A. D., and Wiersma, S. T. (2013). Global epidemiology of hepatitis C virus infection: New estimates of age-specific antibody to HCV seroprevalence. *Hepatology*, 57(4):1333–1342.

Navaneethan, U., Kemmer, N., and Neff, G. W. (2009). Predicting the probable outcome of treatment in HCV patients. *Therapeutic Advances in Gastroenterology*, 2:287–302.

Nouroz, F., Shaheen, S., Mujtaba, G., and Noreen, S.(2015). An overview on hepatitis C virus genotypes and its control. *Egyptian Journal of Medical Human Genetics*, 16:291–298.

Nagayama, K., Kurosaki, M., Enomoto, N., Myasaka, Y., Marumo, F., and Sato,C. (2000). Characteristics of hepatitis C viral genome associated with disease progression. *Hepatology*, 31:745–750.

Pawlotsky, J. M., Prescott, L., Simmonds, P., Pellet, C., Laurent-puig, P., Labonne, C., Darthuy, F., Remire, J., Duval, J., Buffet, C., Etienne, J.P., Dhumeaux, D., and Dussaix, E. (1997). Serological determination of hepatitis C virus genotype: Comparison with a standardized genotyping assay. *Journal of Clinical Microbiology*, 35:1734–1739.

Petruzziello, A., Marigliano, S., Loquercio, G., Cozzolino, A. & Cacciapuoti, C. (2016). Global epidemiology of hepatitis C virus infection: An up-date of the distribution and circulation of hepatitis C virus genotypes. *World Journal of Gastroenterology*, 22:7824–7840.

Petruzziello, A., Sabatinio, R., Lequercio, G., Guzzo, A., Di Capua, L., Labonia, F., Cozzolino, A., Azzaro, R., and Botti, G. (2019). Nine-year distribution pattern of hepatitis C virus (HCV) genotypes in Southern Italy. *PLoS ONE*, 14(2): e0212033.

Rahman, M., Hossain, M. E., Afrad, M. H., Hasan, R., Rahman, M., Sarker, M. S., and Azim, T.(2018). Hepatitis C virus infections among clients attending an HIV testing and counseling center in Dhaka, Bangladesh. *Journal of Medical Virology*, 90: 383–387.

Rockstroh, J., Grint, D., Boesecke, C., Soriano, V., Lundgren, J., d'Arminio Monforte, A., and Peters, L. (2012). Increases in acute hepatitis C (HCV) incidence across Europe: which regions and patient groups are affected. In *11th International Congress on Drug Therapy in HIV Infection (HIV11), Glasgow*, p. 11-15

Sarrazin, C., and Zeuzem, S. (2010). Resistance to Direct Antiviral Agents in Patients with Hepatitis C Virus Infection. *Gastroenterology*, 138: 447–462.

Strader, D. B., Wright, T., Thomas, D. L. and Seeff, L. B. (2004). Diagnosis, Management, and Treatment of Hepatitis C. *Hepatology*, 39:1147–1171.

Tang, Y. W., Sefers, S. E., Li, H., Kohn, D. J., and Procop, G. W. (2005). Comparative evaluation of three commercial systems for nucleic acid extraction from urine specimens. *Journal of Clinical Microbiology*, 43(9): 4830–4833.

Yee, L. J., Weiss, H. L., Langer, R.G., Herrera, J., Kashlo, R. A., and van leewen, D. J. (2001). Risk factors for acquisition of hepatitis C virus infection: A case series and potential implications for disease surveillance. *BMC Infectious Diseases*, 1(1): 1–5.

Zein, N. N. (2000). Clinical significance of Hepatitis C virus genotypes. *Clinical Microbiology Reviews*, 13:223–235.