Original Article

The Natural History of Hepatitis C Viral Infection and HCV Genotypic Distribution in Thai Hemophilia Patients at Siriraj Hospital

Chuenjitkulthaworn T, MD[1], Bandidniyamanon W, MEcon[3], Ruchutrakool T, MD[2], Chainuvati S, MD[3]

[1] Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
[2] Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
[3] Division of Gastroenterology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Background: Hemophilia patients are at risk of hepatitis C viral infection (HCV) from blood transfusions, which can often lead to chronic hepatitis C (CHC). Patients are often excluded from HCV treatments due to the risk of bleeding from a liver biopsy. CHC patients should have access to HCV treatment in Thailand. However, data on HCV in Thai hemophilia patients are limited.

Objective: To study the genotypic and disease progression of chronic HCV infection in hemophilia patients.

Materials and Methods: All hemophilia patients registered at Siriraj Hospital were screened and recruited for the study. Chronic HCV patients were evaluated for liver tests, HCV viral load, genotype and liver stiffness (LS) measurement by transient elastography, and imaging studies.

Results: Of 89 hemophilia patients in hospital database during the study period, 21 patients died, 7 patients had negative anti-HCV, 3 patients had co-infection and 22 patients refused to participate. Hence, 36 male patients signed informed consent, of those, 29 patients had positive anti-HCV (80.6%). Genotype 3a was the most common (42.8%), followed by genotype 1a (28.5%). The mean HCV viral load was 2,416,722 IU/ml. LS were measured on 28 patients (96.6%). Median value of LS was 6.0 kPa (range 4.3 to 31.2). In cirrhosis group (n = 5), the mean LS was 26.3 kPa (range 4.3 to 31.2), which was significantly different from non-cirrhosis group (5.3 [range 4.3 to 8.1] kPa, $p = 0.008$. The median LS value in long-term transfusion time (≥30 years) is higher than the group with a transfusion time of <30 years (6.8 vs. 4.8 kPa, $p = 0.025$). Three patients were successfully treated and achieved sustained virological response.

Conclusion: The most common HCV genotype in Thai hemophilia patients was genotype 3a. LS correlated with the exposure time during blood transfusion.

Keywords: Chronic hepatitis C, Hemophilia, Transient elastrography

J Med Assoc Thai 2019;102(Suppl.10): 81-5
Website: http://www.jmatonline.com

Hemophilia is the most common hereditary bleeding disorder and estimated to affect 1 to 5 individuals per 50,000 male population. Prior to 1990, almost all patients were infected with hepatitis C virus (HCV), with some also being coinfected with the human immunodeficiency virus (HIV). Transfusion-transmitted infections (TTI) are serious complications for hemophiliac patients treated by factor VIII and IX concentrates[1] before 1985. Multi-transfused hemophiliacs with antihemophilic products, including fresh frozen plasma, cryoprecipitate or factor concentrate, are in danger of acquiring viral hepatitis. These infections occurred mostly before 1985. Since 1990, the incidence of HCV infection has increased to 170 million patients worldwide. The root cause stems from the duration of the patient's first blood transfusion[2-5]. In addition, HCV is also one of the causes of liver cirrhosis, end-stage liver disease and hepatocellular carcinoma. Rates of disease progression among patients with hemophilia and chronic HCV infection vary. Currently, 6 main genotypes and more than 80 sub-genotypes were reported, with prevalence varying in different countries. Based on study by Hanafial, et al, compared to the rest of the world, Thailand has a moderate prevalence of HCV infection (1.5% to 3.5%) However, it has the highest prevalence among South-East Asia countries (2%)[6].

The prevalence of HCV infection in hemophilia patients in other countries ranged from 36 to 89.6%[7-9]. There is limited data in terms of the prevalence of HCV infection, liver disease progression, and its impact towards Thai patients. Physicians often used abnormal levels of alanine aminotransferase (ALT) as a surrogate marker to identify the significance of liver disease. Besides bleeding complications,

Correspondence to:
Chainuvati S.
Division of Gastroenterology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.
Phone: +66-2-4197281
E-mail: siwaporn.cha@mahidol.ac.th

How to cite this article: Chuenjitkulthaworn T, Bandidniyamanon W, Ruchutrakool T, Chainuvati S. The Natural History of Hepatitis C Viral Infection and HCV Genotypic Distribution in Thai Hemophilia Patients at Siriraj Hospital. J Med Assoc Thai 2019;102(Suppl.10): 81-5.

chronic hepatitis C (CHC) and cirrhotic complications are major co-morbidities in hemophilia patients[10,11]. The cohort study in 1996 reported that the principal risk factor of HCV infection was from blood transfusions before HCV screening was available in Thailand. The most common genotypic variations being genotype 3a (55%)[12-15].

Currently, HCV treatment has become widely available to Thai patients and is deemed highly effective. These new regimens can offer a 90% cure rate towards the HCV infection regardless of the HCV genotype[20]. The gold standard measurement of liver fibrosis is a liver biopsy, however, physicians often avoid this procedure in hemophilia patients due to a bleeding risk. Determination of liver stiffness (LS) using transient elastography (TE) has been introduced as a non-invasive surrogate to liver biopsy for the assessment of liver fibrosis in patients with chronic viral hepatitis[16,17]. The comparison between cut-off value of liver stiffness and liver fibrosis score in CHC shows: significant fibrosis (≥F2) is equivalent to TE value of 7.10 kPa, F ≥3 is equivalent to TE value of 9.50 kPa and F = 4 or cirrhosis is equivalent to TE value of 12.5 kPa[18,19,21].

This study aims to study the prevalence of HCV genotypic variations in hemophilia patients at Siriraj Hospital and to determine the natural history of HCV in these patients using time of blood transfusion, data on TE and imaging studies.

Materials and Methods

This is a single center, observational and cross-sectional study in all HCV-infected hemophilia patients from hemophilia clinic, Siriraj Hospital. All hemophilia patients from the hospital database were screened and those who were alive were then contacted. The study was conducted between December 2014 and March 2016, after an approval by Siriraj Institutional Review Board (IRB). The study was registered to the Thai Clinical Trial Registry, 680/2557(EC2).

Patients who met the inclusion criteria and consented for the study were enrolled. The inclusion criteria were: 1) hemophilia patients whose age is ≥18 years 2) followed up at Siriraj Hospital 3) gave consent for blood testing for HCV 4) accepted to have TE and/or other imaging studies of liver (ultrasonography or computerized tomography) within 1 year. Exclusion criteria were: 1) patient who had undetected HCV RNA (except the patients who have been cured after treatment), 2) negative anti-HCV, 3) patients with chronic liver disease from other causes aside from HCV, and 4) co-infection with HIV.

All participants were tested for anti-HCV by ELISA technique. Anti-HCV positive patients were seen by hepatologists at the liver clinic. Blood biochemistry performed at the time of enrollment included HCV viral load, HCV genotype, white blood cell count, hemoglobin, platelet count, aspartate aminotransferase (AST), alanine transaminase (ALT), total bilirubin, albumin, and prothrombin time level. HCV genotyping was performed using the reverse transcription-polymerase chain reaction (RT-PCR) and reverse hybridization essay. Serum HCV RNA quantifica-tion was determined with the Cobas Amplicor HCV Monitor, v2.0 (Roche Diagnostics) which has a lower limit of quantification of 15 IU/ml. Imaging study (ultrasound [US], or computed tomography [CT]) was used to evaluate liver cirrhosis. LS was evaluated by TE using FibroScan® Touch-502 which is the value ≥12.5 kilopascal (kPa) was recommended as a cut-off for cirrhosis in direct-antiviral agents (DAA) era.

Statistical analysis

Results are expressed as mean and standard deviation (SD) or median (min, max) for continuous data. Frequencies and percentages were presented for categorical data. Continuous data were compared using independent t-test for normal distribution data and non-parametric Kruskal Willis test for skewed data. Categorical data was compared by Chi-square or Fisher exact test as appropriate and the *p*-value of <0.05 is considered as statistical significance. All analyses were performed by SPSS 18.

Sample size calculation

According to the literature review, we found one study in 1992 reported the incidence of transfusion-transmitted infection in India which the proportion of anti-HCV positive is 27%[24]. Therefore, the estimated proportion of the sample size was calculated power and set at 0.85 with significant level 0.05, 95% confidence interval. The 85 patients were selected to enroll in this study.

Results

There were 89 hemophiliac patients in clinic registry. Sixty-eight patients were alive at the time of consent. Seven patients were anti-HCV negative and 3 patients were HIV-HCV co-infected. During the study period, a total of 36 patients were tested for anti-HCV and 29 patients with HCV Ab positive result were enrolled and tested for HCV viral load and other laboratories and imaging. All patients were analyzed for primary and secondary outcomes.

The baseline demographics data are listed in Table 1. Twenty-nine patients (100%) were male and the mean age was 41 years (range, 30 to 52 years). Twenty-six patients (89.7%) were hemophilia A and 3 patients were hemophilia B. Most of them (82.8%) were severe hemophilia (FVIII:C <1 IU/dL). The mean transfusion duration was 37.3 years (SD 10.4). The HCV genotypes were tested in 21 patients and the main common genotypes were genotype 1 (10 patients; 47.6%) and genotype 3a (9 patients; 42.8%). The mean baseline serum ALT level was 32 IU/L (range, 7 to 80 IU/L), and the mean baseline serum HCV RNA level was 2,416,722 IU/mL (range, 15 to 14,400,000 IU/mL).

US and/or CT was performed on 23 patients (79.3%). Five patients had liver cirrhosis on imaging studies. TE was obtained in 28 of 29 patients (96.6%). The median value of LS was 6.0 kPa (range 4.3 to 31.2). The median value of LS in cirrhosis group (n = 5) was 26.3 kPa (range 4.3 to 31.2) and median value of LS in non-cirrhosis group (n = 18) was 5.3 kPa (range 4.3 to 8.0) (p = 0.008). The median LS

value of the group with transfusion time >30 years was 6.8 kPa (range 4.3 to 31.2), whereas those with transfusion time <30 years was 4.8 kPa (range 4.3 to 8.1) (p = 0.025). The group of patients who had LS value <12.8 kPa (n = 24) had a mean transfusion time of 36.2±10.3 years and in and in patients who had LS value >12.8 kPa (n = 4) had mean transfusion time 43±12.5 years (p = 0.247).

There were 3 patients who received conventional treatment with pegylated-interferon (Peg-IFN) and ribavirin (RBV) and all of them achieved sustained virological response (SVR). One patient had liver cirrhosis and hepatocellular carcinoma. The liver stiffness and transfusion times in patients with and without cirrhosis by imaging were demonstrated in Table 2.

Discussion

The present study is the first study of chronic HCV infection in Thai hemophilia patients who were followed at our tertiary center for several years. The most common genotypic variation of HCV infection is 3a, which was similar to the genotypic variation found in Thai blood donor study[14] whereas the global prevalence of genotypic variation of hepatitis C infection in previous study was genotype 1 (83.4 million cases; 46.2% of HCV cases). According to previous studies, genotype 1 (4.91 million of 8.61 million cases; 57%) was the most common amongst other Southeast Asian countries[22].

Clinical course of CHC was difficult to determine because of uncertain onset of HCV infection. In this study, we estimated HCV infection as the first time of transfusion. Majority of our patients had slow progression of HCV infection as the median of LS was only 6.0 kPa and their ALT, PT, platelet count level were in the normal range. Only 5 of them showed liver cirrhosis and one patient developed HCC and was successfully treated with trans-arterial chemoembolization and is still alive. There were 3 patients who received Peg-IFN/RBV therapy and achieved SVR; all of them had no significant liver fibrosis from the LS measurement.

There were two prior studies which determined that the natural history of HCV infection in inherited bleeding disorder patients was mostly hemophilia. The data from an Israeli group determined the significant time related HCV infection was 25 years of transfusion and the overall 10-year survival of HCV group was 95.3%, which did not significantly differ from the HCV-negative group. Liver-related survival between the two groups (HCV mono-infected vs. non-infected) was not significantly different[23]. The study by Fransen van de Putte[24] showed that the median LS was 7.3 (5.9 to 10) in 84 HCV in bleeding disorder patients. Only 34% of the patients received treatment and 24% of them achieved SVR. Compared to our study, 10% of our patients underwent treatment and all of them achieved SVR. This could be explained in that our patients had HCV genotype 3, which is an easier genotype to treat with Peg-IFN. If we used LS value of >7.5 kPa, 7 of 28 patients (25%) would have significant fibrosis which was indicated for treatment.

TE is a good diagnostic tool for evaluation of liver fibrosis in viral hepatitis patients[18,21]. It is a non-invasive procedure and does not cause bleeding complication in both non-hemophilic and hemophilic patients. Clinicians often use LS value to determine treatment necessity. The median LS values in cirrhosis and non-cirrhosis were significantly different (26.3 vs. and 5.3 kPa). The LS value in long-term transfusion time >30 years) was significantly different from LS value in the group that received blood transfusion <30 years. These results could be explained by the fact that the longer duration of HCV infection in patients, the more severe fibrosis would be established.

Table 1. Demographics data of the 29 patients

Type of hemophilia, n (%)	
Hemophilia A	26 (89.7)
Hemophilia B	3 (10.3)
Severity of hemophilia, n (%)	
Mild	1 (3.4)
Moderate	4 (13.8)
Severe	24 (82.8)
Age (years), mean (SD)	41 (11)
Body mass index (kg/m^2), mean (SD)	22.3 (3.9)
Blood transfusion time (years), mean (SD)	37.3 (10.4)
HCV diagnosis (years), median (range)	5 (1 to 12)
Ultrasonography/CT scan, n (%)	23 (79.3)
HCV genotypes, n (%)	(n = 21)
1a	6 (28.5)
1b	4 (19.3)
3a	9 (42.8)
1a/3a	1 (4.7)
6c	1 (4.7)
HCV viral load (IU/mL), median (range)	2,416,722 (15 to 14,400,000)
Albumin (g/dL), mean (SD)	4.2 (0.6)
ALT (IU/mL), mean (SD)	32 (21)
Total bilirubin (mg/dL), mean (SD)	0.7 (0.3)
PT (seconds), mean (SD)	12.4 (1.0)
Platelets (cell/mm^3), mean (SD)	253,272 (106,615)
Fibroscan (kPa), median (range)	6.0 (4.3 to 31.2)

Table 2. Data of the 23 patients regarding to the presence of cirrhosis by imaging studies, liver stiffness and transfusion time

	Cirrhosis (n = 5)	Non-cirrhosis (n = 18)	p-value
Liver stiffness (kPa), median (range)	26.3 (4.3 to 31.2)	5.3 (4.3 to 8.1)	0.008
Time of transfusion (years), mean (SD)	45.6 (12.3)	35.9 (10.3)	0.025

Limitations of this study were the small number of participants and the difficulty of patients to attend doctor visits.

Conclusion

The most common HCV genotype of Thai hemophilia patients was genotype 3a, which is similar to the genotypic variation found in the Thai population. LS value is correlated with the duration of blood transfusion. Higher value of LS was noted in the patients who received transfusions for longer than 30 years. The majority of HCV infections in hemophilia patients had slow progression of disease. This special group of patients should be counseled about treatment availability and should be monitored for HCV-related complications.

What is already known on this topic?

Hemophilia patients are at risk for transfusion-transmitted infections (TTI). Genotypic distribution among population is varied according to countries.

What this study adds?

In this study, we found that majority of CHC in hemophilia Thai patients was genotype 3a. Prior to the study period, less than 10% of these patients had been seen by hepatologist and less than 10% of them had treatment and evaluation. This study should at least direct our attention to treating these patients who acquired CHC from blood transfusion in the past. Nowadays, hepatitis C treatment with direct-acting antiviral agents yields more than 95% SVR.

Acknowledgements

This study was supported by a research grant from Faculty of Medicine, Siriraj Hospital. We are grateful to the devoted research coordinators and to all of the staff in the Division of Gastroenterology and Hematology at the Siriraj Hospital.

Potential conflicts of interest

The authors declare no conflicts of interest.

References

1. Goedert JJ, Eyster ME, Lederman MM, Mandalaki T, De Moerloose P, White GC, et al. End-stage liver disease in persons with hemophilia and transfusion-associated infections. Blood 2002;100:1584-9.
2. Lee C, Dusheiko G. The natural history and antiviral treatment of hepatitis C in haemophilia. Haemophilia 2002;8:322-9.
3. European Association for the Study of the Liver. EASL Recommendations on treatment of hepatitis C 2018. J Hepatol 2018;69:461-511.
4. Omata M, Kanda T, Wei L, Yu ML, Chuang WL, Ibrahim A, et al. APASL consensus statements and recommendations for hepatitis C prevention, epidemiology, and laboratory testing. Hepatol Int 2016;10:681-701.
5. Bunchorntavakul C, Chavalitdhamrong D, Tanwandee T. Hepatitis C genotype 6: A concise review and response-guided therapy proposal. World J Hepatol 2013;5:496-504.
6. Mohd HK, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology 2013;57:1333-42.
7. Brettler DB, Alter HJ, Dienstag JL, Forsberg AD, Levine PH. Prevalence of hepatitis C virus antibody in a cohort of hemophilia patients. Blood 1990;76:254-6.
8. Khan MM, Tait RC, Kerr R, Ludlam CA, Lowe GD, Murray W, et al. Hepatitis C infection and outcomes in the Scottish haemophilia population. Haemophilia 2013;19:870-5.
9. Yazdani MR, Kassaian N, Ataei B, Nokhodian Z, Adibi P. Hepatitis C virus infection in patients with hemophilia in Isfahan, Iran. Int J Prev Med 2012;3(Suppl 1):S89-93.
10. Zawilska K, Podolak-Dawidziak M. Therapeutic problems in elderly patients with hemophilia. Pol Arch Med Wewn 2012;122:567-76.
11. Zoulim F, Bailly F. New approaches to the management of hepatitis C in haemophilia in 2012. Haemophilia 2012;18 Suppl 4:28-33.
12. Jarvis LM, Ludlam CA, Ellender JA, Nemes L, Field SP, Song E, et al. Investigation of the relative infectivity and pathogenicity of different hepatitis C virus genotypes in hemophiliacs. Blood 1996;87:3007-11.
13. Sistayanarain A, Kunthalert D, Vipsoongnern Y. A shift in the hepatitis C virus genotype dominance in blood donor samples from Thailand. Mol Biol Rep 2011;38:4287-90.
14. Indraprasit S, Damrongtham C. Evaporative water loss in the normal adult Thai. J Med Assoc Thai 1984;67:79-83.
15. Chimparlee N, Oota S, Phikulsod S, Tangkijvanich P, Poovorawan Y. Hepatitis B and hepatitis C virus in Thai blood donors. Southeast Asian J Trop Med Public Health 2011;42:609-15.
16. Wilde JT, Mutimer D, Dolan G, Millar C, Watson HG, Yee TT, et al. UKHCDO guidelines on the management of HCV in patients with hereditary bleeding disorders 2011. Haemophilia 2011;17:e877-83.
17. Gatselis NK, Zachou K, Saitis A, Samara M, Dalekos GN. Individualization of chronic hepatitis C treatment according to the host characteristics. World J Gastroenterol 2014;20:2839-53.
18. Poynard T, Vergniol J, Ngo Y, Foucher J, Munteanu M, Merrouche W, et al. Staging chronic hepatitis C in seven categories using fibrosis biomarker (FibroTest) and transient elastography (FibroScan(R)). J Hepatol 2014;60:706-14.
19. Friedrich-Rust M, Rosenberg W, Parkes J, Herrmann E, Zeuzem S, Sarrazin C. Comparison of ELF, FibroTest and FibroScan for the non-invasive assessment of liver fibrosis. BMC Gastroenterol 2010;10:103.

20. AASLD-IDSA HCV Guidance Panel. Hepatitis C guidance 2018 update: AASLD-IDSA recommendations for testing, managing, and treating hepatitis C virus infection. Clin Infect Dis 2018;67:1477-92.
21. Degos F, Perez P, Roche B, Mahmoudi A, Asselineau J, Voitot H, et al. Diagnostic accuracy of FibroScan and comparison to liver fibrosis biomarkers in chronic viral hepatitis: a multicenter prospective study (the FIBROSTIC study). J Hepatol 2010;53:1013-21.
22. Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, et al. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology 2015;61:77-87.
23. Maor Y, Schapiro JM, Bashari D, Martinowitz U. Survival of hepatitis C-infected haemophilia patients is predicted by presence of cirrhosis but not by anti-viral treatment. Ann Hepatol 2014;13:753-61.
24. Fransen van de Putte DE, Fischer K, de Knegt RJ, Posthouwer D, van Erpecum KJ, Mauser-Bunschoten EP. Liver stiffness measurements to assess progression of fibrosis in HCV-infected patients with inherited bleeding disorders. Haemophilia 2011;17:e975-80.