RESEARCH NOTE

PREVALENCE AND GENOTYPES OF HEPATITIS C VIRUS INFECTION AMONG DRUG ADDICTS AND BLOOD DONORS IN THAILAND

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Abstract. Hepatitis C virus (HCV) is an infectious agent that has the potential to cause chronic liver disease, cirrhosis and hepatocellular carcinoma. We determined the prevalence and genotypes of HCV infection among groups of drug addicts: intravenous drug users (n = 134), methamphetamine users (n = 100), inhaled-drugs users (n = 19) and alcoholics (n = 50); a group of blood donors acted as a control. The control group consisted of 179 randomly-selected anti-HCV positive samples : these were subjected to HCV RNA screening and genotyping. The anti-HCV test was performed by ELISA; HCV RNA screening was by nested RT-PCR that employed primers from the 5′ noncoding region. The genotype assay was based upon analysis of the 5′ NCR amplified sequences and RFLP. Hepatitis C virus was highly prevalent among all groups of drug addicts (12-70%). In 2000, among the new blood donors (n = 66,340) at the National Blood Center, Thai Red Cross, anti-HCV prevalence amounted to 0.98%. The HCV genotype distribution showed that the most prevalent genotype was 3a, followed by 1b and 6a. Our data demonstrated the very high prevalence of HCV infection in IVDUs, a finding that is consistent with the blood-borne nature of the virus. In order to curb HCV infection, a determined effort to educate both the general population and high-risk groups is required; such a program of education would address both general and particular methods of transmission, especially the use of non-sterile needles etc.

Hepatitis C virus (HCV) infection, which can cause chronic liver diseases, cirrhosis, and hepatocellular carcinoma, is a major problem worldwide: approximately 170 million people are already infected and 3-4 million cases of new infection are expected every year (World Health Organization, 1999). HCV is transmitted parenterally or by direct percutaneous exposure to infectious materials, such as contaminated blood products. This is especially true in countries where donated blood is not screened for HCV and the sharing of contaminated needles by intravenous drug users (IVDVs) is common. In order to understand the epidemiology and route of transmission of HCV, we studied the prevalence and genotypes of hepatitis C virus among groups of drug addicts and blood donors in Thailand. HCV can be classified by phylogenetic tree analysis as at least 6 genotypes, of which 1b, 3a and 6 are prevalent in Thailand (Simmonds *et al*, 1993; Mellor *et al*, 1995).

The study protocol was approved by the Ethics Committee of the Ministry of Public

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Table 1
Prevalence of HCV-RNA and genotypes among drug addicts and blood donors.

Groups	No.	HCV-RNA+ve %	Genotypes (%)					
			1a	1b	3a	3b	6a	3a/6a
IVDUs	134	70.2	0	11.7	75.5	0	8.5	4.3
Methamph users	100	12	0	8.3	83.3	0	8.3	0
Inhaled-drugs users	19	21.1	0	0	75	0	25	0
Alcoholics	50	20	0	0	100	0	0	0
Blood donors	179[a]	84.9	5.3	19.1	72.4	0.7	2.6	0

[a]All blood donors were selected from positive anti-HCV specimens.

Health, Thailand. All the participants (drug addicts) were told about the objectives of the study; written consent was obtained from each participant prior to the study. The study population was comprised of intravenous drug users (n=134), methamphetamine users (n=100), inhaled-drugs users (n=19) and alcoholics (n=50); all of the subjects were recruited from Thanyarak Hospital in Pathum Thani, Thailand. As a control, the prevalence of hepatitis C among blood donors was determined (n=66,340) from data collected by the National Blood Center, Thai Red Cross, during the year 2000 (National Blood Center, Thai Red Cross, personal communications).

Blood specimens were obtained from participants at the time of the interview. The sera were separated, kept at -70ºC, and transferred to the Viral Hepatitis Research Unit, Chulalongkorn University and Hospital, Bangkok. All specimens were tested for HCV-RNA by reverse transcriptase polymerase chain reaction (RT-PCR) for the non-coding region. Our serum collection, RNA extraction, applying the guanidinium method (Cha *et al*, 1991), and RT-PCR were carried out as previously described (Theamboonlers *et al*, 1995; 2000) although with minor modifications: the cDNA, representing the core region was amplified using the nested primer pairs 954 and 410 for outer primers, and 953 and 951 for inner primers (Mellor *et al*, 1995); for the 5′ NCR, we designed two outer primers, OC1 (5′-GGC GAC ACT CCA CCA TGA AT-3′) and OC2 (5′-CAT GGT GCA CGG TCT ACG AG-3′); the inner primers were 939 and 211 (Davidson *et al*, 1995). We modified the annealing temperature and time to 48ºC and 0.7 minutes (42 seconds). Upon completion of the second round of PCR, we analyzed the products by electrophoresis on a 2% agarose gel stained with ethidium bromide. Under UV light the amplified core region became visible as a 288 bp, fragment; the 5′ NCR was visible as a 313 bp fragment. We subjected the PCR products to RFLP analysis, using the restriction endonucleases AvaI and SmaI under the reaction conditions described by (Mellor *et al* 1996) in order to differentiate genotypes 1a, 1b, and genotype 6 variants. We analyzed the genotype 6 variants applying the method detailed by Davidson *et al* (1995), which enabled us to isolate genotype 3 from the type 6 variants (using either *Rsa*I + *Hae*III or *Hinf*I + *Mva*I) and then to differentiate genotype 3 into types 3a and 3b using *Scrf*I.

As shown in Table 1, there was a higher rate of HCV infection in the IVDU group (70%) compared with the other groups of drug addicts (12-21%) and the new blood donors (0.98%). (National Blood Center, Thai Red Cross, personal communication). Approximately three out of four HCV-infected individuals were found to have genotype 3a.

Based on our data, and considering that the virus is blood borne, it seems reasonable

to suggest that the risk factor for HCV transmission in the IVDU group is contaminated blood, which is transmitted from person to person by the sharing of needles. HCV genotypes 3a, 1b and 6a are the predominant genotypes in Thailand.

In conclusion, our data indicate that HCV prevalence in the drug addicts is very high and that the preponderance of HCV genotype 3a has increased, particularly among IVDUs, probably as a result of shared needles etc. If HCV transmission to be reduced, then intensive counseling and health education would be of paramount importance; an appropriate means of preventing HCV transmission may be necessary.

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REFERENCES

Cha TA, Kolberg J, Irvine B, *et al*. Use of a signature nucleotide sequence of hepatitis C virus for detection of viral RNA in human serum and plasma. *J Clin Microbiol* 1991; 29: 2528-34.

Davidson F, Simmonds P, Ferguson JC, *et al*. Survey of major genotypes and subtypes of hepatitis C virus using RFLP of sequences amplified from the 5′ non-coding region. *J Gen Virol* 1995; 76: 1197-204.

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. *J Gen Virol* 1995; 76: 2493-507.

Mellor J, Walsh EA, Prescott LE, *et al*. International HCV Collaborative Study Group. Survey of type 6 group variants of hepatitis C virus in Southeast Asia by using a core-based genotyping assay. *J Clin Microbiol* 1996; 34: 417-23.

Simmonds P, Holmes EC, Cha TA, *et al*. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. *J Gen Virol* 1993; 74: 2391-9.

Theamboonlers A, Yatsuhashi H, Hamada R, Fujino T, Poovorawan Y, Yano M. Hepatitis C virus antibodies, viral RNA levels and genotypes in Thailand. *Southeast Asian J Trop Med Public Health* 1995; 26: 506-10.

Theamboonlers A, Kaewin P, Hirsch P, Poovorawan Y. Determination of hepatitis C virus genotypes in Thailand by RFLP. *Ann Trop Med Parasitol* 2000; 94: 525-7.

World Health Organization. Hepatitis C-global prevalence. *WHO Wkly Epidemiolol Rec* 1999; 7:65-72.