HEPATITIS GBV-C INFECTION IN INTRAVENOUS DRUG USERS

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Abstract. Our group has investigated 204 intravenous drug users for the presence of GBV-C-RNA by means of reverse transcriptase polymerase chain reaction (RT-PCR). The majority of the individuals tested were male, their age ranging from 16 to 63 years, and the duration of intravenous drug use from one to 40 years. We detected GBV-C-RNA in 46 of the 204 IVDUs (22.5%) with its prevalence peaking in the age group between 21 to 30 years while decreasing with advancing age. Similarly, its frequency was found in inverted relation to the duration of drug use. The present findings strongly hint at the host's immune system's capacity to clear hepatitis GBV-C as opposed to the other blood-borne hepatitis viruses. From the liver function tests performed we could not detect any statistically significant difference regarding ALT elevation observed in GBV-C-positive as compared to GBV-C-negative individuals. To date, GBV-C in most cases does not appear to cause any serious liver disease.

INTRODUCTION

On the grounds of epidemiological and clinical studies, the existence of as yet undiscovered hepatotropic viruses as the causative agents of non-A-E hepatitis has been postulated (Alter, 1994; Simons *et al*, 1995a). In 1995, two novel viruses designated GBV-A and GBV-B, respectively, were isolated from the serum of a tamarin infected by the human GB hepatitis agent applying the technic of subtractive polymerase chain reaction (PCR) (Simons *et al*, 1995b). A different group of researchers identified a third virus termed GBV-C and closely related to GBV-A by means of reverse transcription PCR (RT-PCR) using degenerate oligonucleotide primers for amplification of a segment of the helicase gene originating from either GBV-A, GBV-B or hepatitis C virus (HCV) (Leary *et al*, 1996). GBV-A and GBV-B have subsequently been identified as belonging to the family of Flaviviridae distinguishable by their single-stranded RNA genome of positive polarity and therefore clearly distinct from HCV (Muerhoff *et al*, 1995). Yet another team of researchers isolated a novel virus termed hepatitis GBV-C (GBV-C) from the plasma of a patient with non-A-E chronic hepatitis (Linnen *et al*, 1996) which due to its close relatedness to GBV-C is now considered a different isolated of the same virus (Zuckerman, 1996) which has become known as parenterally transmitted and has therefore been associated with post-transfusion hepatitis. The prevalence of GBV-C infection detected by RT-PCR in blood donors varied from 1 to 5%,which is not significantly different from the frequencies found among the same population groups in European and other Asian countries, as well as in Australia. (Dawson *et al*, 1996; Fiordalisi *et al*, 1996; Jarvis *et al*, 1996; Masuko *et al*, 1996; Moaven *et al*, 1996; Wu *et al*, 1997; Poovorawan *et al*, 1998a). Numerous studies performed in order to elucidate the clinical significance of GBV-C infection found this new agent capable of infecting humans both acutely and chronically which frequently coincides with an elevation of the liver enzymes. As to GBV-C infection being responsible for ensuing liver disease and regarding the potential severity of the latter, there has been quite some controversy. For example in Italy, GBV-C-RNA could be demonstrated in 35% of acute and 39% of chronic hepatitis patients, (Fiordalisi *et al*, 1996). A report originating from Karachi, Pakistan, also claims having identified GBV-C infection as the underlying cause of chronic liver disease (Moatter *et al*, 1996). Similarly in southern China, GBV-C infection has been claimed responsible for liver disease, specifically, acute or chronic hepatitis, liver cirrhosis, or HCC (Wu *et al*, 1997). The role of GBV-C in fulminant hepatitis remains also somewhat controversial, especially since studies conducted subsequently to the reports from Germany (Heringlake *et al*, 1996), Japan (Yoshiba *et al*,

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1995), and India (Panda *et al*, 1996), respectively, have not been able to confirm those findings (Kuroki *et al*, 1996; Sallie *et al*, 1996; Kao *et al*, 1996).

Furthermore, due to its mode of transmission GBV-C is often detected as a co-infection with HCV and to a lesser extent HBV. Contrasting infection with either of those hepatotropic viruses, however, GBV-C has been shown to be cleared from the host's serum after prolonged exposure, thereby suggesting an effective immune response.

The purpose of the present study was to establish the prevalence of GBV-C infection among intravenous drug users (IVDUs) in Thailand and to correlate the results with the medical history as well as the habits of intravenous drug users, thereby establishing the route of infection by blood borne viruses in members of high risk groups.

MATERIALS AND METHODS

Population study

From December 1997 until mid January 1998, samples of venous blood were collected from altogether 204 IVDUs all of whom had been using drugs intravenously for varying periods of time and who attended at the Drug Addict Center no. 7, Bangkok Metropolitan Health Center no. 7 and Drug Addict Center no. 16, Health Center no. 16, Health Department, Bangkok Metropolitan City, in order to receive methadone therapy. Before drawing blood, each individual was interviewed by means of a standard questionnaire. In addition, everyone of the IVDUs tested was asked to sign a form thereby giving his/her informed consent to participating in the study.

Laboratory methods

GBV-C-RNA Detection: RNA extraction was performed by using the guanidine method (Cha *et al*, 1991). Subsequently, denaturation was performed at 65°C for 5 minutes. The RNA samples were reverse-transcribed into cDNA in a total volume of 20 µl by using 50 U of MuLV Reverse Transcriptase, (Perkin Elmer) 10 µl of RNA, 50 mM KCl, 10 mM Tris-HCl pH 8, 400 µM dNTPs, 10 U RNAse inhibitor, and subsequently incubated at 37°C for one hour. GBV-C-RNA was detected by nested PCR using four primers created from the 5' untranslated region (UTR) of GBV-C. First amplification step: 5 µl of cDNA samples were amplified in a 50 µl reaction volume containing 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl pH 8, 200 µM dNTP, 1 U Ampli Taq DNA Polymerase (Perkin Elmer Cetus), 0.8 µM each of outer sense primer, located at position 108, having the sequence 5' AGG TGG TGG ATG GGT GAT 3' of and the outer anti-sense primer, located at position 531, having the sequence 5' TGC CAC CCG CCC TCA CCC GAA 3', 1.5 mM $MgCl_2$. The reaction was then performed for 30 cycles at 94°C for 0.6 minutes, at 55°C for 0.7 minutes and at 72°C for 1.5 minutes. Second amplification step: 20 µl reaction was performed as described above for the first amplification step, using 1 µl of first step PCR product. The inner sense primer, located at position 134, had the sequence 5' TGG TAG GTC GTA AAT CCC GGT 3' and the anti-sense primer, located at position 476, had the sequence 5' GGR GCT GGG TGG CCY CAT GCWT 3' (R = A or G, W = A or T, Y = C or T). Both primers were originally created at the University of Edinburgh, as has been published elsewhere (Jarvis *et al*, 1996). The above primers were employed for the second step, consisting of 30 cycles of amplification. The 10 µl of amplified product were fractionated by 2% Nusieve gel electrophoresis in Tris borate buffer at 120 volt for 50 minutes and visualised by UV fluorescence after staining with ethidium bromide. The product band will show at 421 base pairs for the first amplification step and at 343 base pairs for the second step. Serum obtained from a patient previously diagnosed GBV-C-RNA positive was used as a positive control and autoclaved, DEPC-treated water as a negative control, respectively.

ALT determination: Serum ALT was determined by automated chemical analyser (Hitachi 911) at the Central Laboratory, Chulalongkorn Hospital. The normal value for healthy individuals was 0-38 µ/l.

Data analysis

We expressed the data by determining their respective arithmetic mean values along with the standard deviation (SD), using the unpaired Student's *t*-test and chi-square test for statistical analysis.

RESULTS

We obtained venous blood from altogether 204 IVDUs, 11 of whom are female, the remaining 193 male, their age ranging from 16 to 63 years with a mean age of between 28 and 33 years, and the duration of intravenous drug use ranging from one to forty years with a mean duration of between 111 and 155 months in the GBV-C-positive and GBV-C-negative subjects, respectively. The drug injected by all of them has been heroin, between one and ten times per day. Approximately 50% smoked marijuana in addition and about 20% took various tranquillizers, in few cases opium (four) and morphine (two). In 46 (22.5%) of the 204 IVDUs GBV-C-RNA was detected by RT-PCR with its prevalence peaking in the age group between 21 and 30 years while successively decreasing in the age groups above 30 years (Table 1, Fig 1). The duration of drug use demonstrated an inverted relation to the percentage of detectable GBV-C-RNA (Table 1, Fig 2). Our data also show the average values obtained with regard to the liver function test performed in GBV-C-positive and GBV-C-negative individuals, demonstrating an elevation of the liver enzymes in those IVDUs positive for GBV-C (Table 1). No statistically significant difference regarding, ALT level and ALT elevation was observed between the IVDU groups with and without detectable GBV-C-RNA. Of the 204 IVDUs 121 were also tested for anti-HIV antibody, which found 33 (27.3%) positive versus 88 (72.7%) negative.

Table 1

Demographic data of IVDUs separated into groups with and without detectable GBV-C-RNA.

	GBV-C-RNA	
	Positive	Negative
Number	46	158
Sex (M:F)	45:1	148:10
Age in years		
< 20	8	25
21-30	24	44
31-40	10	50
41-50	4	32
> 50	0	7
Mean age in years and SD[a]	28.26 ± 8.04	33.15 ± 10.62
(range)	(16-47)	(16-63)
Duration of IVDU in months		
< 60	16	38
60-120	16	46
121-240	6	30
> 240	8	44
Mean duration of IVDU in months and SD[a]	111.48 ± 91.63	155.43 ± 113.56
(range)	(24-324)	(12-480)
Mean ALT in μ/l and SD[b]	51.65 ± 70.85	35.22 ± 79.78
(range)	(5-354)	(2-373)
Number of IVDUs with ALT elevation[c] (> 38 μ/l)	16	38

[a] Unpaired Student's *t*-test ($p < 0.05$)
[b] Unpaired Student's *t* test ($p > 0.05$)
[c] Chi-square test ($p > 0.05$)

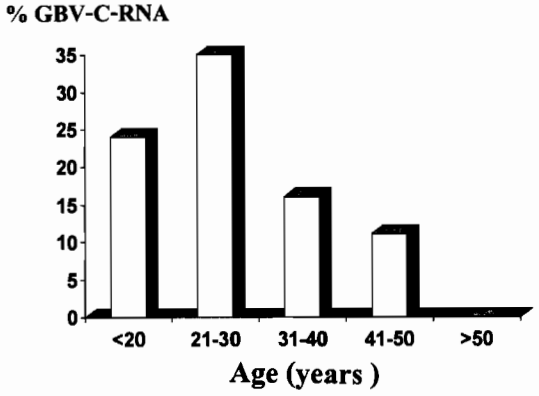

Fig 1–The percentage of GBV-C-RNA in relation to the respective age of the individuals tested.

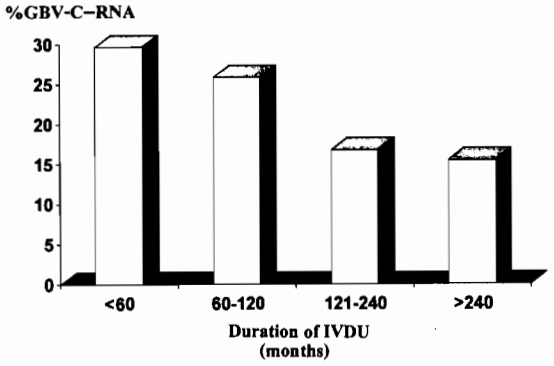

Fig 2–The percentage of GBV-C-RNA in relation to the duration of intravenous drug use.

DISCUSSION

The present study has been performed with 204 IVDUs all of whom, due to their habit of intravenous drug use accompanied by needle sharing, are at an increased risk of exposure to parenterally transmitted viruses, such as HIV, HBV, and HCV. Accordingly, our results also show a high prevalence of hepatitis GBV-C infection, another parenterally transmissible agent, among the members of this particular high-risk group. The results obtained in the present study furthermore show a rather strong similarity to those garnered in thalassemic children having undergone multiple blood transfusions (Poovorawan *et al*, 1998a) in whom the prevalence of GBV-C infection amounted to 32.6% compared to that determined for healthy subjects supposed to be at low risk regarding infection by blood borne viruses, *eg* 1.2% in adolescents, 5% in voluntary blood donors and pregnant women. Among the 204 IVDUs tested in this study, 45 (22%) were found positive for GBV-C-RNA, a finding correlating well with the subjects comprising the members of a high risk group. On average, those found negative for GBV-C-RNA are approximately five years older and likewise, have been using drugs intravenously for close to four more years compared to the ones tested GBV-C negative. Both findings strongly hint at a clearance of the virus by the host's immune system after prolonged exposure, even in individuals who, due to their habit of intravenous drug use combined with needle sharing, are at least to some extent immuno-compromised, a discovery previously reported in the case of thalassemic children having undergone multiple blood transfusions (Poovorawan *et al*, 1998b). This is further corroborated by the decrease in percent GBV-C-RNA in direct relation to the duration of intravenous drug use, as shown in Fig 2, as well as by the peak incidence of percent GBV-C-RNA detected within the age group between 21 and 30 years, who can be surmised to have taken the drugs already for several years, contrasted by its ascent within the age group below 20 years and its sharp decline within both age groups between 31 and 40, as well as 41 and 50 years, respectively, as depicted in Fig 1. Our data thereby confirm those obtained by the group of Thomas *et al*, (1997) in Baltimore, USA. The availability of the newly developed GBV-C antibody detection test will further confirm our assumption in this regard.

As to the status of the liver enzymes, 16 of the 46 (34.8%) GBV-C-positive IVDUs showed elevated ALT, compared to 38 of the 158 (24%) of the GBV-C-negative ones. On the one hand, this difference is not statistically significant, yet, despite various studies reporting GBV-C to be the causative agent of clinical liver disease, an elevated ALT level has also been shown not to be a reliable marker for GBV-C infection (Wang and Jin, 1997). In most cases of GBV-C infection investigated to date it has actually been found as a co-infection with HCV, without any effect on the severity of the simultaneous HCV infection (Alter *et al*, 1997), and to a lesser extent with HBV. Our group has actually

established the presence of GBV-C-RNA in 20% of asymptomatic HCV carriers contrasted by its low prevalence of 0 and 5% found in cirrhosis and chronic hepatitis patients, respectively (Poovorawan *et al*, 1998a). Considering that both viruses are blood borne, thereby sharing their route of transmission, the high prevalence of simultaneous infection is not surprising. For example in Thailand, 96.7% of IVDUs were found positive for anti-HCV (Apichartpiyakul *et al*, 1995). Yet contrasting HCV infection, which frequently develops into a chronic carrier state with the possible sequelae of cirrhosis and hepatocellular carcinoma, GBV-C can be eliminated more readily by the respective host's immune response, at least in immuno-competent individuals, as has been demonstrated by the much higher prevalence of GBV-C-RNA among immuno-suppressed versus immuno-competent patients (Kudo *et al*, 1996). On the one hand, and on the other by the much higher prevalence of anti-HCV along with anti-HIV compared to that of GBV-C-RNA among IVDUs. This is further corroborated by the observation that GBV-C-RNA is usually detected in a younger age group compared to chronic hepatitis C, cirrhosis or hepatocellular carcinoma and interestingly, by the present identical results obtained among IVDUs, although at least in some cases, their immune status might be compromised. In order to confirm this hypothesis a clinical trial of the serological test for anti-GBV-C antibody would be worthwhile.

In addition, GBV-C seems to exist rather frequently in an asymptomatic carrier state with viremia having persisted for up to 16 years as shown by retrospective testing of stored sera from GBV-C-positive patients (Masuko *et al*, 1996). As far as GBV-C infection can be associated with elevated liver enzyme levels, those patients solely infected with GBV-C showed normal ALT and of those with enzyme elevation, about half had levels raised just above the upper limit of normal. Hence, as we could not unequivocally associate any serious liver disease with GBV-C infection, be that in IVDUs or in any of the other population groups examined previously (Poovorawan *et al*, 1998a, b) future studies for determining the clinical significance of this hepatotropic virus are required.

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