Epidemiology of Hepatitis B, C, D and G Viruses and Cytokine Levels among Intravenous Drug Users*

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Summary: To investigate the features of various hepatitis virus infection in intravenous drug users (IVDU), we conducted an epidemiological survey of hepatitis viruses including hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV) and hepatitis G virus (HGV) in IVDU. The correlation of T_H lymphocyte cytokine and hepatitis virus infection was examined. A study population of 406 IVDU consisted of 383 males and 23 females. HBV-DNA and HCV-RNA were detected by fluorescence quantitative polymerase chain reaction. HBsAg, HBeAg, anti-HBc, anti-HCV, HDV-Ag and anti-HGV were assayed by ELISA. The levels of cytokines of T_H1 and T_H2 were measured by ELISA. The similar indices taken from 102 healthy persons served as controls. The infection rate of each virus among IVDU was 36.45 % for HBV, 69.7 % for HCV, 2.22 % for HDV, and 1.97 % for HGV, respectively. The co-infection rate of HBV and HCV was detected in 113 of 406 (27.83 %). In contrast, among controls, the infection rate was 17.65 % for HBV and 0 % for the other hepatitis viruses. The levels of PHA-induced cytokines (IFN-γ and IL-4) and the level of serum IL-2 were obviously decreased in IVDU. On the other hand, the level of serum IL-4 was increased. The IFN-γ level was continuously decreased when the IVDU was infected with HBV/HCV. In conclusion, HBV and HCV infection were common in this population of IVDU and they had led to a high incidence of impaired T_H1 cytokine levels.
Key words: hepatitis virus; co-infection; intravenous drug users; epidemiology; cytokine

Viral hepatitis presents a major global public health concern. HBV and HCV, in particular, were the causative agents responsible for parenterally transmitted diseases[1,2]. IVDU represent a special subgroup of the population who often shared contaminated needles for intravenous drugs injection[3]. Opiate use was known to alter immune function and had immunosuppressive effects that may modify T-lymphocyte subpopulations. IVDU were subject to transmittable blood-borne pathogens. The pathogens may resulte in the immune system dysfunction[4]. With IVDU, reports on the relationship between the infection of hepatitis viruses and changes in T_H cytokine levels are not reported although a few epidemiological studies of had been reported in drug addicted users in China[5,6]. In the present study, we conducted an epidemiological survey of HBV, HCV, HDV and HGV and examined the association of hepatitis virus infection with T_H cytokine levels in IVDU in southwestern China.

1 MATERIALS AND METHODS

1.1 Study Population

In this study, 508 serum samples, including samples from 406 IVDU (383 males and 23 females, with a mean age of 32.4 y, a range of 17—61 y who had a history of drug use for 1—18 y) and 102 controls (64 males and 38 females, with a mean age of 30.5 y and a range of 18—58 y) in southwestern China. All subjects had no clinical manifestation of hepatitis. 199 cases had shared needles with other drug users. Informed consent for inclusion in this study was obtained from each individual. The blood samples were collected from 2002 to 2003 and sera were stored at −40 ℃ or below until use.

1.2 Viral Marker Detection

Samples were detected for markers of hepatitis virus infection (HBsAg, HBeAg, anti-HBc, anti-HCV, HDV-Ag and anti-HGV) by enzyme linked immunosorbent assay kit (ELISA, Kehua, China). Serum samples were also tested for HCV-RNA and HBV-DNA by fluorescence quantitative polymerase chain reaction (FQ-PCR technologies, China).

1.3 Assay for PHA-induced Cytokines and Serum Cytokines

Peripheral blood mononuclear cells (PBMC) were isolated from 3 mL fresh heparinized blood sample obtained from each subject by Ficoll centrifugation. The PBMCs were suspended in RPMI 1640 (PHA-M add to 20 μg/mL) and were placed in culture plates at 1×10^6 cells per well for proliferation and cytokine assays. Supernatants were removed after 60-h incubation for determination of IFN-γ and IL-4. The levels of IFN-γ and IL-4 were determined by commercial ELISA kit (ELISA,

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Jingmei, Guangzhou). The serum levels of IL-2 and IL-4 were determined by radioactive immune assay (RIA, 3V Co., China). All commercial kits were used by following the manufacturer's instructions.

1.4 Statistical Analysis

The results were analyzed by Student's *t*-test and *Chi*-square test. A *P* value less than 0.05 was considered to be significant.

2 RESULTS

2.1 Prevalence and Co-infection of Hepatitis Virus

Among IVDU, 283/406 (69.7 %) cases were anti-HCV positive. 54/283 (19.08 %) of these sera were positive for HCV- RNA ($2.98\times10^2-2.25\times10^5$ copies per mL). 148/406 (36.45 %) IVDU suffered from HBV infection (anti-HBc positive), 12/148 (8.11 %) and 15/148 (10.14 %) of these sera had detectable HBeAg and HBV-DNA ($4.59\times10^3->5\times10^7$ copies per mL) respectively. The rate of HDV/HGV seroprevalence was lower than that of HCV/HBV. Hepatitis virus seropositivity among IVDU was associated with needle sharing. Among 102 controls, HBV infectious rate was 17.65 %. No other hepatitis viruses were detected (table 1). The findings of co-infection of hepatitis viruses are given in table 2. Addicts infected with the hepatitis B and C viruses had been taking drugs longer than those who were not infected with these two viruses.

Table 1 Prevalence of HBV, HCV, HDV and HGV in IVDU and controls

Groups	HBsAg	HBeAg	Anti-HBc	HBV-DNA	Anti-HCV	HCV-RNA	HDV-Ag	Anti-HGV
IVDU	24 (5.91)	12 (3.96)	148 (36.45)	15 (3.69)	283 (69.7)	54 (13.3)	9 (2.22)	8 (1.97)
Control	1 (0.98)	2 (1.96)	18 (17.65)	1 (0.98)	0 (0)	0 (0)	0 (0)	0 (0)
χ^2	4.20	0.30	13.11	1.97	160.53	15.18	2.26	2.04
P	<0.05	>0.05	<0.01	>0.05	<0.01	<0.01	>0.05	>0.05

Table 2 Infection and co-infection of hepatitis viruses in IVDU (n=406)

Category	Positive	(n) %
HBV+	27	6.65
HCV+	165	40.64
HBV+HCV+	109	26.85
HBV+HDV+	8	1.97
HBV+HCV+HDV+	1	0.25
HBV+HCV+HGV	3	0.74
HCV+HGV	5	1.23
Total	318	78.33

2.2 Cytokine Levels

The levels of PHA-Induced IFN-γ and IL-4 were lower in IVDU than in healthy controls ($P<0.01$). The level of serum IL-2 was lower, but IL-4 was higher (table 3).

2.3 HBV/HCV Infection and Cytokine Levels

In IVDU, co-existence of HBV/HCV infection was related to the decreased level of PHA-induced IFN-γ. But higher virus load was not correlated with lower IFN-γ level. Serum cytokines showed no significant difference between HBV/HCV-infected IVDU and non-HBV/HCV-infected IVDU ($P>0.05$, table 4, 5).

Table 3 Cytokine levels of PHA-induced and serum

Groups	n	PHA-induced cytokine		Serum cytokine	
		IFN-γ (pg/mL)	IL-4 (pg/mL)	IL-2 (ng/mL)	IL-4 (ng/mL)
IVDU	406	273.7±63.4	20.7±1.43	1.73±1.68	9.43±6.22
Control	102	785.3±21.3	28.9±1.08	3.82±1.59	0.78±0.33
t		23.97	7.65	11.37	27.03
P		<0.01	<0.01	<0.01	<0.01

Table 4 Cytokine levels during HBV-infection

Groups	n	PHA-induced cytokine		Serum cytokine	
		IFN-γ (pg/mL)	IL-4 (pg/mL)	IL-2 (ng/mL)	IL-4 (ng/mL)
Negative△	40	288.01±40.03	20.76±1.87	1.69±1.65	9.46±7.12
Anti-HBc$^+$	148	266.02±54.58*	20.59±1.33	1.81±1.69	9.38±5.53
HBV-DNA$^+$	15	263.14±55.12	20.24±2.16	1.78±1.92	9.33±8.39

* $P<0.05$ as compared with negative cases; △ Viral infection markers were not discovered in IVDU

Table 5 Cytokine levels during HCV-infection

Groups	*n*	PHA-induced cytokine		Serum cytokine	
		IFN-γ (pg/mL)	IL-4 (pg/mL)	IL-2 (ng/mL)	IL-4 (ng/mL)
Negative△	40	288.01±40.03	20.76±1.87	1.69±1.65	9.46±7.12
Anti-HCV$^+$	283	266.4±61.1**	21.23±1.42	1.67±1.49	9.02±5.84
HCV-RNA$^+$	54	264.2±56.3*	20.6±1.49	1.68±1.73	9.32±6.47

* $P<0.05$, ** $P<0.01$ as compared with the negative cases;
△ Viral infection markers were not discovered in IVDU

3 DISCUSSION

HBV, HCV, HDV and HGV share transmission routes. HBV and HCV are major causes of acute and chronic liver disease worldwide. Persistent infection with these viruses often leads to chronic liver diseases, including cirrhosis or primary hepatocellular carcinoma. However, HBV is transmitted easily via both percutaneous and mucosal exposures, and HCV is transmitted predominantly by percutaneous exposures. Most participants of high-risk practices (e. g. drug use through injection) had hepatitis virus infection. The infection rate in IVDU with infection of hepatitis viruses or other blood-borne pathogens infection was considerably higher than in that the non-IVDU population. Repeated co-infection with different viruses probably is common in IVDU[7]. In this study, a higher percentage (69.7 %) of HCV infection was observed. And 19.08 % of their sera were positive for HCV-RNA. HCV seroprevalence rates ranged from 3.2 % to 90.1 % in other studies of addicted users. Variations in seroprevalence may well reflect geographic differences. However, part of the variation was likely due to the difference in serologic methodologies, and co-infection with other viruses might prevent the detection of HCV-RNA in sera. 36.54 % of the population had HBV. 10.14 % of these sera had detectable HBV-DNA. Only 88 of the subjects (21.67 %) had no hepatitis virus infection, whereas 318 (78.33 %) were infected with one or more than one viruses. The results of our research suggested that intravenous drug use often leads to the infection and co-infection of more than one hepatitis viruses, especially HCV and HBV. These results were consistent with the findings reported recently[8, 9].

Gilson *et al* believed that the co-infection of HCV and HBV could increase the expression of anti-HBs and DNA polymerase[10]. They proposed that co-infection of both HCV and HBV in IVDU might augment the level of HBV replication and aggravate the damage to the liver. But our reports concerning co-infection of HCV and HBV yielded different results and no significant correlation was observed between HBV-DNA and other viral infection (HCV, HDV or HGV). The discrepancy might be due to the small number of our samples (15 HBV-DNA positive cases). Epidemiologically, the population shared the same route of infection therefore, the HBV/HCV-infected population have a higher incidence of both HDV and HGV than those without infection of HBV/HCV[11]. Our investigation showed HDV patients tend to have HBV infection. Anti-HGV was detected in IVDU who were positive for anti-HCV and anti-HBc.

Opiate use was known to alter immune function. Opiate suppresses cell-mediated immunity and humoral immunity, and impairs the activity of natural killer cells. Therefore, the drugs injected into body, by modulating the immune response, may lead to immune tolerance to viruses and entry of the virus into the host. It is known that the subsets of T_H cell could be distinguished by the pattern of cytokine co-expression. T_H1 cells produce IFN-γ and IL-2, and T_H2 cells produce IL-4, IL-5, and IL-10. IFN-γ and IL-2 were cytokines that play important roles in the development of CD4$^+$/CD8$^+$ lymphocyte activity and cellular immune response such as enhancing activities of cytotoxic T lymphocytes (CTL), NK and T_H1. IL-4 inhibited the production of T_H1 cells[12]. The imbalance of T_H1 and T_H2 might affect cell-mediated immunity and humoral immunity. Our investigation showed that the levels of PHA- induced IFN-γ, IL-4 and serum IL-2 were decreased in IVDU, but the levels of serum IL-4 were increased. IVDU have the imbalance of T_H1 and T_H2. Virus entry into blood might also be facilitated due to the dysfunction of T lymphocyte subpopulation in IVDU[13].

Patients with chronic HCV infection usually display weak CD4$^+$ T cell response. It was commonly believed that a strong CD4$^+$ T cell response with secretion of T_H1 cytokines was required for spontaneous elimination of HCV[14]. CD4$^+$ T_H lymphocyte may also contribute to the clearance of HBV infection by secreting cytokines that inhibit viral replication. In addition T_H cell response may play a role in supporting the induction and proliferation of HBV-specific CTL and B cells[15]. It had been shown that hepatotropic viruses could be eliminated or controlled by different mechanisms such as destruction of infected cells by cytotoxic CD8$^+$ T cells or secretion of antiviral cytokines [16] The induced IFN-γ level was much lower in IVDU with HBV/HCV-infection than in non-HBV/HCV IVDU. There was no statistically significant difference in the level of IL-4 between IVDU with or without HBV/HCV-infection. The activation of

virus-specific CTL was critical for the elimination of virus-infected cells. Virus-specific CTL were significantly diminished in patients with chronic HCV infection as compared with those observed for persistent infection of other viruses, such as HIV[17]. This apparent suppression of CTL responses suggested that HCV may interfere with the activation of $CD4^+$ helper T cells, thus allowing for the establishment of chronic infection. HCV-induced immune suppression was found to result from inhibition of IFN-mediated CTL activation. When T_H1/T_H2 balance was disturbed, IFN-γ is diminished, and T_H1 dominance switches to T_H2 phenotype. The host might also be more susceptible to viral infection. And viral replication was enhanced. IFN is a factor produced by cells in response to viral infection that protects other cells of the same species from attack by a wide range of viruses. But viral replication can cause immune system dysfunction. These results suggested that HBV/HCV infection impairs the cellular immune response by decreasing T_H1 type cytokines. T_H1 cells seemed to be more severely destroyed in active infection of HBV/HCV than in latent infection. No difference in serum cytokine levels was found between HCV-RNA positive cases and HCV-RNA negative one. There was no significant correlation between cytokines and HBV-DNA.

In conclusion, our study showed that the prevalence of HCV/HBV infection was high in IVDU. The present study also confirmed the observation of other scholars that HCV and HBV were more common in IVDU and that hepatitis virus infection was a factor responsible for immune system dysfunction. In IVDU, the balance of T_H lymphocyte subsets was upset, and this is especially true of HBV and HCV infection.

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