BACKGROUND

Vibrio vulnificus (*V. vulnificus*), a gram-negative bacterium of the family *Vibrionaceae*, is a worldwide inhabitant of salt water [1,2]. These bacteria tend to be more common in warmer waters (17–20°C) [3,4]. *V. vulnificus* causes serious illness including necrotizing fasciitis and septicemia, and death in persons with preexisting liver disease or compromised immune systems [5–7]. People with chronic liver disease, particularly liver cirrhosis, are more prone to developing infection, and are at greatest risk for an adverse outcome [8,9]. Other predisposing factors are iron overload and hemochromatosis, and immunosuppression caused by steroid treatment, malignancy, human immunodeficiency virus (HIV) infection, renal failure and organ transplantation [10,11].

V. vulnificus infection was first reported by Roland in 1970 in a case of endotoxic shock with leg gangrene [12]. In Japan, Matsuo et al. reported the first case of *V. vulnificus* infection in 1978 [13]. There have since been case reports of approximately 200 patients over a period of about 30 years [14]. However, because the 200 cases represent only those that were published, the actual number of *V. vulnificus* infections is considered to be higher [14]. The annual number of *V. vulnificus* septicaemia cases in Japan has been estimated at 425 (95% CI 238–752) [15]. The prevalence of *V. vulnificus* septicaemia is estimated at 3.3 per million in Japan. The annual number of *V. vulnificus* infection in Japan is notably higher than in other countries, such as Korea and the USA [15]. The prevalence of *V. vulnificus* septicaemia is low in the general population, and estimated at 0.6 per million in USA [8]. A study of the epidemiological and clinical characteristics of *V. vulnificus* infections reported in Japan from 1975 to 2005 [14] found that about 90% of Japanese patients with *V. vulnificus* infection had liver disease such as liver cirrhosis, hepatocellular carcinoma (HCC), and chronic hepatitis.

It is estimated that approximately 2 million Japanese people are chronically infected with hepatitis C virus (HCV) [16]. Approximately 35,000 patients died due to HCC in Japan, and the number of deaths in Japan from HCC continues to increase. In Japan, approximately 80% of HCCs are caused by HCV and about 10% by hepatitis B virus (HBV). The increase in the number of HCC patients due to HCV in turn contributes to the increase in the number of deaths in Japan from HCC.

In Japan, patients with liver disease are not provided adequate educational opportunities. Therefore, in this study, we assessed knowledge about *V. vulnificus* infection in patients with chronic liver disease.

MATERIAL AND METHODS

Subjects

Between August 1, 2008 and October 31, 2008, anonymous questionnaires relating to general knowledge of *V. vulnificus* infections were given to all patients with chronic liver diseases who had been treated at 14 geographically-distinct institutions in Japan, as well as to their attending physicians. A physician at each participating institution completed a questionnaire with the patient's medical information and handed the questionnaire to the patient. Next the patient was interviewed about *V. vulnificus* infection. The questionnaire was conducted in one-to-one interview style by patient and physician. A physician at each medical institution returned the completed questionnaires to Kurume University of Medicine; 1,336 completed questionnaires were recovered, and the collection rate was 97.3% (1,336/1,373). The 14 medical organizations were those where many liver specialists authorized by the Japan Association for the Study of the Liver work full-time.

We mailed questionnaires directly to these 14 medical institutions through a collaborative study. A database for the results of our investigation was compiled at the Department of Digestive Disease Information & Research, Kurume University School of Medicine.

Items of investigation

Anonymous questionnaires asked patients and their attending physicians to respond to the following items; patient background (age, gender, diagnosis of liver diseases, comorbidities, and steroid use), patient awareness and understanding of *V. vulnificus* infection, frequency of eating raw fish and shellfish, raw shrimp and sushi, the season in which raw fish was eaten, and frequency of bathing in the sea and shellfish gathering. After the patients answered the questionnaires, we provided them with literature containing basic information about *V. vulnificus* infection.

The investigation was conducted in accordance with the "ethical guidelines on epidemiological studies" of the Ministry of Education and Science and the Ministry of Health, Labour and Welfare, and observed the spirit of the Helsinki Declaration. Physicians at study facilities explained to patients the content and significance of the study and obtained consent in accordance with each facility's regulations.

Statistical analysis

All data are expressed as mean ± standard error. Differences between the 2 groups were analyzed using the Welch's test and the Mann-Whitney U test. Differences were judged significant for $p<0.05$ (2-tailed). All statistical analyses were conducted using JMP Version 6 (SAS Institute, Cary, NC, USA).

RESULTS

Patient's background

We analyzed 1,336 questionnaires in which 656 indicated they were males, 670 females, and 10 did not specify gender. Mean age was 61.4±12.3, as shown in Table 1.

Among the 1,336 patients, the distribution of diagnoses of liver disease was as follows: HCV-related liver diseases 760 (56.9%), HBV-related liver diseases 266 (19.9%), HCV & HBV-related liver diseases (simultaneous infection) 4 (0.3%), non-B non-C-related liver diseases 19 (1.4%), other liver diseases 273 (20.4%), and no answer 14 (1.0%). Some institutions differed significantly in patients' age, gender distribution, or liver diseases, compared to the overall averages (Table 1).

Table 1. Clinical information for 1,336 patients from whom questionnaires returned.

Prefecture	Medical institution	n	Collection rate of questionnaire	Age			Sex				Liver diseases												
				Mean	SD	P value	Male	Female	No answer	P value	HCV-related liver disease		HBV-related liver disease		HCV & HBV-related liver disease		NBNC-related liver disease		The other		No answer		P value
			(%)	year			n	n	n		n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	
Hokkaido	Sapporo Medical University School of Medicine	100	(100.0)	59.4	13.4	NS	44	55	1	NS	48	(48.0)	27	(27.0)	0	(0.0)	4	(4.0)	19	(19.0)	2	(2.0)	NS
Fukushima	Fukushima Medical University School of Medicine	97	(97.0)	63.6	12.2	NS	38	52	7	NS	42	(43.3)	12	(12.4)	0	(0.0)	2	(2.1)	34	(35.1)	7	(7.2)	<0.05
Chiba	Chiba University Graduate School of Medicine	97	(97.0)	58.8	13.5	NS	47	50	0	NS	63	(65.0)	15	(15.5)	0	(0.0)	1	(1.0)	18	(18.6)	0	(0.0)	NS
Shizuoka	ELM Medical Clinic	100	(100.0)	57.2	12.2	0.001	71	29	0	<0.0001	38	(38.0)	36	(36.0)	0	(0.0)	0	(0.0)	26	(26.0)	0	(0.0)	<0.001
	Miyazaki Clinic	100	(100.0)	51.0	15.3	<0.000000001	53	47	0	NS	40	(40.0)	37	(37.0)	0	(0.0)	0	(0.0)	23	(23.0)	0	(0.0)	<0.001
Aichi	Social Insurance Chukyo Hospital	100	(100.0)	61.4	14.1	NS	44	55	1	NS	59	(59.0)	14	(14.0)	0	(0.0)	1	(1.0)	25	(25.0)	1	(1.0)	NS
Fukuoka	Kurume University School of Medicine	213	(100.0)	60.6	11.6	NS	86	127	0	0.01	135	(63.4)	38	(17.8)	0	(0.0)	1	(0.5)	39	(18.3)	0	(0.0)	NS
Saga	Inuzuka Hospital	100	(100.0)	64.4	11.0	<0.05	47	52	1	NS	85	(85.0)	6	(6.0)	1	(1.0)	0	(0.0)	8	(8.0)	0	(0.0)	<0.00001
Nagasaki	Narao Hospital	122	(81.3)	66.5	10.8	<0.00001	68	54	0	NS	71	(58.2)	42	(34.4)	0	(0.0)	1	(0.8)	7	(5.7)	1	(0.8)	<0.0001
	National Nagasaki Medical Center	59	(98.3)	64.5	10.5	NS	29	30	0	NS	47	(79.7)	6	(10.2)	1	(1.7)	0	(0.0)	5	(8.5)	0	(0.0)	<0.01
Oita	Oita University	100	(100.0)	59.6	13.4	NS	41	59	0	NS	53	(53.0)	16	(16.0)	2	(2.0)	3	(3.0)	25	(25.0)	1	(1.00	<0.05
	National Hospital Organization Oita Medical Center	48	(96.0)	64.9	12.5	<0.05	23	25	0	NS	31	(64.6)	8	(16.7)	0	(0.0)	4	(8.3)	4	(8.3)	1	(2.1)	0,001
	Oita Cardiovascular Hospital	50	(100.0)	67.0	10.9	<0.001	29	21	0	NS	36	(72.0)	8	(16.0)	0	(0.0)	2	(4.0)	4	(8.0)	0	(0.0)	NS
	Abe Diabetes Clinic	50	(100.0)	62.0	10.6	NS	36	14	0	0.001	12	(24.0)	1	(2.0)	0	(0.0)	0	(0.0)	36	(72.0)	1	(2.0)	<0.00000000000001
Total		1336	(97.3)	61.4	12.3		656	670	10		760	(56.9)	266	(19.9)	4	(0.3)	19	(1.4)	273	(20.4)	14	(1.0)	

Liver cirrhosis was observed in 304 (22.8%) patients, including those with HCV-related liver cirrhosis (177 cases), HBV-related liver cirrhosis (66), HCV & HBV-related liver cirrhosis (1), non-B non-C-related liver cirrhosis (11), and other liver diseases such as primary biliary cirrhosis (PBC) and autoimmune hepatitis (AIH) (49).

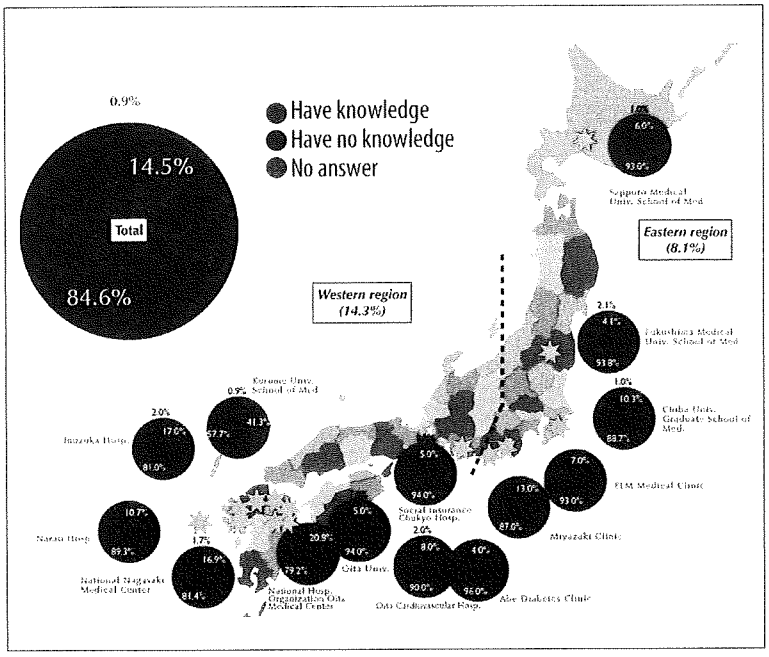

Figure 1. Knowledge of *V. vulnificus* infections among all patients with liver diseases. Only 14.5% of such patients had knowledge of this infection. Fourteen red stars indicate the location of each medical institution. Japan consists of 47 prefectures. Half of east of Japan, including Tokyo, where Japan is metropolitan, is called eastern Japan, and the western half of Japan is called western Japan. The broken line indicates the boundary between the 2 areas.

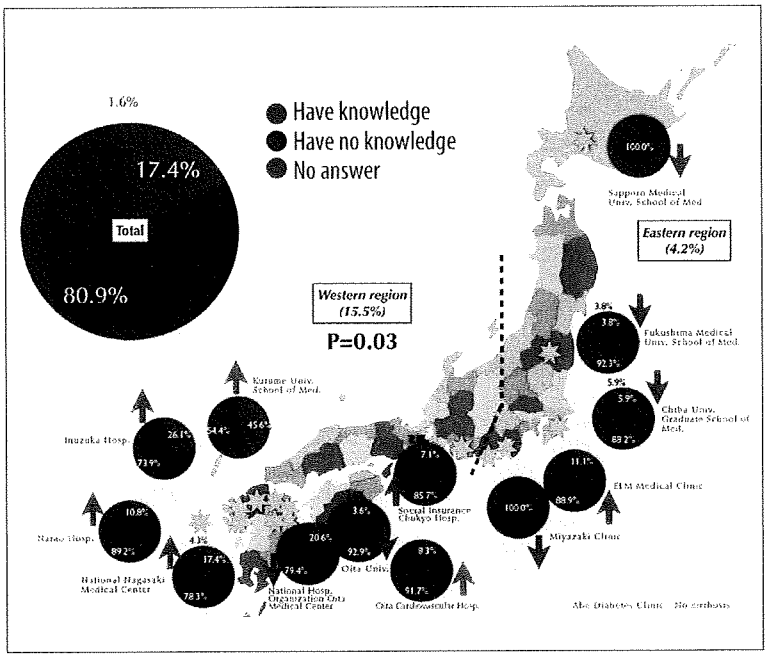

Figure 2. Knowledge of *V. vulnificus* infections in patients with liver cirrhosis. The rate of patient knowledge in the western region of Japan was significantly higher than in the eastern region. The upward pointing arrow indicates an increase in the rate of *V. vulnificus* infections in a given institution compared to Figure 1. A down-pointing arrow indicates a decrease compared to Figure 1.

There were associated comorbidities in 732 (54.8%) of all patients with liver disease. These were classified using International Classification of Diseases (ICD) criteria: diseases of the circulatory system (372 cases), endocrine, nutritional and metabolic diseases (316), diseases of the digestive system (73), malignant neoplasms (54), diseases of the genitourinary system (33), diseases of the nervous system (23), diseases of the musculoskeletal system and connective tissue (18), diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism (17), diseases of the respiratory system (16), mental and behavioral disorders (7), diseases of the skin and subcutaneous tissue (4), diseases of the eye and adnexa (4), certain infectious and parasitic diseases (2), and other diseases (6).

There were 563 patients (42.1%) with no comorbidities, 40 patients provided no answer about comorbidities, and 1 patient was unassessable. There were 60 patients who took oral or topical steroids for their liver disease or comorbidities.

Knowledge of *V. vulnificus* infection in patients with liver diseases

Only 14.5% (194/1,336) of patients with liver disease had general knowledge regarding *V. vulnificus* infections. The level of patient knowledge varied widely among medical institutes, ranging from 4.0% to 41.3%. The mean rate (14.3%) of knowledge among patients who resided in the western re-

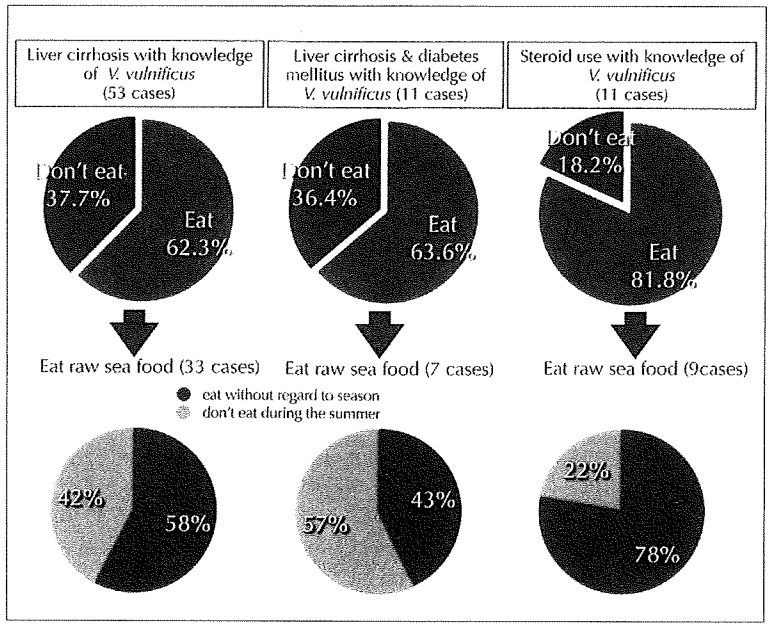

Figure 3. Frequency of eating raw seafood. Even if patients had high risk factors for infection, such as liver cirrhosis, diabetes mellitus, or steroid use, and had knowledge of *V. vulnificus* infections, most ate raw seafood without regard to season.

gion of Japan was higher than that (8.1%) in the eastern region (Figure 1).

Of 304 patients with liver cirrhosis, 17.4% (53/304) (minimum 0%, maximum 45.6%) had knowledge of *V. vulnificus* infection (Figure 2). This rate (17.4%) was higher than the mean rate (14.5%) of knowledge among all patients with liver diseases, but the proportion of those with knowledge was lower in 6 institutes. The rate (15.5%) of knowledge of *V. vulnificus* infection among those with liver cirrhosis in the western region was significantly higher than those (4.2%) in the eastern region (P=0.03).

Knowledge of *V. vulnificus* infection among patients with liver cirrhosis and diabetes mellitus

Sixty patients had liver cirrhosis and diabetes mellitus. Of these, 11 (18.3%) had knowledge of *V. vunificus* infections. Patients with liver cirrhosis and diabetes mellitus in 7 institutes had no knowledge of the infection.

Frequency of intake of raw seafood

A total 1,170 (87.6%) of 1,336 patients answered that they often eat raw seafood. Most (1,002 cases, 85.6%) of the patients answered that they eat raw seafood without regard to season. There was significant difference between patients with knowledge and without knowledge who eat raw seafood (P<0.00001).

Thirty-three of 53 patients who suffered from liver cirrhosis and who had knowledge of *V. vulnificus* infection ate raw seafood (19 cases ate raw seafood without regard to season; 14 did not eat raw seafood during the summer). Seven of 11 patients, who suffered from liver cirrhosis and diabetes mellitus and with knowledge of *V. vulnificus* infection, ate raw seafood (3 cases ate raw seafood without regard to season; 4 cases did not eat raw seafood during the summer). Nine of 11 patients who took steroids and who had knowledge of *V. vulnificus* infection ate raw seafood (7 cases ate raw seafood without regard to season, 2 cases did not eat raw seafood during the summer).

In these cases, even if patients with high risk factors, such as liver cirrhosis and diabetes mellitus, had knowledge of *V. vulnificus* infections, most ate raw seafood without regard to season (Figure 3). However, the rate of the patients with liver cirrhosis who did not eat raw seafood and who had knowledge was significantly lower than that of the patients with liver cirrhosis and without knowledge who did not eat raw seafood (37.7% vs. 14.8%, P=0.0001).

Frequency of bathing in the sea and shellfish gathering

The results of the patients who answered questionnaires about bathing in the sea and shellfish gathering were as follows: often (18 cases, 1.3%), sometimes (122, 9.1%), rarely (394, 29.5%), never (768, 57.5%), unassessable (4, 0.3%), and no answer (30, 2.2%). Most of the patients does not swim in the sea and did not go clamming.

Discussion

V. vulnificus causes severe human infections, and is acquired through wounds or contaminated seafood. In Japan, many cases of *V. vulnificus* infection have been reported to occur in the western region and more than half of the infections were reported to occur in Kyusyu [14,17]. Inoue et al. did a retrospective survey in which 1,693 hospitals from across Japan were surveyed, including advanced life saving emergency centers and dermatology institutions [17]. Ninety-four cases were confirmed as *V. vulnificus* infections over 5 years. The authors reported that many *V. vulnificus* infections occurred in Kyusyu, especially in the coastal areas of the Ariake and Yatsushiro Seas.

One reason for the high incidence of *V. vulnificus* infection in the western region in Japan is thought to be higher seawater temperature. *V. vulnificus* proliferates in areas where, or during months when, the water temperature exceeds

17–20°C [3,4]. The other reason is the greater number of HCV carriers in Kyusyu. Geographically, HCC is more frequent in western than eastern Japan [16].

The awareness of *V. vulnificus* infections among Japanese physicians is reported to be low [15]. Only 15.7% of emergency-physicians were reported to have a basic knowledge of *V. vulnificus* infections. In 2004, Osaka et al. reported that emergency-room physicians who work in the western region of Japan had more knowledge of *V. vulnificus* infections [15]. The Ministry of Health, Labour and Welfare warned of the risk of *V. vulnificus* infection on their website in 2006.

Our study demonstrates that awareness of *vulnificus* infections among patients with chronic liver diseases is low. Medical institutions in Japan, except for Kurume University of Medicine, did not provide educational opportunities for learning about *V. vulnificus* infections. Although the 15.5% rate of knowledge among patients with liver cirrhosis in the western region was significantly higher than that in the eastern region (P=0.03), this rate is far from adequate.

The most significant host factor contributing to virulence is chronic liver disease [8,9]. This may act in several ways including: portal hypertension, causing shunting of the bacteria around reticuloendothelial cells in the liver [18,19]; decreased clearance of bacteria from the portal circulation by Kupffer's cells in the diseased liver [19]; increased iron in the serum, as seen in patients with cirrhosis and hemochromatosis, which promotes growth of *V. vunificus* [7,20]; and achlorhydria occurring naturally or induced by medications [8,19,21].

Factors conferring high risk include: liver disease and other diseases with possible hepatic involvement or elevated serum iron levels (including cirrhosis, alcoholism, malignancy, hemochromatosis, or thalassemia major) [8,9,19,20]; therapeutically induced or naturally low gastric acid (achlorhydria or antacid or H2 blocker use) [8,19,21]; and conditions that compromise the immune system (HIV infection, diabetes mellitus, renal disease, or steroid dependency) [10,11,19].

Primary liver cancer, 95% of which is HCC, is ranked third among men and fifth among women as a cause of death from malignant neoplasms in Japan [22,23]. The number of deaths and death rate of HCC has been increasing. Geographically, HCC is more frequent in western than eastern Japan. Meanwhile, according to the Ministry of Internal Affairs and Communications, yearly per capita fish consumption in Japan was 63.2 kilograms on average for 2003–2005, about 4 times higher than the world average. The Japanese custom of eating raw fish and shellfish such as sashimi or sushi has become widely known throughout the world. Their traditional eating habits are attributed to the fact that patients with knowledge about *V. vulnificus* infections still ate raw seafood.

Therefore, it is important for physicians in Japan to expand their knowledge of *V. vulnificus* infections and become familiar with prevention methods. It is also important for patients with liver diseases to acquire the necessary knowledge of *V. vulnificus* infections and prevention methods, such as avoidance of eating raw seafood during the summer. Because of rapid aggravation and high mortality, patients should also keep an emergency contact number handy.

Conclusions

In conclusion, standardized guidelines for prevention of *V. vunificus* infections and education of patients with liver diseases should be required.

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＜Editorial＞

わが国におけるB型肝炎ウイルス遺伝子型の分布

四柳　宏[1)*]　小池　和彦[2)]

はじめに

わが国におけるB型肝炎ウイルス（HBV）遺伝子型（genotype）の分布に関してはこれまで主として急性肝炎，慢性肝炎患者を対象にした検討がなされてきた．その概略は本誌49巻12号に松浦らによってまとめられている．これらの成績は医療機関を訪れた患者という大きなバイアスが入っており，本邦におけるHBV genotypeの実際の分布を反映しているかどうかは不明であった．また，地域における特徴に関しても十分にはわかっていなかった．

本号に報告されている田中らの「わが国の献血者におけるHBV-genotypeの都道府県別分布」は，本邦の献血者におけるHBV genotypeをまとめたものである[1]．同じ著者らにより，わが国の献血者におけるHBV-genotypeの分布を年齢，性別にまとめた論文も最近公表され[2]，これら2本の論文により，本邦のHBV genotypeの分布に関して興味深い事実が明らかにされた．本稿ではこれらの論文を中心に，わが国におけるHBV Genotypeの疫学に関してまとめてみたい．

地域別に見た慢性肝疾患例及び献血者のGenotype分布に関して

本邦における慢性肝疾患例におけるHBV genotypeの分布は2001年にOritoらにより最初に報告されているが[3]，この報告では沖縄県と山形県でGenotype Bの割合が多いとされている．その後秋田県でもgenotype Bの割合が多いことが判明した[4]．今回の田中論文でもこの3県におけるGenotype Bの割合は70%以上を占めている．田中論文は献血者が対象であり，IgM-HBc抗体陽性例は少ないことから，慢性肝疾患患者に比較的近いgenotype分布が示されていると考えられる[2]．

今回Genotype Bの割合が50%を超えたのは前述の3県の他に埼玉県，新潟県が挙げられる．新潟県はOritoらの調査時はGenotype Bの慢性肝疾患に占める割合は4.6%であり，その原因に関しては今後検討が必要と思われる．ただし，Genotype Bの症例はGenotype Cの症例に比べて早くHBe-seroconversionが起こるため，肝病変の進展速度が緩やかであり，医療機関を受診する機会が少ない可能性がある[5]．この点も考慮する必要があろう．

いずれにしても，Genotype Bは山形，秋田の2県を中心とした東北地方と沖縄県に多く分布していること，逆に中国地方や九州地方では少ないことが確認されたと言えよう．

本邦のB型肝炎患者におけるHBV subgenotype分布

HBV genotypeには現在AからHまでの8つがあるが，Genotype A，B，C，D，Fの5つはさらに細かなsubgenotypeに細分化できる．外来株であるgenotype AにはA1/Aa（アジア・アフリカ型），A2/Ae（欧米型）など5つのsubgenotypeが知られている．Genotype BにはB1/Bj，中国株であるB2/Baをはじめ7つのsubgenotypeが知られている．Genotype CにはC1/Cs，本邦，韓国などの主な株であるC2/Ceをはじめ5つのSubgenotypeが知られている．Genotype Dは5つの，Genotype Fは4つのSubgenotypeに細分化される[6]．

これまでの本邦からの報告でSubgenotypeまで取り扱ったものは少ない．Hayashiらは中京地方の急性肝炎の症例に関してSubgenotypeまで含めた解析を行っている[7]．A1/Aaのgenotype Aに占める割合は約10%，B2/Baのgenotype Bに占める割合は約25%，C1/CsのGenotype Cに占める割合は約5%と報告している．また，Kobayashiらは急性及び慢性肝疾患のGenotype分布の変遷を報告しているが[8]，A1/Aaのgenotype Aに占める割合は約10%，B2/Baのgenotype Bに占める割合は約20%，C1/Csの症例は存在しなかったとしている．今回の田中らの報告によれば献血者においてA1/Aaのgenotype Aに占める割合は約28%，B2/

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Table I

		著者	発表年	地域	人数		Genotype及びsubgenotype								その他及び重複感染
						A	A1/Aa	A2/Ae	B	B1/Bj	B2/Ba	C	C1/Cs	C2/Ce	
献血者	HBs抗原陽性者（全例）	Yoshikawa et al.*2	2009	全国47都道府県	1887	5.6	28.3	70.8	30.8	66.6	23.8	62.6			1
	初回献血者				1349	5			31			62.3			1.7
	反復献血者				538	6.9			30.3			62.3			0.5
	IgM-HBc抗体陽性者				61	21.7			15			63.3			0
	NAT陽性				68	19.1			16.2			63.2			1.5
患者	慢性肝疾患	Orito et al.*3	2001	全国12都道府県	720	1.7			12.2			84.7			1.4
		Kobayashi et al.*9	2002	東京，神奈川	1077	1.9			9.4			87.7			1
		Takeda et al.*10	2006	愛知・岐阜	80	0			6.3			84.7			9
		Hayashi et al.*7	2007	愛知・岐阜	123	3.3			15.4			81.3			0
		Kobayashi et al.*8	2008	東京，神奈川	4129	3	9.5**	76.6**	12.3	74.5**	19.6**	84.5	0**	100**	0.2
		Matsuura et al.*11	2009	全国14都道府県	1271	3.5			14.1			82.2			0.2
	急性肝炎	Yotsuyanagi et al.*12	2005	全国9都道府県	145	19			5			75			1
		Suzuki et al.*13	2005	東京，神奈川	97	32			9			45			14
		Ozasa et al.*14	2006	全国14都道府県	301	14.3			14.6			67.4			3.7
		Sugauchi et al.*15	2006	全国19都道府県	485	19			12			46			23
		Takeda et al.*10	2006	愛知・岐阜	98	18.4			4.1			74.5			3
		Hayashi et al.*7	2007	愛知・岐阜	123	21.1			8.1			67.5			3.3
		Hayashi et al.*16	2008	愛知・岐阜	139	22.3	9.6	90.4	8.6	75	25	64.7	5.6	93.3	4.2
		Kobayashi et al.*8	2008	東京，神奈川	126	28.6	9.5**	76.6**	10.3	74.5**	19.6**	59.5	0**	100**	1.6
		Yamada et al.*17	2008	東京，神奈川	146	46.5			11			41.1			1.4

**急性・慢性の合計

Ba の genotype B に占める割合は約 23% と算出され，Genotype A1/Aa の割合が献血者で高いことがわかる．

田中らの報告で割合の高い Genotype A1/Aa の分布は，関東，中京，関西地方の中核都市に加えて岡山/広島に限られており，Genotype A2/Ae が四国以外の全地域に広がってきているのとは対照的である．急性肝炎における Genotype A の症例の多くには不特定パートナーとの性交渉歴が認められることが既に指摘されているが，Genotype A1/Aa と A2/Ae の分布の違いが異なる原因については今後疫学調査を中心とした更なる検討が必要である．

田中らの報告では B2/Ba の症例の genotype B に占める割合は約 4 分の 1 であった．B2/Ba の症例の割合が高いのは A1/Aa 同様関東，中京，関西，そして九州の地方中核都市であった．B2/Ba は中国株であり，これらの都市に中国出身者が増加しつつある現状と関連があるものと推察される．同様の傾向は Kobayashi らによっても指摘されている．しかしながら，Kobayashi らは，B2/Ba の genotype B に占める割合は 1971 年から 1996 年までの間は変化がなかったとも報告しており，Subgenotype Ba も本邦にかなり古くから存在していたことが示唆される[8]．

HBV genotype の分布に関して現在まで報告されている主な成績を Subgenotype まで含めて Table 1 にまとめた．

年齢による HBV genotype 分布の違い

慢性肝疾患の HBV genotype 分布を年齢別に解析するためには多くの症例が必要である．Matsuura らは全国 14 都道府県から 1271 例の慢性肝疾患（genotype A 44 例，genotype B 179 例，genotype C 1046 例）における Genotype 分布を年齢別に解析している[11]．Genotype A の症例は 40 歳未満の症例，Genotype B の症例は 60 歳以上の症例，Genotype C の症例は 40 歳以上 60 歳未満の症例に多く認められる傾向があった．この傾向は献血者でも全く同様であった．また，Genotype A（Ae）に関しては感染例のほとんどは男性であり，不特定の同性，異性との性交渉が感染の原因であることと一致しているものと考えられた．

Matsuura らは病型分布も報告している．上述の通り Genotype C は Genotype C に比べて進展慢性肝疾患の合併率は低いものの，50 歳以上の症例を中心に肝硬変/肝細胞癌の症例が認められた．また，genotype A の症例でも 50 歳以上の症例に肝硬変/肝細胞癌の症例が認められた．Genotype C 以外でも高齢になると進展慢性肝疾患の合併があることを忘れてはいけない．

おわりに

本邦における HBV 感染の疫学は，居住民族及び性風俗の多様化に伴い，急速に変化してきている．HBV genotype の測定により，今後多くの点が明らかにされていくものと期待される．

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Distribution of hepatitis B viral genotypes in Japan

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Key words: acute hepatitis　chronic hepatitis　blood donor　HBV　genotype

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Original Article

Chronic hepatitis C in patients co-infected with human immunodeficiency virus in Japan: a retrospective multicenter analysis

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Aim: A nationwide survey in Japan revealed that nearly one-fifth of human immunodeficiency virus (HIV)-positive patients are co-infected with hepatitis C virus (HCV). We conducted a study to further analyze the features of liver disease in HIV–HCV co-infected patients.

Methods: We analyzed 297 patients from eight hospitals belonging to the HIV/AIDS Network of Japan.

Results: HCV genotypes 1, 2, 3, 4 and mixed genotypes were detected in 55.2, 13.7, 18.9, 0.9 and 11.3% of patients, respectively, in contrast to the fact that only genotypes 1 and 2 are detected in HCV mono-infected patients in Japan. This is compatible with the transmission of HCV through imported blood products contaminated by HCV. Sixteen of 297 HIV–HCV co-infected patients had advanced liver disease accompanied by ascites, hepatic encephalopathy or hepatocellular carcinoma. The average age of such patients was 41.1 ± 14.0 years, which was much younger than that of HCV mono-infected patients with the same complications. The progression speed of liver disease estimated from the changes in the levels of serum albumin, bilirubin, or platelet was slower in patients who achieved sustained virological response with interferon treatment than in those who did not receive it. The overall sustained virological response rate to interferon treatment was 43.3%.

Conclusions: Our findings suggest that liver disease is more advanced in HIV–HCV co-infected patients than in HCV mono-infected patients, and interferon treatment may retard the progression of liver disease in such patients.

Key words: acquired immunodeficiency syndrome, chronic liver disease, genotype, interferon therapy

INTRODUCTION

THE PROGNOSIS OF human immunodeficiency virus (HIV) infection has markedly improved since the introduction of hyperactive anti-retroviral therapy (HAART).[1,2] Opportunistic infection has been prevented or properly managed, resulting in lower mortality rates. Liver disease, in particular related to hepatitis C virus (HCV) infection, has now become the main cause of mortality among HIV-infected patients on HAART in Western countries.[3,4] A national survey among Japanese HIV-infected patients with coagulation disorders has shown that the mortality rate related to HCV-related liver disease after 1997 was twofold that before 1997.[5] In Japan, therefore, HCV infection may also be a major cause of death in HIV–HCV co-infected patients. However, there has been no extensive analysis of liver disease in HIV–HCV co-infected patients in Japan.

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Interferon (IFN) treatment in combination with ribavirin administration, which is now the first choice for HCV mono-infected patients,[6] is also a standard treatment for chronic hepatitis in HIV–HCV co-infected patients. Eradication of HCV is assumed to improve liver function, and normalization of serum aminotransferase (ALT) levels by IFN treatment may retard the progression of liver disease in HIV–HCV co-infected patients, even if they are on HAART. However, in general, the response rate to IFN treatment is lower in HIV–HCV co-infected patients than in HCV mono-infected patients.[7] The effects of IFN treatment on liver function and prognosis in HIV–HCV co-infected patients in Japan are yet undefined.

In 2004, we conducted a nationwide survey to determine the prevalence of HCV infection in HIV-infected patients by distributing a questionnaire to the hospitals in the HIV/AIDS Network of Japan, which revealed that 935 (19.2%) of 4877 HIV-positive patients were also positive for anti-HCV antibody.[8] In this study, we analyzed the progression of liver diseases and the impact of IFN treatment on the parameters of liver function in HIV–HCV co-infected patients in a multicenter retrospective study.

METHODS

Registry of patients with HIV–HCV co-infection

THE QUESTIONNAIRE REGARDING the current state of HIV–HCV co-infection was sent to the 366 hospitals in the HIV/AIDS Network of Japan in 2004, sponsored by the Japanese Ministry of Health, Labour and Welfare. One hundred seventy-six hospitals (48.1%) responded. The results, already published,[8] showed that HIV–HCV co-infected patients are concentrated in particular hospitals in big cities around Japan. Among these hospitals, we chose three hospitals in the Tokyo metropolitan area, and one each in the Hokkaido, Chubu, Osaka, Chugoku and Kyushu areas. These eight hospitals belong to the HIV/AIDS Network and had more HIV–HCV co-infected patients than other hospitals.

In the study, the following information was obtained from the hospitals regarding each HIV–HCV co-infected patient who visited the hospitals at least once between January and December in 2004: (1) age and sex of HIV-positive patients with anti-HCV; (2) possible transmission routes of HIV; (3) history of habitual alcohol intake; (4) date of the first and last visits; (5) counts of white blood cells, CD4-positive lymphocytes and platelets at the first and last visits; (6) levels of serum albumin and bilirubin at the first and last visits; (7) levels of HIV-RNA and HCV-RNA at the first and last visits; (8) history of IFN treatment with or without ribavirin; (9) history of HAART; and (10) history of jaundice, ascites, hepatic encephalopathy and hepatocellular carcinoma (HCC). The study sheets were completed by the physicians in charge and sent to the Department of Internal Medicine, University of Tokyo.

Ethical issues

The protocol of the current survey was approved by the ethical committee of each institution, and written informed consent was obtained from each patient.

Statistical analysis

The collected data were analyzed using Mann–Whitney's *U*-test whenever appropriate. *P*-values less than 0.05 were regarded as statistically significant.

RESULTS

Clinical backgrounds of registered patients

FROM THE EIGHT hospitals, 297 patients were registered. The number, age, sex, estimated transmission routes and history of habitual alcohol intake are shown in Table 1. Two hundred and ninety (97.6%) were male patients. The mean age of the patients was 37.9 ± 10.3.

HCV genotype was determined in 212 patients. One hundred seventeen (55.2%) patients were infected by genotype 1 HCV. Infection by genotypes 2, 3 or 4 HCV was found in 29 (13.7%), 40 (18.9%) and 2 (0.9%) patients, respectively. Twenty-four (11.3%) patients were infected by HCV of mixed genotypes. In the remaining 85 patients, the genotype was indeterminable or undetermined. The mean ages of patients infected by different HCV genotypes were similar (Table 1).

In 259 (87.2%) of 297 registered patients, HIV was most probably transmitted through the administration of blood products. Other transmission routes were sexual contacts among men who have sex with men (MSM) (4.0%), heterosexual contacts (3.0%) and intravenous drug use (IDU) (0.3%). Habitual alcohol consumption was noted in only one patient with genotype 1 HCV (0.6%).

Outcomes of IFN treatment in HIV–HCV co-infected patients

Serum HCV-RNA levels were available both at the first visit and registry to the study (i.e. the end of observa-

Table 1 Demography, transmission route and HCV genotypes in HIV–HCV co-infected patients

HCV genotype	Number (%)	HCV sub-genotypes	Viral load† (High : Low)	Age	Sex (Male : Female)	Transmission route				
						Transfusion	MSM	Hetero-sexual	IDU	Others
1	117 (55.2)	1a 31, 1b 43, 1a+1b 31, undetermined 12	31:11	38.3 ± 10.4	114:3	102	7	1	0	7
2	29 (13.7)	2a 16, 2b 11, undetermined 2	5:5	39.8 ± 9.5	29:0	24	1	1	0	3
3	40 (18.9)	3a 40	12:2	36.1 ± 8.9	40:0	38	0	0	0	2
4	2 (0.9)	4a 2	2:0	38.5 ± 2.1	2:0	2	0	0	0	0
Mixed	24 (11.3)	2a+3a 6, 1b+3a 3, others 15	11:0	38.7 ± 8.7	24:0	24	0	0	0	0
Others	85	Undetermined 85	6:1	36.2 ± 11.5	81:4	69	4	7	1	4
Total	297		67:19	37.9 ± 10.3	290:7	259 (87.2%)	12 (4.0%)	9 (3.0%)	1 (0.3%)	16 (5.5%)

†Viral loads are available in only a subset of patients. High viral load: more than 1 Meq/mL by branched DNA-probe assay or more than 100 KIU/mL by Amplicor monitor assay.
HCV, hepatitis C virus; HIV, human immunodeficiency virus; IDU, intravenous drug users; MSM, men who have sex with men.

tion) in 158 patients. Of these 158, 60 patients (38.0%) received IFN treatment for HCV, and 35 of these 60 patients did it in combination with ribavirin. Those who did not complete the scheduled treatment were excluded from the current analysis.

As shown in Table 2, 26 (43.3%), 11 (18.4%) and 23 (38.3%) of the treated patients achieved sustained virological response (SVR), end-of-treatment virological response (ETR) and no virological response (NR), respectively. The SVR rate in patients with each genotype is shown in Table 2. The SVR rate in the patients who underwent IFN treatment in combination with ribavirin was 31.4% in total. The SVR rate in patients with each genotype who underwent IFN/ribavirin combination therapy is shown in Table 2.

All of the 26 patients who achieved SVR remained negative for serum HCV-RNA in the further follow-up periods. In contrast, none of the patients with ETR or NR became negative for serum HCV-RNA in the follow-up periods. In five patients who did not receive IFN treatment, HCV-RNA was negative at the end of the observation period, although it was positive at least twice before the registry. The profiles of the five patients are shown in Table 3.

Changes in liver function and associated complications (Table 4)

As mentioned above, the data on liver function and serum HCV-RNA positivity were available both at the first visit and registry (end of observation) in 158 of the 297 registered patients. The mean observation period was 9.5 ± 5.0 and 8.2 ± 8.2 years in the IFN-treated and IFN-untreated patients, respectively. Unfortunately, few, if any, patients underwent liver biopsy, because most HIV–HCV co-infected patients had coagulation disorders.

The annual change in the serum albumin concentration was +0.05 ± 0.42 g/dL in the IFN-treated patients, and −0.80 ± 0.82 g/dL in the non-IFN-treated patients. The annual change in the serum bilirubin concentration was +0.08 ± 0.38 mg/dL in the IFN-treated patients, while it was +0.15 ± 0.15 mg/dL in the non-IFN-treated patients. Among the IFN-treated patients, the serum bilirubin concentration decreased by 0.02 ± 0.08 mg/dL in the patients who achieved SVR, which was significantly larger than that in the non-IFN-treated patients at the end of the observation ($P < 0.05$). The annual changes in platelet counts were +0.06 ± 1.13 ($\times 10^4/\mu l$) in the IFN-treated patients and −0.94 ± 0.95 ($\times 10^4/\mu l$) in the non-IFN-treated patients. The change in platelet

Table 2 Virological response to interferon treatment in HIV–HCV co-infected patients

Genotype	Viral load (High : Low)†	Response			Total
		SVR	ETR	NR	
(a) Response to interferon treatment in total (with or without ribavirin)					
1	9:6	7 (33.3%)	1	13	21
2	5:3	4 (40.0%)	2	4	10
3	5:1	5 (62.5%)	1	2	8
4	1:0	0	1	0	1
Mixed	5:1	2 (33.3%)	3	1	6
Others	6:2	8 (57.1%)	3	3	14
Total	31:13	26 (43.4%)	11	23	60
(b) Response to ribavirin/interferon combination therapy including peginterferon					
1	8:2	2 (15.3%)	0	11	13
2	1:2	1 (25.0%)	0	3	4
3	4:1	4 (66.7%)	1	1	6
4	1:0	0	1	0	1
Mixed	4:1	1 (20.0%)	3	1	5
Others	3:0	3 (50.0%)	1	2	6
Total	21:6	11 (31.4%)	6	18	35

†Viral loads are available in only a subset of patients. High viral load: more than 1 Meq/mL by Branched DNA-probe assay or more than 100 KIU/mL by Amplicor monitor assay.
ETR, end of treatment virological response; NR, no virological response; SVR, sustained virological response.

counts in the patients who achieved SVR was significantly larger than that in the non-IFN-treated patients ($P < 0.05$, Table 4).

No symptoms of hepatic failure (ascites or hepatic encephalopathy) were observed in the 60 IFN-treated patients while they were observed in six of the 98 non-IFN-treated patients. HCC was found in one IFN-treated patient after SVR, while it was found in two non-IFN-treated patients (Table 4).

Impact of HAART on liver function and associated complications (Table 5)

Information on HAART was available in 292 patients. The mean observation periods were 8.4 ± 4.2 years in 234 patients on HAART, and 9.8 ± 6.0 years in 58 patients not on HAART. Changes in the levels of albumin, bilirubin or platelet were similar between the two groups (statistically not significant). The morbidities of hepatic decompensation symptoms (ascites and hepatic encephalopathy) and HCC were not significantly different between the two groups. In total, nine patients had hepatic decompensation and seven had HCC, and the average age of such patients was 41.1 ± 14.0 years, which was much younger than that of HCV mono-infected patients with the same complications.[9]

DISCUSSION

IN THE CURRENT study, the features of liver disease in HIV–HCV co-infected patients in Japan were analyzed. The determination of HCV genotypes revealed that genotype 3 or 4, which is rarely seen in HCV mono-infected patients in Japan,[10] was found in a substantial fraction of HIV-infected patients. In addition, some of these patients were infected with HCV of mixed genotypes. These results are compatible with the fact that HCV is transmitted through imported blood products that were contaminated by HCV, as is the case with HIV infection.[11] Infection by HCV of mixed genotypes may reflect frequent administrations of blood products of different lots.

We evaluated the response rate to IFN treatment in HIV–HCV co-infected patients in Japan. Because the IFN treatment protocol varied between facilities, it was not easy to evaluate the effects of the treatments including IFN in this cohort. However, the regimen of ribavirin/IFN combination therapy was similar between the hospitals: the treatment period was 24 weeks in patients with HCV genotypes 2 and 3, and 48 weeks in those with HCV of other genotypes when either pegylated or standard IFN in combination with ribavirin was used.[12] Therefore, it may be possible to estimate the effect

Table 3 Clinical backgrounds of patients who spontaneously cleared HCV in HIV-infected patients

Patient no.	Age	Sex	Transmission route	Observation period (years)	HCV-RNA (KIU/mL)	HCV genotype	HIV-RNA ($\times 10^3$/mL)	WBC (/μL)	CD4+ T cells (/μL)	Platelets ($\times 10^4$/mL)	ALT (U/l)	HAART
1	33	M	Transfusion	8.8	290	ND	200 000	4500	5	26.3	21	Yes
2	31	M	MSM	2.3	Positive†	ND	13 000	5760	931	22.7	29	Yes
3	27	M	Transfusion	9.3	>850	3a	180 000	4000	51	10.1	84	Yes
4	53	M	Transfusion	4.5	Positive†	1a	20 000	4800	296	35.4	24	No
5	22	M	Transfusion	7.8	220	ND	990	5500	125	33.1	44	Yes

†Positive: HCV-RNA was positive by qualitative PCR, but was not quantitatively determined.
ALT, aminotransferase; HAART, highly active anti-retroviral therapy; HCV, hepatitis C virus; HIV, human immunodeficiency virus; MSM, men who have sex with men; ND, not determined; WBC, white blood cells.

Table 4 Changes in clinical parameters and IFN treatment in HIV–HCV co-infected patients

	Outcome of IFN treatment	Number	Observation period (years)	⊿Albumin†	⊿Bilirubin‡	⊿Platelet§	Ascites/ encephalopathy	HCC
IFN-treated patients		60	9.5 ± 5.0	0.05 ± 0.42	0.08 ± 0.38*	0.06 ± 1.13	0	1
	SVR	26	9.1 ± 4.4	0.13 ± 0.59	(−) 0.02 ± 0.08*	0.14 ± 0.76*	0	1
	ETR	11	14.6 ± 7.0	(−) 0.07 ± 0.14	0.51 ± 1.04	0.07 ± 1.50	0	0
	NR	23	7.4 ± 2.0	0.01 ± 0.30	0.09 ± 0.30	(−) 0.18 ± 0.32	0	0
Non-IFN-treated patients		98	8.2 ± 8.2	(−) 0.80 ± 0.82	0.15 ± 0.15	(−) 0.94 ± 0.95	6	2
All		158	8.7 ± 4.7	(−) 0.45 ± 2.93	0.13 ± 0.52	(−) 0.59 ± 3.78	6	3

*$P < 0.05$ versus patients without IFN treatment.
†⊿Albumin: changes in albumin concentration (g/dL)/observation period (years).
‡⊿Bilirubin: changes in bilirubin concentration (mg/dL)/observation period (years).
§⊿Platelet: changes in platelet count ($\times 10^4$/μL)/observation period (years).
ETR, end of treatment virological response; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IFN, interferon; NR, no virological response; SVR, sustained virological response.

Table 5 Changes in clinical parameters and HAART in HIV–HCV co-infected patients

	Number	Age	Sex (M : F)	Observation period (years)	⊿Albumin†	⊿Bilirubin‡	⊿Platelet§	IFN	Ascites/ encephalopathy	HCC
HAART (+)	234	37.8 ± 10.4	227:7	8.4 ± 4.2	(−) 0.002 ± 0.18	0.13 ± 0.53	(−) 0.40 ± 3.71	143 (61.1%)	6	5
HAART (−)	58	38.1 ± 10.5	58:0	9.8 ± 6.0	(−) 0.14 ± 0.18	0.03 ± 0.25	(−) 1.40 ± 3.30	30 (51.7%)	3	2

†⊿Albumin: changes in albumin concentration (g/dL)/observation period (years).
‡⊿Bilirubin: changes in bilirubin concentration (mg/dL)/observation period (years).
§⊿Platelet: changes in platelet count ($\times 10^4$/[L)/observation period (years).
HAART, highly active anti-retroviral therapy; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

of ribavirin/IFN combination therapy in HIV–HCV co-infected patients in this study.

The response rate to ribavirin/IFN combination therapy was 31.4% in total, and 15.3% in patients with HCV genotype 1, which are comparable rates to those achieved in previous studies on HIV–HCV co-infected patients in Western countries.[7] The low response rate in HIV–HCV co-infected patients compared with HCV mono-infected patients[12] may be attributed to several factors: impaired immune response, high HCV loads and viral quasi-species caused by frequent chances of transmission. Of these, high viral loads may be essential, because Table 2 shows that patients with genotype 1 HCV achieved SVR even by IFN monotherapy if their viral loads were low. In the era of IFN monotherapy, patients with favorable conditions were treated first of all: pretreatment viral loads in patients who received IFN monotherapy were lower than those who received PEG-IFN–ribavirin combination therapy. This may be the reason why the efficacy of PEG-IFN–ribavirin combination therapy was lower than that with IFN monotherapy in this study.

The serum bilirubin concentrations and platelet counts were improved in the patients who achieved SVR by IFN treatment. Although the response rate to IFN treatment is lower in HIV–HCV co-infected patients than in HCV mono-infected patients, the overall benefit of IFN treatment on liver function may be similarly expected in the patients who achieved SVR. HAART showed no impact on the liver function in HIV–HCV co-infected patients. Improvement of liver function can be expected only in IFN-treated patients, although there is a possibility that only patients with preserved liver function were able to receive IFN treatment. Given that liver disease is the major life-threatening factor in HIV-infected patients, IFN treatment should be considered in the early stage of HIV–HCV co-infection.

It should be noted that nine patients had hepatic decompensation and seven had HCC, and the average age of such patients was much younger than that of HCV mono-infected patients with the same complications.[9] This finding is compatible with reports from Western countries showing a faster progression of fibrosis[13] and earlier development of HCC.[14] A possibly interesting finding is that five patients (approximately 3% of patients whose serum HCV-RNA level was serially determined) cleared HCV-RNA from the serum without IFN treatment. Previous reports showed that some HIV-infected patients could spontaneously clear HCV-RNA.[15–17] The clearance of HCV among patients with chronic HCV infection is rare, although it has been

reported in Japan.[18] Three of the five patients had high HCV loads and low CD4$^+$ T-lymphocyte counts, which are generally thought to be unfavorable for spontaneous HCV clearance. A difference in immune status of HIV-infected patients from HCV mono-infected patients may be involved in such an observation, although further studies are awaited.

In summary, our study demonstrated that approximately 20% of HIV-infected patients are co-infected with HCV. Some of the HIV–HCV co-infected patients had advanced liver disease such as ascites, encephalopathy or HCC at a younger age than HCV mono-infected patients, suggesting that the progression of liver disease may be more rapid in HIV–HCV co-infected patients than in HCV-mono-infected ones. Treatments with regimens including IFN, which may improve liver function and decrease liver-related death, should be considered in HIV–HCV co-infected patients.

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Original Article

Short-term prolongation of pegylated interferon and ribavirin therapy for genotype 1b chronic hepatitis C patients with early viral response

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Aim: We tailored extended treatments using pegylated interferon (PEG IFN) and ribavirin (RBV) to viral responses after initiation of therapy and investigated the efficacy and safety of its therapy for chronic hepatitis C (CHC) patients.

Methods: Eighty-two genotype 1b CHC patients were enrolled in the present study. All patients received PEG IFN-α-2b and weight-based RBV therapy. We defined a viral response in which serum HCV-RNA is undetectable at week 4 as rapid viral response (RVR), detectable at week 4 and undetectable by week 12 as early viral response (EVR), and detectable at week 12 and undetectable by week 24 as late viral response (LVR). We set the treatment duration depending on viral response; 48 weeks for RVR patients and 72 weeks for LVR. Furthermore, EVR patients received a short-term extension of treatment duration to 52–60 weeks. We prospectively investigated sustained viral response (SVR) rates of these groups.

Results: Overall SVR rate for the total patient group was 57.3%. SVR rates of the RVR, EVR and LVR patients were 100%, 80.5% and 40.0%, respectively. Nine patients could not complete this treatment protocol. Baseline platelet count and mutation in the interferon sensitivity-determining region of NS5A were significant independent predictors of SVR, and amino acid substitution of the core region was a significant independent predictor of non-viral response by multivariate logistic regression analyses.

Conclusion: The results indicate that short-treatment extension of PEG IFN plus RBV treatment protocols in EVR patients can improve overall SVR rates.

Key words: chronic hepatitis C, extended treatment, pegylated interferon, ribavirin

INTRODUCTION

SUSTAINED VIRAL RESPONSE (SVR) rates have improved with the development of combined pegylated interferon (PEG IFN) plus ribavirin (RBV) therapy for patients with chronic hepatitis C (CHC). However, the SVR rate for genotype 1 CHC patients is around 50% when this treatment is used for 48 weeks, which is the current standard duration of treatment.[1,2] Various drugs are now undergoing clinical trials to improve the SVR rate.[3,4] However, these drugs will not be available for a few years. As a result, modification of current therapeutic protocols is being attempted worldwide.

The SVR rate varies depending on the viral response after initiating treatment and patients with late viral response display the lower therapeutic efficacy.[5]

Pearlman *et al.* reported that extended treatment from 48 to 72 weeks increases SVR rates among patients in whom serum HCV-RNA is detected at week 12 and becomes undetectable by week 24.[6] Additionally, Sanchez-Tapias *et al.* compared SVR rates at 48 weeks

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with those at 72 weeks of treatment in patients with serum HCV-RNA detected at week 4.[7] Their results suggested that extended treatment to 72 weeks is also useful for increasing the SVR rate in CHC. However, extended treatment for all patients in whom serum HCV-RNA is detected at week 4 is difficult, given the associated decrease in medication tolerability and the increased medical expense.

In the present study, serum HCV-RNA was measured every 4 weeks and a rapid viral response (RVR) was defined by a viral response in which serum HCV-RNA was undetectable at week 4, an early viral response (EVR) was defined by a viral response in which serum HCV-RNA was detectable at week 4 and undetectable by week 12, and a late viral response (LVR) was defined by a viral response in which serum HCV-RNA was detectable at week 12 and undetectable by week 24. We set the treatment duration depending on the viral response; 48 weeks for RVR patients and 72 weeks for LVR patients. Furthermore, EVR patients received a short-term extension of treatment duration to 52–60 weeks, as EVR patients with PEG IFN plus RBV therapy have made up about 50% of subjects in several Japanese trials.[8,9]

The aim of the present study was to prospectively investigate the efficacy and safety of extended treatment using PEG IFN and RBV depending on the week in which serum HCV-RNA became undetectable, and to clarify whether a short-term extension of treatment in EVR patients would increase overall SVR for Japanese genotype 1 CHC patients. In addition, we investigated refractory factors for extended treatment to clarify prediction of treatment efficacy.

METHODS

Patients

SUBJECTS COMPRISED OF 82 consecutive Japanese genotype 1b CHC patients treated with PEG IFN-α-2b and RBV between December 2004 and March 2006 (Table 1). All patients were positive for anti-HCV antibodies, genotype 1b and high viral load (> 100 KIU/mL) according to HCV-RNA level. We excluded patients with other causes of liver disease, co-infection with HIV or hepatitis B virus, evidence of decompensated liver disease, or a history of hepatocellular carcinoma.

Treatment

All patients received PEG IFN-α-2b (1.5 μg/kg/week) and weight-based RBV (600 mg for < 60 kg; 800 mg for > 60 kg and < 80 kg; 1000 mg for > 80 kg). Duration of treatment was prospectively determined by the time at which serum HCV-RNA became undetectable. The detailed treatment protocol is shown in Figure 1. Concerning the patient group with undetectable HCV-RNA at 12 weeks treatment, the initial study design, which was to assign those patients to two subgroups randomly with treatment duration of 56 weeks or 60 weeks, was later changed to assign them to a single treatment duration of 60 weeks. Patients with positive serum HCV-RNA at 24 weeks finished treatment without prolongation of PEG IFN plus RBV therapy. We defined this group as non-viral response (NR). Serum HCV-RNA was estimated every 4 weeks, defining response by the time at which serum HCV-RNA became undetectable, as follows: RVR, by 4 weeks; EVR by 8–12 weeks; and LVR, by 16–24 weeks. SVR rates were prospectively investigated in these groups.

Table 1 Characteristics of patients at baseline

Sex (male/female)	45/37
Age (years)	57.1 ± 10.6
Height (cm)	164.7 ± 9.3
Bodyweight (kg)	59.2 ± 9.7
Body mass index (kg/m^2)	22.3 ± 2.7
History of treatment with interferon (naïve/retreatment)	60/22
Laboratory data	
ALT (IU/L)	64.5 ± 30.2
GGT (IU/L)	30.8 ± 24
LDL cholesterol (mg/dL)	58.2 ± 13.0
Hyaluronic acid (ng/mL)	94.6 ± 116
Leukocytes (mm^3)	4350 ± 1090
Hemoglobin (g/dL)	14.1 ± 1.3
Platelets (10^4/mm^3)	14.6 ± 4.1
HCV RNA level (KIU/L)	2032 ± 1590
Histological findings	
Activity (1/2/3/ND)	18/16/2/46
Staging (1/2/3/4/ND)	12/14/9/1/46
Amino acid substitutions in HCV gene	
ISDR in the NS5A, mutant-type/non-mutant-type/ND	14/62/6
Aa 70 and/or Aa91 in the core region, Double-wild/Non-double-wild/ND	38/40/4

Continuous variables are mean ± standard deviation.
ALT, alanine aminotransferase; GGT, γ-glutamyl transpeptide; ISDR, interferon sensitivity-determining region; LDL, low density lipoprotein; ND, not determined.

Evaluation of serum HCV-RNA

The HCV-RNA level was measured quantitatively by polymerase chain reaction (PCR) before and during

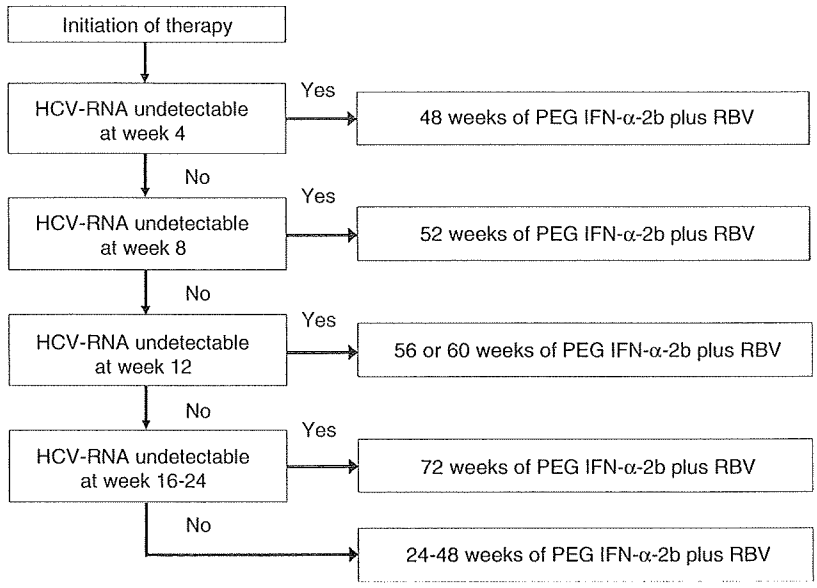

Figure 1 Treatment protocol. Serum hepatitis C virus ribonucleic acid (HCV-RNA) was measured every 4 weeks and treatment duration was set according to viral response. PEG IFN, pegylated interferon; RBV, ribavirin.

therapy (Cobas Amplicor HCV monitor v2.0 using the 10-fold dilution method; Roche Diagnostics, Branchburg, NJ, USA). The lower limit of the assay was 5×10^3 IU/mL. When HCV-RNA was quantitatively undetectable, HCV-RNA levels were also checked by qualitative PCR assay with a limit of 50 IU/mL. Qualitative PCR assay was used to decide the time at which serum HCV-RNA became undetectable.

Evaluation of amino acid substitutions in interferon sensitivity-determining region of NS5A and core region

Part of the amino acid sequence for NS5A in HCV genotype 1b, the interferon sensitivity-determining region (ISDR), reportedly correlates with the response to IFN therapy in Japanese patients.[10] Amino acid substitution patterns in the core region of HCV genotype 1b have also recently been correlated with the response to interferon therapy in Japanese patients.[11,12] We examined amino acid substitutions in the ISDR of NS5A and at aa71 and aa90 in the core region. Whether these factors are predictive of SVR or NR was analyzed. The present study split amino acid substitutions in the ISDR of NS5A into two patterns: mutant type (> 4 mutations) and non-mutant type (0–3 mutations), according to the number of amino acid substitutions. Wild-type pattern at both aa71 and aa90 was evaluated as double wild-type, whereas all other patterns were evaluated as non-double wild-type.

Statistical analysis

SVR was analyzed on an intention-to-treat basis. Uni- and multivariate logistic regression analyses were used to determine predictive factors for SVR and NR. We also calculated odds ratios and 95% confidence intervals (95% CI). Variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent factors. Potential predictive factors associated with SVR included the following variables: sex; age; body mass index; history of treatment with interferon; alanine aminotransferase; gamma-glutamyl transferase; low-density lipoprotein cholesterol; hyaluronic acid; leukocytes; hemoglobin; platelet count; HCV-RNA level; and amino acid substitutions at the ISDR of NS5A and aa71 and aa90 of the HCV core region. We evaluated predictive factors of SVR for patients with undetectable HCV-RNA by 24 weeks after initiation of therapy.

RESULTS

Tolerability of treatment

THE TREATMENT COURSE was completed in 73 of the 82 patients, with 43 patients completing treatment without requiring any reduction in drug dose. Five patients completed treatment with reduced dosages of