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Knowledge of *Vibrio vulnificus* infection among Japanese patients with liver diseases: A prospective multicenter study

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Summary

Vibrio vulnificus (*V. vulnificus*) is a seafood-borne infectious pathogen that can be lethal to humans. The infection has been correlated with pre-existing liver disease, particularly liver cirrhosis. Awareness of *V. vulnificus* infection among Japanese citizens is low, despite the increasing number of patients with hepatocellular carcinoma (HCC). The present study was conducted to assess the level of knowledge of patients with liver disease regarding *V. vulnificus* infection.

Questionnaires were sent to patients with chronic liver disease who had been treated by liver specialists at 14 medical institutes.

Of 1,336 patients, 304 (22.8%) had liver cirrhosis, and 732 (54.8%) had comorbidities of this disease. Only 14.5% (194/1,336) of patients had knowledge of *V. vulnificus* infection. Of 304 patients with liver cirrhosis, 17.4% (53/304) of the patients had knowledge of *V. vulnificus* infection. Of 60 patients with liver cirrhosis and diabetes mellitus, 11 (18.3%) patients had knowledge of *V. vulnificus* infections. Even when the 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.

Patients with chronic liver diseases and their physicians need to be better educated about *V. vulnificus* infection and its prevention.

key words: *Vibrio vulnificus* • liver diseases • hepatitis C virus (HCV) • hepatocellular carcinoma (HCC)

Abbreviations: *V. vulnificus* – *Vibrio vulnificus*; HCV – Hepatitis C virus; HBV – Hepatitis B virus;

HCC – Hepatocellular carcinoma; PBC – primary biliary cirrhosis; AIH – autoimmune hepatitis;

ICD – International Classification of Diseases

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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* septicemia cases in Japan has been estimated at 425 (95% CI 238–752) [15]. The prevalence of *V. vulnificus* septicemia 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* septicemia 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 \pm 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	Age			Sex				Liver diseases							P value
			Collection rate of questionnaire			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		
			(%)	Mean	SD											P value	
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 (1.0)	<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)	<0.000000000000001
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).



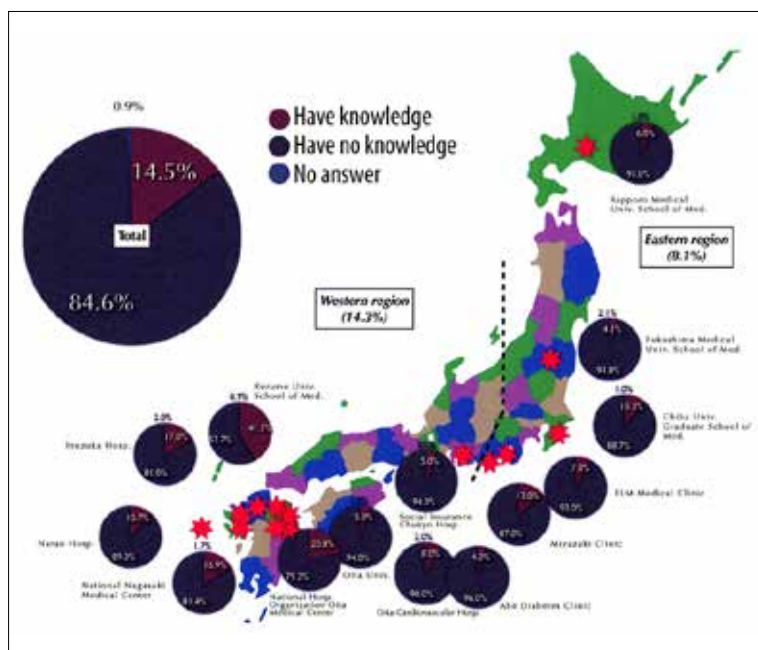


Figure 1. Knowledge of *V. vulnificus* infections among all patients with liver diseases. Only 14.5% of such patients had knowledge of this infection. Fourteen red stars indicate the location of each medical institution. Japan consists of 47 prefectures. Half of east of Japan, including Tokyo, where 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.

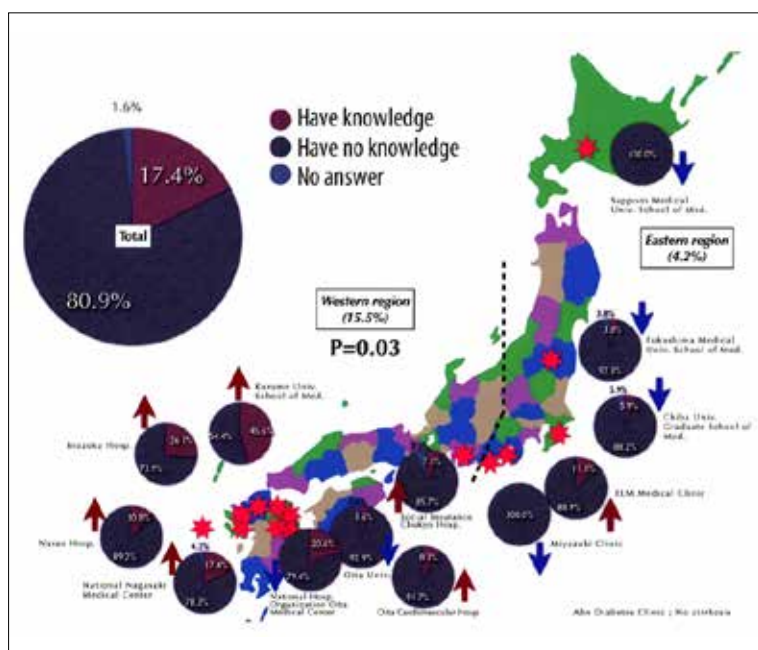


Figure 2. Knowledge of *V. vulnificus* infections in patients with liver cirrhosis. The rate of patient knowledge in the western region of Japan was significantly higher than in the eastern region. The upward pointing arrow indicates an increase in the rate of *V. vulnificus* infections in a given institution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

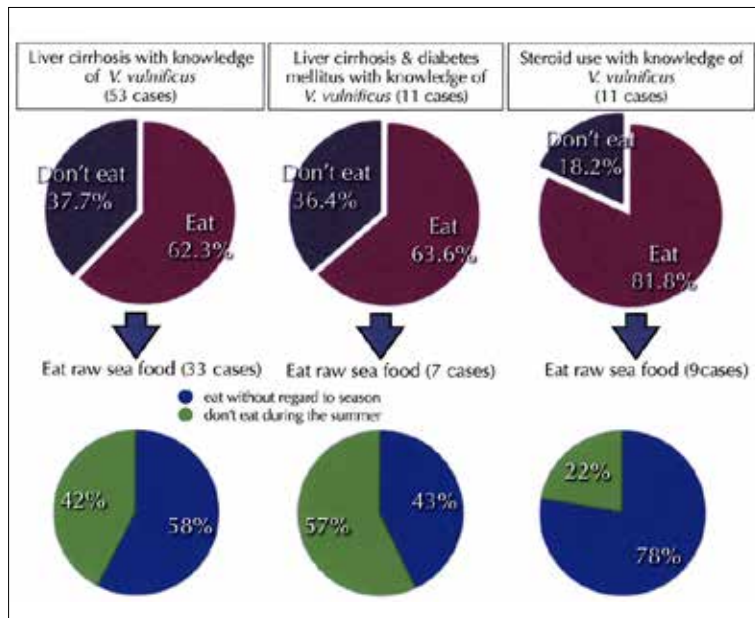


Figure 3. Frequency of eating raw seafood. Even if patients had high risk factors for infection, such as liver cirrhosis, diabetes mellitus, or steroid use, and had knowledge of *V. vulnificus* infections, most ate raw seafood without regard to season.

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. vulnificus* 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. vulnificus* [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. vulnificus* infections and education of patients with liver diseases should be required.

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