Characteristics of the 14 patients withdrawing the treatment

length	reasons	HCJ6	PSLRATCTTHGKAYDVDMVDANLFMGGDVTRIESES	Rapid virologic response	effect
3wks	economic	none	////////////////////////////////////	no	relapse
4wks	fatigue	none	////////////////////////////////////	yes	Sustained virologic response
5wks	economic	ah	----------T----------	no	No virologic response
9wks	rash	bu	----------D----------R----W-----	yes	Sustained virologic response
10wks	ALT elevate	G	S------A----T----LT----------I------	yes	Sustained virologic response
10wks	moving	ca	----------	yes	dropout
11wks	ALT elevate	ec	----------R----------	no	relapse
12wks	unknown	fv	----------	yes	dropout
13wks	fatigue	aq	----------M----S--S-------G-	yes	Sustained virologic response
15wks	ineffective	Y	----T-------T----------	no	No virologic response
20wks	unknown	cs	----T--A---------A----------I------	yes	dropout
27wks	depression	L	A----------V----------G-V------	yes	Sustained virologic response
28wks	moving	h	----------YC----------	yes	dropout
40wks	pneumonia	r	--------YG---H-M----------	yes	Sustained virologic response

Fig. 3. Clinical characteristics of the 14 patients who withdrew from pegylated-interferon-alpha 2a therapy. Reasons for discontinuing therapy, length of therapy, alignment of the amino acid sequence of the ISDR, rapid virologic response, and response to IFN therapy are shown. ISDR, interferon sensitivity-determining region.

and adjusted for genotype and IFN protocol. Adjustment for racial differences, diversity between the HCV strains with respect to genotype and ISDR sequence, and IFN regimen would be needed to use the ISDR as a simple diagnostic tool to predict sustained virologic response. Nevertheless, the present study had a few limitations. Only the correlation between mutations within the ISDR and sustained virologic response was analyzed, although other parts of NS5A have been reported to be associated with IFN response [Nousbaum et al., 2000; Murphy et al., 2002]. The approach of counting the number of mutations to the chosen consensus sequence in the ISDR, originally reported by Enomoto, was used for the present analysis; however, this method may not be the best way to measure sequence variation. Phylogenetic analyses of the ISDR were used to evaluate the diversity of the ISDR sequence, but distinctive clustering was not found in the wild types with A2205 and with T2205 and with V2205. The ISDR interacts with PKR and inactivates replication of HCV in vitro [Gale et al., 1998]. However, some reports have not confirmed the interaction between PKR and NS5A [Podevin et al., 2001; Tan and Katze, 2001]. PKR-independent effects of NS5A have been reported [Polyak et al., 2001; Evans et al., 2004]. Although the effect of amino acid substitutions of the ISDR was unclear, the ISDR system could be used clinically as a simple diagnostic tool to predict sustained virologic response in patients infected with genotype 2a who received pegylated-IFN-alpha 2a monotherapy.

The current recommended therapy for patients with HCV genotype 2 is a combination of pegylated-IFN and ribavirin for 24 weeks [Strader et al., 2004]. However, pegylated-IFN-alpha 2a monotherapy in patients with HCV genotype 2a resulted in a high sustained virologic response rate (77.3%). Most reports dealing with pegylated-IFN-alpha and ribavirin combination therapy did not differentiate between HCV genotypes 2 and 3 or did not classify subgenotypes 2a and 2b [Zeuzem et al., 2004; Mangia et al., 2005; von Wagner et al., 2005; Shiffman et al., 2007]. There is also limited information regarding sustained virologic response in patients with HCV genotype 2a treated with pegylated-IFN-alpha and ribavirin combination therapy. Thus, it is difficult to compare the present results to those obtained with pegylated-IFN-alpha and ribavirin combination therapy. Large, randomized, prospective studies of pegylated-IFN-alpha with or without ribavirin for patients with genotype 2a, especially ISDR mutant, are needed to clarify these issues. The present study combined two predictive factors: rapid virologic response and the amino acid variations in ISDR compared to the reference sequence. Rapid virologic response is considered to be a strong indicator of progression to sustained virologic response for patients with HCV genotype 2a. Knowledge of both the ISDR sequence and rapid virologic response would be useful for individualization of IFN regimens for chronic hepatitis C patients, but rapid virologic response cannot be assessed before treatment. In the present study, there were no predictive factors associated with rapid virologic response on multivariate analyses (data not shown). Thus, it is impossible to predict which patients will be rapid virologic responders before IFN therapy. With respect to assessment before starting treatment, the number of mutations in the ISDR is a better predictor than rapid virologic response.

In conclusion, the present results indicate that pegylated-IFN-alpha 2a monotherapy is effective for achieving sustained virologic response in Japanese patients with HCV genotype 2a, particularly in those with rapid virologic response and mutant-type ISDR.

The ISDR sequence variation of HCV genotype 2a is useful for predicting IFN responsiveness.

ACKNOWLEDGMENTS

The authors thank Ms. Miho Kawaguchi, Ms. Mika Torii, and Ms. Yukiyo Hasegawa for their assistance.

REFERENCES

Akuta N, Suzuki F, Suzuki Y, Sezaki H, Hosaka T, Someya T, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kobayashi M, Kumada H. 2005. Hepatocyte steatosis is an important predictor of response to interferon (IFN) monotherapy in Japanese patients infected with HCV genotype 2a: Virological features of IFN-resistant cases with hepatocyte steatosis. J Med Virol 75:550–558.

Chung RT, Monto A, Dienstag JL, Kaplan LM. Mutations in the NS5A region do not predict interferon-responsiveness in American patients infected with genotype 1b hepatitis C virus.1999. J Med Virol 58:353–358.

Enomoto N, Takada A, Nakao T, Date T. 1990. There are two major types of hepatitis C virus in Japan. Biochem Biophys Res Commun 170:1021–1025.

Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C. 1996. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 334:77–81.

Evans MJ, Rice CM, Goff SP. 2004. Phosphorylation of hepatitis C virus nonstructural protein 5A modulates its protein interactions and viral RNA replication. Proc Natl Acad Sci USA 101:13038–13043.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783–791.

Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL, Jr., Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. 2002. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 347:975–982.

Gale M, Jr., Blakely CM, Kwieciszewski B, Tan SL, Dossett M, Tang NM, Korth MJ, Polyak SJ, Gretch DR, Katze MG. 1998. Control of PKR protein kinase by hepatitis C virus nonstructural 5A protein: Molecular mechanisms of kinase regulation. Mol Cell Biol 18:5208–5218.

Hayashi K, Fukuda Y, Nakano I, Katano Y, Toyoda H, Yokozaki S, Hayakawa T, Morita K, Nishimura D, Kato K, Urano F, Takamatsu J. 2003. Prevalence and characterization of hepatitis C virus genotype 4 in Japanese hepatitis C carriers. Hepatol Res 25:409–414.

Herion D, Hoofnagle JH. 1997. The interferon sensitivity determining region: All hepatitis C virus isolates are not the same. Hepatolgy 25:769–770.

Kobayashi M, Watanabe K, Ishigami M, Murase K, Ito H, Ukai K, Yano M, Takagi K, Hattori M, Kakumu S, Yoshioka K. 2002. Amino acid substitutions in the nonstructural region 5A of hepatitis C virus genotypes 2a and 2b and its relation to viral load and response to interferon. Am J Gastroenterol 97:988–998.

Mangia A, Santoro R, Minerva N, Ricci GL, Carretta V, Persico M, Vinelli F, Scotto G, Bacca D, Annese M, Romano M, Zechini F, Sogari F, Spirito F, Andriulli A. 2005. Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. N Engl J Med 352:2609–2617.

Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. 2001. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: A randomised trial. Lancet 358:958–965.

Murakami T, Enomoto N, Kurosaki M, Izumi N, Marumo F, Sato C. 1999. Mutations in nonstructural protein 5A gene and response to interferon in hepatitis C virus genotype 2 infection. Hepatology 30:1045–1053.

Murphy MD, Rosen HR, Marousek GI, Chou S. 2002. Analysis of sequence configurations of the ISDR, PKR-binding domain, and V3 region as predictors of response to induction interferon-alpha and ribavirin therapy in chronic hepatitis C infection. Dig Dis Sci 47:1195–1205.

Nakano I, Fukuda Y, Katano Y, Nakano S, Kumada T, Hayakawa T. 1999. Why is the interferon sensitivity-determining region (ISDR) system useful in Japan? J Hepatol 30:1014–1022.

Nousbaum J, Polyak SJ, Ray SC, Sullivan DG, Larson AM, Carithers RL, Jr., Gretch DR. 2000. Prospective characterization of full-length hepatitis C virus NS5A quasispecies during induction and combination antiviral therapy. J Virol 74:9028–9038.

Otagiri H, Fukuda Y, Nakano I, Katano Y, Toyoda H, Yokozaki S, Hayashi K, Hayakawa T, Fukuda Y, Kinoshita M, Takamatsu J. 2002. Evaluation of a new assay for hepatitis C virus genotyping and viral load determination in patients with chronic hepatitis C. J Virol Methods 103:137–143.

Pascu M, Martus P, Hohne M, Wiedenmann B, Hopf U, Schreier E, Berg T. 2004. Sustained virological response in hepatitis C virus type 1b infected patients is predicted by the number of mutations within the NS5A-ISDR: A meta-analysis focused on geographical differences. Gut 53:1345–1351.

Podevin P, Sabile A, Gajardo R, Delhem N, Abadie A, Lozach PY, Beretta L, Bréchot C. 2001. Expression of hepatitis C virus NS5A natural mutants in a hepatocytic cell line inhibits the antiviral effect of interferon in a PKR-independent manner. Hepatology 33:1503–1511.

Polyak SJ, Khabar KS, Paschal DM, Ezelle HJ, Duverlie G, Barber GN, Levy DE, Mukaida N, Gretch DR. 2001. Hepatitis C virus nonstructural 5A protein induces interleukin-8, leading to partial inhibition of the interferon-induced antiviral response. J Virol 75:6095–6106.

Saitou N, Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425.

Saiz JC, Lopez LF, Ampurdanes S, Dopazo J, Forns X, Sanchez TJ, Rodes J. 1988. The prognostic relevance of the nonstructural 5A gene interferon sensitivity determining region is different in infections with genotype 1b and 3a isolates of hepatitis C virus. J Infect Dis 177:839–847.

Seeff LB. 2002. Natural history of chronic hepatitis C. Hepatology 36:S35–S46.

Shiffman ML, Suter F, Bacon BR, Nelson D, Harley H, Sola R, Shafran SD, Barange K, Lin A, Soman A, Zeuzem S, ACCELERATE Investigators. 2007. Peginterferon alfa-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 or 3. N Engl J Med 357:124–134.

Simmonds P, Bukh J, Combet C, Deleage G, Enomoto N, Feinstone S, Halfon P, Inchauspe G, Kuiken C, Maertens G, Mizokami M, Murphy DG, Okamoto H, Pawlotsky JM, Penin F, Sablon E, Shin-I T, Stuyver LJ, Thiel HJ, Viazov S, Weiner AJ, Widell A. 2005. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology 42:962–973.

Squadrito G, Raffa G, Restuccia T, Pollicino T, Brancatelli S, Raimondo G. 2002. Is investigation of hepatitis C virus NS5A gene heterogeneity a tool for predicting long-lasting response to interferon therapy in patients with HCV-1b chronic hepatitis? J Viral Hepat 9:360–369.

Strader DB, Wright T, Thomas DL, Seeff LB, American Association for the Study of Liver Diseases. 2004. Diagnosis, management, and treatment of hepatitis C. Hepatology 39:1147–1171.

Takeuchi T, Katsume A, Tanaka T, Abe A, Inoue K, Tsukiyama-Kohara K, Kawaguchi R, Tanaka S, Kohara M. 1999. Real-time detection system for quantification of hepatitis C virus genome. Gastroenterology 116:636–642.

Tan SL, Katze MG. 2001. How hepatitis C virus counteracts the interferon response: The jury is still out on NS5A. Virology 284:1–12.

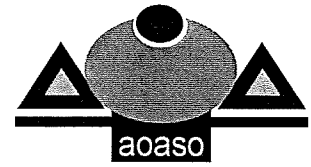
von Wagner M, Huber M, Berg T, Hinrichsen H, Rasenack J, Heintges T, Bergk A, Bernsmeier C, Haussinger D, Herrmann E, Zeuzem S. 2005. Peginterferon-alpha-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. Gastroenterology 129:522–527.

Zeuzem S, Lee JH, Roth WK. 1997. Mutations in the nonstructural 5A gene of European hepatitis C virus isolates and response to interferon alfa. Hepatology 25:740–744.

Zeuzem S, Hultcrantz R, Bourliere M, Goeser T, Marcellin P, Sanchez-Tapias J, Sarrazin C, Harvey J, Brass C, Albrecht J. 2004. Peginterferon alfa-2b plus ribavirin for treatment of chronic hepatitis C in previously untreated patients infected with HCV genotypes 2 or 3. J Hepatol 40:993–999.

Obesity Research & Clinical Practice (2009) 3, 29—34

ELSEVIER



http://www.iotf.org/oonet/aoaso.htm

ORIGINAL ARTICLE

Heterozygosity for leptin receptor (fa) accelerates hepatic triglyceride accumulation without hyperphagia in Zucker rats

Katsuro Himeno, Masataka Seike, Satoshi Fukuchi, Takayuki Masaki, Tetsuya Kakuma, Toshiie Sakata, Hironobu Yoshimatsu*

Department of Internal Medicine I, Faculty of Medicine, Oita University, Idaigaoka, Yuhu, Oita 879-5593, Japan

Received 11 January 2008; received in revised form 17 September 2008; accepted 14 October 2008

KEYWORDS
Leptin receptor;
Zucker fatty rat;
Fatty liver;
Fatty acid

Summary Leptin, *ob* gene product, and its receptors are involved in the regulation of peripheral lipid and glucose metabolism. The present study sought to clarify the functional role of peripheral leptin receptors in hepatic lipid metabolism through analysis of Zucker rats (fa/fa, +/fa), as complete or partial leptin receptor insufficiency models, respectively. In Zucker fa/fa rats, calorie intake, body weight, liver weight, hepatic triglyceride content and serum insulin, triglycerides, FFA, and leptin were elevated compared to lean littermates (+/+ rats). In contrast, Zucker +/fa rats showed no remarkable changes in calorie intake, body weight and serum FFA compared with +/+ rats. Nevertheless, hepatic triglyceride content, liver weight and other serum parameters such as insulin, triglyceride and leptin were higher than in +/+ rats. In the representation of fatty acids component in the liver, there were no changes in +/fa rats relative to +/+ rats. Thus, in Zucker +/fa rats, fatty liver may develop in the absence of hyperphagia, obesity or changes in hepatic fatty acid metabolism. These results indicate that partial insufficiency of leptin receptor rather than changes in serum insulin, triglyceride and leptin may contribute to the increase in hepatic triglyceride content observed in +/fa rats.
© 2008 Asian Oceanian Association for the Study of Obesity. Published by Elsevier Ltd. All rights reserved.

Introduction

Reflecting its wide range of physiological functions, receptors for leptin are distributed widely throughout the body. Notably, leptin receptors have been shown to be expressed at relatively high levels in the hypothalamus, the choroid plexus, the lung and

* Corresponding author. Tel.: +81 97 586 5790; fax: +81 97 549 4480.
E-mail address: omu_jp@yahoo.co.jp (H. Yoshimatsu).

1871-403X/$ — see front matter © 2008 Asian Oceanian Association for the Study of Obesity. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.orcp.2008.10.003

the kidney, and at lower levels in the liver, adipose tissue, skeletal muscle and the pancreas [1]. The abundance of leptin receptors in the brain and the dramatic effects of centrally administered leptin on feeding behavior and body weight indicate that the hypothalamus is the major target of leptin [2–4]. On the other hand, *in vivo* and *in vitro* studies have demonstrated functional significance of peripheral adipocytokines action including leptin in the regulation of metabolic disorder [5–7]. Especially in the liver, leptin has been shown to inhibit intracellular lipid concentration by reducing synthesis of triglyceride and concomitantly increasing β-oxidation of fatty acids [7]. This indicates that leptin receptor insufficiency induced by genetic defect or acquired leptin resistance, even at the peripheral level, may induce abnormal peripheral lipid metabolism, such as hyperlipidemia and fatty liver, independent of a distortion of central leptin action, which would lead to hyperphagia.

Zucker fatty (fa/fa) rats, which are leptin receptor defective animals, are useful for analyzing various metabolic disorders induced by disruption of leptin signaling [8]. In fact, hypertriglyceridemia, hyperinsulinemia, hyperleptinemia and severe fatty liver have been observed in this obese animal. In Zucker fa/fa rats, however, it is difficult to distinguish the relative contributions of peripheral and central leptin signaling because disruption of hypothalamic leptin signaling induces hyperphagia, which in turn affects peripheral lipid metabolism. Furthermore, inactivation of efferent sympathetic outflow in Zucker fa/fa rats [9], which is normally activated by centrally mediated leptin action [9,10], may also affect peripheral lipid metabolism as well as energy expenditure, as regulated by uncoupling proteins (UCP) [11,12].

On the other hand, Zucker +/fa rats, lean heterozygotes with partial leptin receptor defects, are non-obese and non-hyperphagic, indicating partial leptin receptor insufficiency is not sufficient to cause the fa/fa phenotype. There have been very few studies that address whether this partial leptin signaling insufficiency affects other metabolic parameters. It has been reported that adult mice heterozygous for the ob or db allele display increased body fat compared with their lean +/+ littermates [13]. Thus, these previous findings indicate that even partial insufficiency of leptin signaling can affect peripheral lipid metabolism. Based on this background, we used Zucker +/fa rats to clarify whether or not hepatic lipid metabolism is affected in these animals, and whether this was due to either disruption of peripheral leptin signaling, hyperphagia, or a metabolic disorder.

Materials and methods

Animals and diet

The animals used in this study were male Zucker lean (+/+ and +/fa) and obese (fa/fa) rats. Homozygous lean rats (+/+) were obtained from mating of homozygous (+/+) parents. Lean heterozygotes (+/fa) and obese (fa/fa) rats were produced from crosses of lean heterozygous (+/fa) females and obese (fa/fa) males that received adrenalectomy at 6 weeks of age. After weaning at age 4 weeks, they were allowed free access to standard solid rodent food (CE-2, CLEA Japan) and tap water. Animals were housed in a room-illuminated daily from 0700 to 1900 (a 12:12-h light–dark cycle) and maintained at 21 ± 1 °C with humidity at 55 ± 5%. All studies were conducted in accordance with Oita Medical University Guidelines based on the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Measurement and sampling

Body weight and total calorie intake, calculated by measuring consumption of the rodent food, were recorded weekly. At 18 weeks, they were sacrificed by decapitation. For each rat, after a blood sample was collected, the liver and left epididymal fat were surgically removed and weighed, and a liver tissue sample was taken. Expressions of SREBP-1 and ACC in the liver were investigated by real-time PCR methods. The detailed methods of RNA extraction and PCR were described in previous our studies [14,15].

Assay of serum parameters

All blood samples were immediately centrifuged at 1500 rpm for 15 min at 5 °C and the plasma was stored at −20 °C until each assay. Serum concentrations of glucose, triglyceride and FFA were determined by automatic analyzer using an enzymatic method (SRL, Tokyo, Japan). Serum concentrations of insulin, leptin and adiponectin were assayed with an insulin radioimmunoassay kit (Amersham Pharmatica Bioteck, Little Chalfont, UK), a murine leptin enzyme immunoassay kit (Immune Biological Laboratory, Gunma, Japan) and a murine adiponectin enzyme immunoassay kit (Otsuka pharm, Tokushima, Japan).

Table 1 Calorie intake, body weight, and liver weight in Zucker rats.

	+/+	+/fa	fa/fa
Calorie intake (kcal/day)	75.7 ± 1.8	77.4 ± 2.1	110.1 ± 3.1**
Body weight (g)	450.0 ± 10.8	465.0 ± 10.9	663.7 ± 11.8**
Epididymal fat weight (g)	1.2 ± 0.3	1.4 ± 0.4	10.5 ± 0.9**
Liver weight (g)	13.8 ± 0.3	15.4 ± 0.6*	24.3 ± 0.7**

Values are means ± S.E. for six animals in each group.
* Significant difference from +/+ with $p < 0.05$.
** Significant difference from +/+ with $p < 0.001$.

Assay for triglyceride content and fatty acid composition of liver

All the liver tissue samples were immediately frozen in liquid nitrogen and stored at −80 °C until assayed for triglyceride content and fatty acid composition. About 50 mg of each frozen tissue specimen was homogenized in 4.5 ml of phosphate-buffered saline (PBS). Chloroform methanol mixture (2:1 vol/vol) and H_2O were added to the homogenate solutions, which were then centrifuged at 3000 rpm for 5 min. Following centrifugation, the lower chloroform layers were collected, and triglyceride concentrations were assayed by the GPO *p*-chlorophenol method (triglyceride *G*-test, WAKO, Osaka, Japan). From this data, the triglyceride contents of the liver samples were calculated.

In order to assay the fatty acid composition of the samples, the lipid extracts were subjected to methanolysis in 1.37 M HCl in methanol at 100 °C for 2 h, and were evaporated under a stream of nitrogen. Following evaporation, fatty acid methyl esters were extracted with petroleum ether and analyzed by gas chromatography (Shimadzu GC-17A, Shimadzu, Kyoto, Japan) with a 70% sianoplopyl polisilphenirene-ciroxan capillary column (0.25 mm × 25 m; BPX-70, SGE, Ringwood, Austria). Helium was used as the carrier gas and the oven temperature was programmed to hold at 100 °C for the first 2 min, and then to increase from 100 °C to 240 °C at a rate of 5 °C/min and to hold for a final 5 min. The identification and quantification of each fatty acid were made with authentic standard mixture (Funakoshi, Tokyo, Japan) using a Class 5000 (Shimadzu, Kyoto, Japan).

Statistical methods. Results are expressed as mean ± S.E.M. Two-way and one-way ANOVA followed by *post hoc* test were used to determine group difference, with $p < 0.05$ considered to be significant.

Results

Changes in calorie intake, body weight and liver weight. The calorie intake, body and liver weight were significantly increased in Zucker fa/fa rats compared with +/+ rats ($p < 0.001$) (Table 1). There was no difference observed between +/+ and +/fa rats in calorie intake, epididymal fat weight and body weight, but +/fa rats displayed considerably increased liver weights compared with +/+ rats (Table 1).

Changes in serum and hepatic parameters In Zucker fa/fa rats, serum insulin, triglyceride, FFA, and leptin levels were markedly increased in comparison with +/+ rats ($p < 0.001$ for each except FFA, $p < 0.01$ for FFA). With the exception of FFA, the levels of all of these parameters were slightly but significantly elevated in +/fa rats in comparison with +/+ rats ($p < 0.01$ for triglyceride and

Table 2 Serum parameters in Zucker rats.

	+/+	+/fa	fa/fa
Glucose (mg/dl)	107.8 ± 2.1	115.3 ± 2.9	107.3 ± 4.2
Insulin (ng/ml)	1.2 ± 0.1	2.1 ± 0.3*	29.2 ± 3.5***
Triglyceride (mg/dl)	57.0 ± 5.9	87.5 ± 4.5**	388.0 ± 26.5***
FFA (mequiv./l)	0.36 ± 0.03	0.37 ± 0.07	0.56 ± 0.04**
Leptin (ng/ml)	30.7 ± 5.3	57.9 ± 5.8**	859.0 ± 88.4***

Values are means ± S.E. for six animals in each group.
* Significant difference from +/+ with $p < 0.05$.
** significant difference from +/+ with $p < 0.01$.
*** significant difference from +/+ with $p < 0.001$.

Table 3 Hepatic triglyceride content in Zucker rats.

	Hepatic triglyceride content (mg/g liver)
+/+	11.0 ± 1.3
+/fa	19.5 ± 0.8*
fa/fa	41.0 ± 1.7**

Values are means ± S.E. for six animals in each group.
* Significant difference from +/+ with $p < 0.001$.
** significant difference from +/+ with $p < 0.0001$.

leptin, $p < 0.05$ for insulin). Serum glucose levels were not significantly different between the three groups (Table 2). The level of adiponectin was not significantly changed in +/fa rats in comparison with +/+ rats (+/fa 11.1+/−0.3 μg/ml vs. +/fa 10.9+/−0.5 μg/ml; $p > 0.1$). Hepatic SREBP-1 and ACC expressions were both tended to increase but not significantly increased in +/fa rats compared with +/+ rats (SREBP-1: +/+ 100+/−18%; +/fa 125+/−27%, $p > 0.1$; ACC: +/+ 100+/−12%; +/fa 112+/−19%, $p > 0.1$).

Changes in hepatic triglyceride content. Hepatic triglyceride content was 3.7 times higher in Zucker fa/fa rats than in +/+ rats. Interestingly, Zucker +/fa rats displayed hepatic triglyceride levels, which were 1.8 times higher than in +/+ rats (Table 3). In addition, the positive correlation was observed between the levels of circulating and hepatic triglyceride ($r = 0.78$: $p < 0.01$).

Changes in fatty acid composition in the liver. Fig. 1 shows the individual fatty acid composition of fa/fa, +/fa and +/+ liver samples. The major constituents of liver fatty acid were found to be the saturated fatty acids (SFA) palmitate (16:0) and stealinic acid (18:0), the mono-unsaturated fatty acids (MUFA) palmitoleic acid (16:1) and oleic acid (18:1), and the poly unsaturated fatty acids (PUFA) linoleic acid (18:2) and arachidonic acid (20:4). Of the SFAs, the 16:0 fatty acid increased and the 18:0 fatty acid was found to be decreased in fa/fa rats compared with +/+ rats ($p < 0.001$). Of the MUFAs, both the 16:1 and 18:1 fatty acids were present at higher levels in fa/fa rat livers compared with livers from +/+ rats ($p < 0.001$). Of the major PUFAs, the 18:2 and 20:4 fatty acids were found at lower levels in the livers of fa/fa rats compared with those of +/+ rats ($p < 0.001$). In contrast, no remarkable differences in fatty acid composition were detected between +/fa and +/+ rats, except for a slight decrease in 20:4 fatty acids.

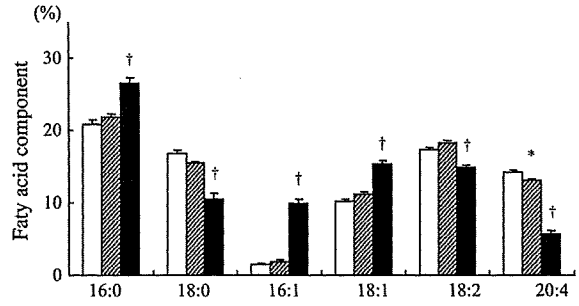


Figure 1 Major fatty acid representation in total liver lipid from 18-week-old +/+ (open bars), +/fa (hatched bars), and fa/fa (filled bars) Zucker rats. Values are means ± S.E. for six animals in each group. *Significant difference from +/+ with $p < 0.05$; †significant difference from +/+ with $p < 0.001$.

Discussion

In this study, we demonstrated that liver triglyceride content was increased, but to different degrees, in Zucker fa/fa rats and +/fa rats, models of complete and partial leptin receptor status, respectively. The most striking difference between these animals was in regard to food intake and body weight, as the fa/fa rats were hyperphagic and obese, while the +/fa rats were normophagic and non-obese. This indicates that the pathogenesis of the increased hepatic triglyceride content in +/fa rats may be different from that of fa/fa rats. At the very least, hyperphagia or obesity per se does not contribute to the development of fatty liver in +/fa rats [16].

Among the serum parameters commonly observed to be elevated in these animal models, hyperleptinemia is not likely to be an inducer of fatty liver, because leptin has been shown to inhibit synthesis of triglyceride in the liver [7]. The positive correlation was observed between the levels of circulating and hepatic triglyceride in the present study. It is reasonable to consider that hypertriglyceridemia is not a cause of fatty liver, but instead results from increased synthesis of hepatic triglyceride and its following secretion from the liver into the circulation. On the other hand, it is highly probable that hyperinsulinemia increases hepatic triglyceride content, since insulin has been shown to accelerate the synthesis of fatty acids and triglycerides through upregulation of sterol regulatory element-binding protein 1c (SREBP1c), a transcriptional factor for fatty acid and triglyceride synthetic enzymes [17,18]. In fact, marked hyperinsulinemia in fa/fa and mild hyperinsulinemia in +/fa rats were observed in present study. Taken together, it is suggested that insufficiency of leptin action due to partial leptin receptor abnormality in Zucker +/fa rats may enhance the development of insulin resistance through abnormal glucose and/or lipid metabolism

in the liver. This would lead to the eventual mild hyperinsulinemia observed in this animal.

Next, we analyzed liver fatty acid composition profiles of the rats since metabolism of hepatic fatty acid was easily affected by dietary factors or insulin action [17–19]. The results revealed changes in lipid profile specific to fa/fa rats, i.e. an increase in monounsaturated fatty acids such as palmitoleic acid and oleic acid, and a concomitant decrease in polyunsaturated fatty acids such as linoleic acid and arachidonic acid. In contrast to fa/fa rats, hepatic fatty acid composition in +/fa rats was not significantly different than that of +/+ rats. These results indicate that neither dietary factors nor mild hyperinsulinemia affect fatty acid metabolism in the liver of +/fa rats. Taken together, partial leptin receptor insufficiency per se rather than changes in several serum parameters including hyperinsulinemia is most likely to induce the increase in hepatic triglyceride content observed in +/fa rats. Of course, complete leptin receptor insufficiency in fa/fa rats must promote the development of fatty liver in conjunction with the influence of hyperphagia, obesity and hyperinsulinemia. In fact, more sever fatty liver was observed in fa/fa rats compared with +/fa rats in this study.

Finally, we must discuss how leptin receptor insufficiency affects lipid metabolism in the liver. Leptin has been shown to upregulate acyl CoA oxidase and carnitine phosphotransferase 1, the enzymes for peroxisomal and mitochondrial β-oxidation, respectively, and to downregulate acyl CoA carboxylase (ACC) and glycerol phosphate acyltransferase, lipogenetic enzymes, in the pancreatic islets [20]. In the liver, leptin has been shown to decrease the expression of SREBP1c mRNA, leading to a concomitant decrease in the levels of its downstream enzymes, such as fatty acid synthase and ACC [21]. These findings indicate that leptin and its peripheral receptors play a role in the prevention of triglyceride accumulation by activation of fatty acid β-oxidation and reduction of triglyceride formation. From this viewpoint, it may be concluded that disruption of leptin action due to leptin receptor insufficiency, even if partial, may promote triglyceride accumulation in the liver. However, in the present study, hepatic SREBP-1 and ACC expression were not significantly increased in +/fa rats compared with +/+ rats. Various kinds of TG synthetic enzymes than SREBP-1 and ACC might play roles in regulating hepatic fat metabolism in +/fa rats. Further studies are needed to be clarified that point.

In summary, this study demonstrates that even partial leptin receptor insufficiency promotes the development of fatty liver and mild insulin resistance independently of hyperphagia and its resultant metabolic disorder. This indicates that low-level insufficiency of leptin action induced by genetic defect or acquired leptin resistance at central as well as peripheral receptors may induce a distortion of peripheral energy metabolism without an overt influence on food intake.

References

[1] Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, et al. Identification and expression cloning of a leptin receptor OB-R. Cell 1995;83:1263–71.
[2] Ahima RS, Qi Y, Singhal NS, Jackson MB, Scherer PE. Brain adipocytokine action and metabolic regulation. Diabetes 2006;55:S145–54.
[3] Kahn BB, Alquier T, Carling D, Hardie DG. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab 2005;1:15–25.
[4] Schwartz MW, Woods SC, Porte Jr D, Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature 2000;404:661–71.
[5] Siegrist-Kaiser CA, Pauli V, Juge-Aubry CE, Boss O, Pernin A, Chin WW, et al. Direct effect of leptin on brown and white adipose tissue. J Clin Invest 1997;100:2858–64.
[6] Masaki T, Chiba S, Tatsukawa H, Yasuda T, Noguchi H, Seike M, et al. Adiponectin protects LPS-induced liver injury through modulation of TNF-alpha in KK-Ay obese mice. Hepatology 2004;40:177–84.
[7] Shimabukuro M, Koyama K, Chen G, Wang MY, Trieu F, Lee Y. Direct antidiabetic effect of leptin through triglyceride depletion of tissues. Proc Natl Acad Sci USA 1997;94:4637–41.
[8] Chua Jr SC, Chung WK, Wu-Peng XS, Zhang Y, Liu SM, Tartaglia L, et al. Phenotypes of mouse diabetes and rat fatty due to mutation in the OB(leptin) receptor. Science 1996;271:994–6.
[9] Shiraishi T, Sasaki K, Niijima A, Oomura Y. Leptin effects on feeding-related hypothalamic and peripheral neuronal activities in normal and obese rats. Nutrition 1999;15:576–9.
[10] Dunbar JC, Hu Y, Lu H. Intracerebroventricular leptin increases lumbar and renal sympathetic nerve activity and blood pressure in normal rats. Diabetes 1997;46:2040–3.
[11] Costford S, Gowing A, Harper ME. Mitochondrial uncoupling as a target in the treatment of obesity. Curr Opin Clin Nutr Metab Care 2007;10:671–8.
[12] Masaki T, Yoshimatsu H. The hypothalamic H1 receptor: a novel therapeutic target for disrupting diurnal feeding rhythm and obesity. Trends Pharmacol Sci 2006;27:279–84.
[13] Chung WK, Belfi K, Chua M, Wiley J, Mackintosh R, Nicolson M, et al. Heterozygosity for Lep(ob) or Lepr(db) affects body composition and leptin homeostasis in adult mice. Am J Physiol 1998;274:R985–90.
[14] Masaki T, Yoshimatsu H, Kakuma T, Hidaka S, Kurokawa M, Sakata T. Enhanced expression of uncoupling protein 2 gene in rat white adipose tissue and skeletal muscle following chronic treatment with thyroid hormone. FEBS Lett 1997;418:323–6.
[15] Masaki T, Chiba S, Tatsukawa H, Noguchi H, Kakuma T, Endo M, et al. The role of histamine H1 receptor and H2 receptor in LPS-induced liver injury. FASEB J 2005;19:1245–52.

[16] Durham HA, Truett GE. Development of insulin resistance and hyperphagia in Zucker fatty rats. Am J Physiol Regul Integr Comp Physiol 2006;290:R652–658.
[17] Shimomura I, Bashmakov Y, Ikemoto S, Horton JD, Brown MS, Goldstein JL. Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. Proc Natl Acad Sci USA 1999;96:13656–61.
[18] Foretz M, Guichard C, Ferre P, Foufelle F. Sterol regulatory element biding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. Proc Natl Acad Sci USA 1999;96:12737–42.
[19] Shimano H, Yahagi N, Amemiya-Kudo M, Hasty AH, Osuga J, Tamura Y, et al. Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. J Biol Chem 1999;274:35832–9.
[20] Zhou YT, Shimabukuro M, Koyama K, Lee Y, Wang MY, Trieu F, et al. Induction by leptin of uncoupling protein-2 and enzymes of fatty acid oxidation. Proc Natl Acad Sci USA 1997;94:6386–90.
[21] Kakuma T, Lee Y, Higa M, Wang Z, Pan W, Shimomura I, et al. Leptin, troglitazone, and the expression of sterol regulatory element binding proteins in liver and pancreatic islets. Proc Natl Acad Sci USA 2000;97:8536–41.

Available online at www.sciencedirect.com
ScienceDirect

MEDICAL SCIENCE
M * O * N * I * T * O * R
International Medical Journal for Experimental and Clinical Research

Indexed in: Current Contents/Clinical Medicine • SCI Expanded • ISI Alerting System • ISI Journals Master List
Index Medicus/MEDLINE • EMBASE/Excerpta Medica • Chemical Abstracts/CAS • Index Copernicus

IF=1.514

Volume 15 • Number 10 WWW.MEDSCIMONIT.COM October 2009

REPRINT

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

Yumiko Nagao, Hisako Matsuoka, Masataka Seike, Kazumi Yamasaki, Junji Kato, Takeyuki Nakajima, Yutaka Miyazaki, Tomoyoshi Ohno, Sadataka Inuzuka, Hiromasa Ohira, Osamu Yokosuka, Hiroshi Yatsuhashi, Tetsu Mori, Koichi Honda, Takumi Kawaguchi, Tatsuya Ide, Michio Sata

Med Sci Monit, 2009; 15(10): PH115-120

International Medical Association for Experimental and Clinical Research

ISSN 1234-1010

© Med Sci Monit, 2009; 15(10): PH115-120
PMID: 19789520

www.MEDSCIMONIT.COM
Public Health

Received: 2009.05.29
Accepted: 2009.07.06
Published: 2009.10.01

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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

Yumiko Nagao[1ABCDEF], Hisako Matsuoka[1B], Masataka Seike[2,3B], Kazumi Yamasaki[4B], Junji Kato[5B], Takeyuki Nakajima[6B], Yutaka Miyazaki[7B], Tomoyoshi Ohno[8B], Sadataka Inuzuka[9B], Hiromasa Ohira[10B], Osamu Yokosuka[11B], Hiroshi Yatsuhashi[12B], Tetsu Mori[13B], Koichi Honda[14B], Takumi Kawaguchi[1B], Tatsuya Ide[1,15B], Michio Sata[1,15ABDEG]

[1] Department of Digestive Disease Information & Research, Kurume University School of Medicine, Kurume, Fukuoka, Japan
[2] Department of Internal Medicine 1, Faculty of Medicine, Oita University, Yufu, Oita, Japan
[3] Abe Diabetes Clinic, Oita, Japan
[4] Narao Hospital, Nagasaki, Japan
[5] 4th Department of Internal Medicine, Sapporo Medical University School of Medicine, Sapporo, Hokkaido, Japan
[6] ELM Medical Clinic, Hamamatsu, Shizuoka, Japan
[7] Miyazaki Clinic, Fuji, Shizuoka, Japan
[8] Department of Gastroenterology, Social Insurance Chukyo Hospital, Nagoya, Aichi, Japan
[9] Inuzuka Hospital, Kashima, Saga, Japan
[10] Department of Gastroenterology and Rheumatology, Fukushima Medical University School of Medicine, Fukushima, Japan
[11] Department of Medicine and Clinical Oncology, Chiba University Graduate School of Medicine, Chiba, Japan
[12] Clinical Research Center, National Nagasaki Medical Center, Nagasaki, Japan
[13] Department of Medicine, Oita Cardiovascular Hospital, Oita, Japan
[14] Department of Gastroenterology, National Hospital Organization Oita Medical Center, Oita, Japan
[15] Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Kurume, Fukuoka, Japan

Source of support: This study was supported in part by Health and Labour Sciences Research Grants for Research on Hepatitis from the Ministry of Health, Labour and Welfare of Japan

Summary

Background: *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.

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

Results: 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.

Conclusions: 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

Full-text PDF: http://www.medscimonit.com/fulltxt.php?ICID=878196

Word count: 2251
Tables: 1
Figures: 3
References: 23

Author's address: Yumiko Nagao, Department of Digestive Disease Information & Research, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan, e-mail addresses: nagao@med.kurume-u.ac.jp

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).

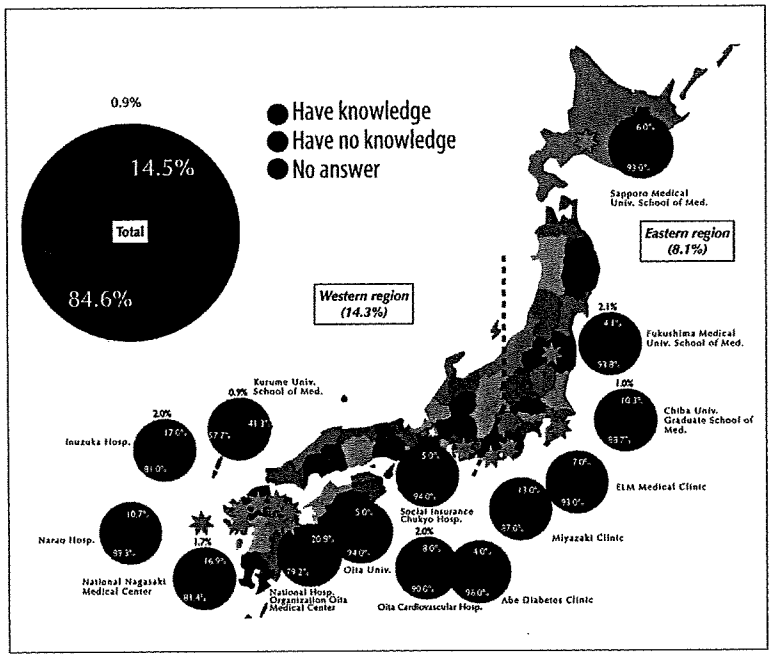


Figure 1. Knowledge of *V. vulnificus* infections among all patients with liver diseases. Only 14.5% of such patients had knowledge of this infection. Fourteen red stars indicate the location of each medical institution. Japan consists of 47 prefectures. Half of east of Japan, including Tokyo, where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

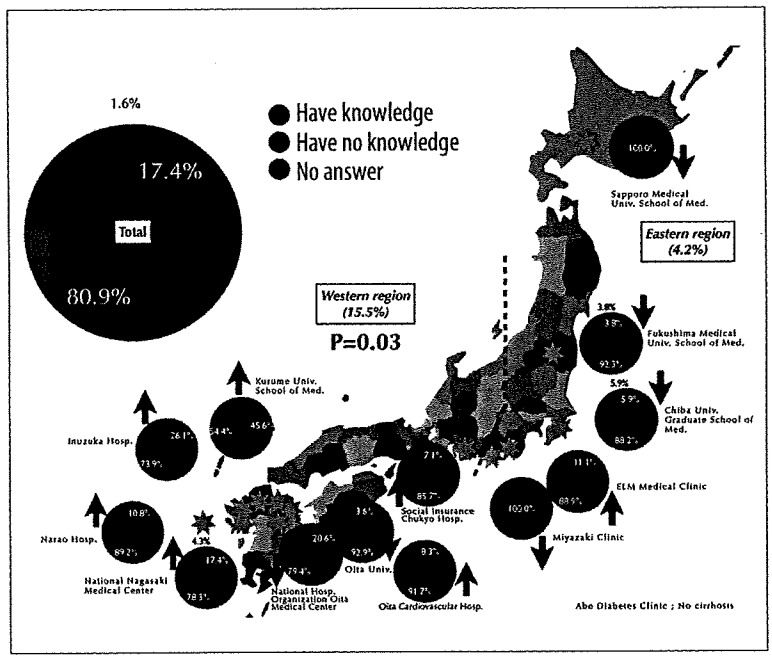


Figure 2. Knowledge of *V. vulnificus* infections in patients with liver cirrhosis. The rate of patient knowledge in the western region of Japan was significantly higher than in the eastern region. The upward pointing arrow indicates an increase in the rate of *V. vulnificus* infections in a given institution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

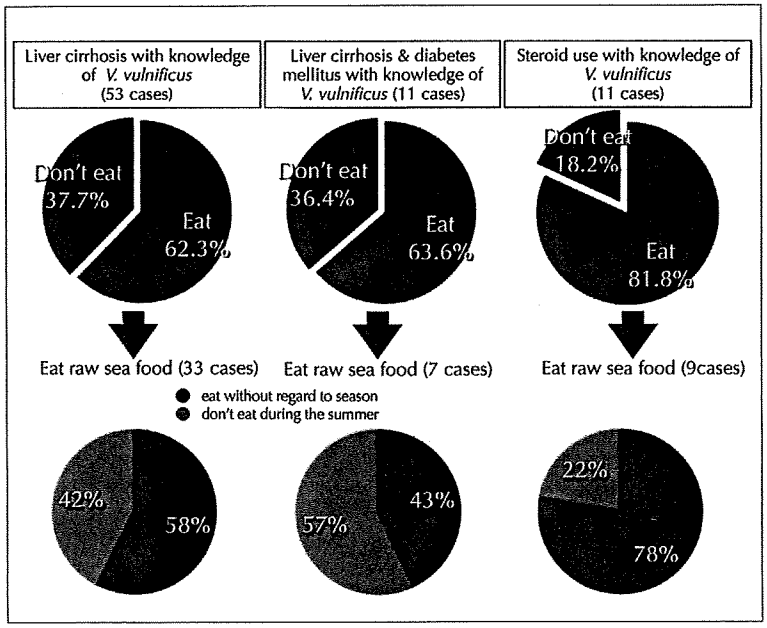


Figure 3. Frequency of eating raw seafood. Even if patients had high risk factors for infection, such as liver cirrhosis, diabetes mellitus, or steroid use, and had knowledge of *V. vulnificus* infections, most ate raw seafood without regard to season.

PH

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.

References:

1 Blake PA, Merson MH, Weaver RE et al: Disease caused by a marine Vibrio. Clinical characteristics and epidemiology. N Engl J Med, 1979; 300: 1–5

2 Morris JG Jr, Black RE: Cholera and other vibrioses in the United States. N Engl J Med, 1985; 312: 343–50

3 Wright AC, Hill RT, Johnson JA et al: Distribution of Vibrio vulnificus in the Chesapeake Bay. Appl Environ Microbiol, 1996; 62: 717–24

4 Heidelberg JF, Heidelberg KB, Colwell RR: Seasonality of Chesapeake Bay bacterioplankton species. Appl Environ Microbiol, 2002; 68: 5488–97

5 Centers for Disease Control and Prevention (CDC). Vibrio vulnificus infections associated with raw oyster consumption – Florida, 1981–1992. MMWR Morb Mortal Wkly Rep, 1993; 42: 405–7

6 Hlady WG, Klontz KC: The epidemiology of Vibrio infections in Florida, 1981–1993. J Infect Dis, 1996; 173: 1176–83

7 Wright AC, Simpson LM, Oliver JD: Role of iron in the pathogenesis of Vibrio vulnificus infections. Infect Immun, 1981; 34: 503–7

8 Haq SM, Dayal HH: Chronic liver disease and consumption of raw oysters: a potentially lethal combination – a review of Vibrio vulnificus septicemia. Am J Gastroenterol, 2005; 100: 1195–99

9 Shapiro RL, Altekruse S, Hutwagner L et al: The role of Gulf Coast oysters harvested in warmer months in Vibrio vulnificus infections in the United States, 1988–1996. Vibrio Working Group. J Infect Dis, 1998; 178: 752–59

10 Strom MS, Paranjpye RN: Epidemiology and pathogenesis of Vibrio vulnificus. Microbes Infect, 2000; 2: 177–88

11 Morris JG Jr: Cholera and other types of vibriosis: a story of human pandemics and oysters on the half shell. Clin Infect Dis, 2003; 37: 272–80

12 Roland FP: Leg gangrene and endotoxin shock due to vibrio parahaemolyticus – an infection acquired in New England coastal waters. N Engl J Med, 1970; 282: 1306

13 Matsuo T, Kohno S, Ikeda T et al: Fulminating lactose-positive Vibrio septicemia. Acta Pathol Jpn, 1978; 28: 937–48

14 Oishi H, Ura Y, Mitsumizo S, Nakashima M: A collective review of Vibrio vulnificus infection in Japan. Kansenshogaku Zasshi, 2006; 80: 680–89

15 Osaka K, Komatsuzaki M, Takahashi H et al: Vibrio vulnificus septicaemia in Japan: an estimated number of infections and physicians' knowledge of the syndrome. Epidemiol Infect, 2004; 132: 993–96

16 Yoshizawa H: Hepatocellular carcinoma associated with hepatitis C virus infection in Japan: projection to other countries in the foreseeable future. Oncology, 2002; 62(Suppl.1): 8–17

17 Inoue Y, Ono T, Matsui T et al: Epidemiological survey of Vibrio vulnificus infection in Japan between 1999 and 2003. J Dermatol, 2008; 35: 129–39

18 Blake PA, Merson MH, Weaver RE et al: Disease caused by a marine Vibrio. Clinical characteristics and epidemiology. N Engl J Med, 1979; 300; 1–5

19 Koenig KL, Mueller J, Rose T: Vibrio vulnificus. Hazard on the half shell. West J Med, 1991; 155: 400–3

20 Hor LI, Chang TT, Wang ST: Survival of Vibrio vulnificus in whole blood from patients with chronic liver diseases: association with phagocytosis by neutrophils and serum ferritin levels. J Infect Dis, 1999; 179: 275–78

21 Johnston JM, Becker SF, McFarland LM: Gastroenteritis in patients with stool isolates of *Vibrio vulnificus*. Am J Med, 1986; 80: 336–38

22 Kiyosawa K, Uemura T, Ichijo T et al: Hepatocellular Carcinoma: Recent Trends in Japan. Gastroenterology, 2004; 127(5 Suppl.1): 17–26

23 Umemura T, Ichijo T, Yoshizawa K et al: Epidemiology of hepatocellular carcinoma in Japan. J Gastroenterol, 2009; 44(Suppl.19): 102–7

MEDICAL SCIENCE

M * O * N * I * T * O * R

International Medical Journal for Experimental and Clinical Research

Indexed in: Current Contents/Clinical Medicine • SCI Expanded • ISI Alerting System • ISI Journals Master List
Index Medicus/MEDLINE • EMBASE/Excerpta Medica • Chemical Abstracts/CAS • Index Copernicus

Editor-in-Chief:	George B. Stefano (New York)
Assistant Editors:	Thomas V. Bilfinger (New York)
	Gregory L. Fricchione (Boston)
Executive Publisher:	Mark R. Graczyński (New York)
Scientific Editors:	Stanisław J. Konturek (Cracow)
	John McGiff (New York)
	Reza Nassiri (Pittsburgh)
Publisher:	Mark R. Graczyński, International Scientific Literature, Inc.

Manuscript preparation: see 'Editorial Policy, Instructions for Authors'
at *www.MedSciMonit.com*

Submisions:

Manuscript submissions only via journal's website at *www.MedSciMonit.com*
Go to Authors Pathway –> Submit Manuscript and follow the instructions.
Please note: you have to register before first submission.

For advertising and reprints contact:
Izabela Pranga,
e-mail: *iza.pranga@medscimonit.com*
Beata Kopak,
e-mail: *b.kopak@medscimonit.com*

Medical Science Monitor is indexed in: Current Contents/Clinical Medicine • IF(2008)=1.514 •
Index Medicus/MEDLINE • EMBASE/Excerpta Medica • Chemical Abstracts • Index Copernicus.
See our website: www.MedSciMonit.com

Copyright:
© International Scientific Literature, Inc., USA. All rights reserved; no part of this publication may be reproduced, stored in retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise without the prior written permission of the Publisher.
Printed on acid-free paper.

肝臓　50巻6号　269-272（2009）　

＜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には日本株である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には南/東南アジア株である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/

1）東京大学医学部生体防御感染症学
2）東京大学医学部消化器内科学
*Corresponding author: hyotsu-tky@umin.ac.jp

Table 1

		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．

文　献

1) 田中昌子，鈴木雅治，吉川　昭，他．わが国の献血者における HBV-genotype の都道府県別分布．肝臓 2009；50：320—323
2) Yoshikawa A, Gotanda Y, Suzuki Y, et al, and the Japanese Red Cross HBV Genotype Research Group. Age- and gender-specific distributions of hepatitis B virus (HBV) genotypes in Japanese HBV-positive blood donors. Transfusion 2009
3) Orito E, Ichida T, Sakugawa H, et al. Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV infection in Japan. Hepatology 2001; 34: 590—594
4) 倉光智之，小松眞史，戸堀文雄．本邦における HBV genotype の変遷とその臨床的意義　当地域における HBV 持続感染例の genotype の年代別の推移．肝臓　2005；46（Suppl. 2）：A384
5) Orito E, Mizokami M, Sakugawa H, et al. A case-control study for clinical and molecular biological differences between hepatitis B viruses of genotypes B and C. Japan HBV Genotype Research Group. Hepatology 2001; 33: 218—223
6) Schaefer S. Hepatitis B virus taxonomy and hepatitis B virus genotypes. World J Gastroenterol 2007; 13: 14—21
7) Hayashi K, Katano Y, Takeda Y, et al. Association of hepatitis B virus subgenotypes and basal core promoter/precore region variants with the clinical features of patients with acute hepatitis. J Gastroenterol 2008; 43: 558—564
8) Kobayashi M, Ikeda K, Arase Y, et al. Change of hepatitis B virus genotypes in acute and chronic infections in Japan. J Med Virol 2008; 80: 1880—1884
9) Kobayashi M, Arase Y, Ikeda K, et al. Clinical characteristics of patients infected with hepatitis B virus genotypes A, B, and C. J Gastroenterol 2002; 37

(1): 35—39
10) Takeda Y, Katano Y, Hayashi K, et al. Difference of HBV genotype distribution between acute hepatitis and chronic hepatitis in Japan. Infection 2006; 34: 201—207
11) Matsuura K, Tanaka Y, Hige S, et al. Distribution of hepatitis B virus genotypes among patients with chronic infection in Japan shifting toward an increase of genotype A. J Clin Microbiol 2009; 47: 1476—1483
12) Yotsuyanagi H, Okuse C, Yasuda K, et al; Japanese Acute Hepatitis B Group. Distinct geographic distributions of hepatitis B virus genotypes in patients with acute infection in Japan. J Med Virol 2005; 77: 39—46
13) Suzuki Y, Kobayashi M, Ikeda K, et al. Persistence of acute infection with hepatitis B virus genotype A and treatment in Japan. J Med Virol 2005; 76: 33—39
14) Ozasa A, Tanaka Y, Orito E, et al. Influence of genotypes and precore mutations on fulminant or chronic outcome of acute hepatitis B virus infection. Hepatology 2006; 44: 326—334
15) Sugauchi F, Orito E, Ohno T, et al. Spatial and chronological differences in hepatitis B virus genotypes from patients with acute hepatitis B in Japan. Hepatol Res 2006; 36: 107—114
16) Hayashi K, Katano Y, Takeda Y, et al. Association of hepatitis B virus subgenotypes and basal core promoter/precore region variants with the clinical features of patients with acute hepatitis. J Gastroenterol 2008; 43: 558—564
17) 山田典栄，四柳　宏，小板橋優，他．首都圏におけるB型急性肝炎の実態と変遷—Genotype Aに焦点をあてて—．肝臓　2008；49：553—559

Distribution of hepatitis B viral genotypes in Japan

Hiroshi Yotsuyanagi[1]*, Kazuhiko Koike[2]

Key words: acute hepatitis　chronic hepatitis　blood donor　HBV　genotype

Kanzo 2009; 50: 269—272

1) Department of Infectious Diseases, Graduate School of Medicine and Faculty of Medicine, University of Tokyo
2) Departmrnt of Gastroenterology, Graduate School of Medicine and Faculty of Medicine, University of Tokyo
*Corresponding author: hyotsu-tky@umin.ac.jp

© 2009 The Japan Society of Hepatology