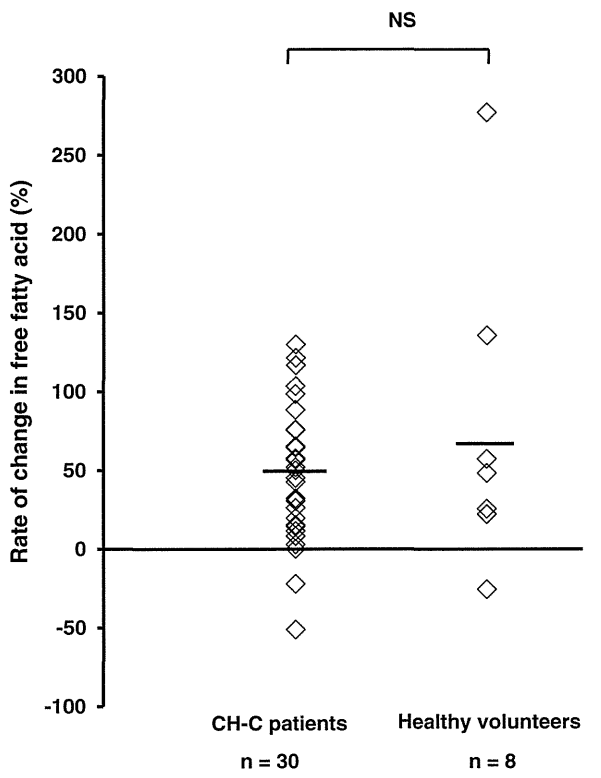

Figure 1 Free fatty acids concentration during fasting in patients with chronic hepatitis C and healthy volunteers. The rate of change in free fatty acid concentration between 12 h and 15 h after fasting was similar in both groups. The line represents a mean value. Welch's *t*-test.

concentration between 12 h and 15 h in patients with a serum HCV core protein level of 10000 fmol/L or higher was significantly lower than that in patients with a level of less than 10000 fmol/L (54.8% (8.5-304.3%) vs. 153.6% (17.1-577.3%); P <0.05) (Figure 5). In addition, the rate of change in total ketone body concentration in patients with a higher HOMA-IR value (2.5 or greater) was significantly lower than that in patients with a value of less than 2.5 (56.7% (8.5-186.7%) vs. 156.4% (33.3-577.3%); P <0.01) (Figure 6). The patients with biopsy-proven steatosis had a relatively low rate of change in total ketone body concentration between 12 h and 15 h in comparison with those without steatosis, although the rate was not significantly different between them (Figure 7). There was no significant difference in the rate of change in total ketone body concentration among the HCV genotypes (1b 120.2% (8.5-577.3%), 2a 129.9% (91.7-304.3%), 2b 135.8% (56.7-253.3%)). No significant difference in the rate of change in total ketone body concentration was demonstrated among the stages of fibrosis (F1 91.7% (17.1-436%), F2 133% (8.5-283.3%), F3 88.6% (34.2-577.3%)).

Discussion

Hepatitis C virus (HCV) is the leading cause of chronic hepatitis, subsequent liver cirrhosis and hepatocellular carcinoma. Hepatic steatosis is commonly seen in patients with chronic HCV infection having a high viral load, and it is in part associated with the development of insulin resistance [1], hepatic fibrosis [11] and hepatocarcinogenesis [12] during infection. Steatosis is also associated with a lower rate of sustained response to anti-viral therapy [13], and shows improvement after successful eradication of HCV by anti-viral therapy [14].

In general, fat accumulation in hepatocytes can result from several causes; increase of fatty acid uptake by hepatocyte, increase of fatty acid synthesis in hepatocyte, decrease of hepatic fatty acid oxidation, decrease of very-low density lipoprotein secretion. The mechanisms of steatosis in HCV infection are not fully understood. In the previous study using liver biopsy specimens of patients with HCV infection, it is shown that expression of peroxisome proliferator-activated receptor (PPAR)-α is impaired, which is an important factor in the regulation of mitochondrial β-oxidation [15]. Therefore, impaired mitochondrial β-oxidation is

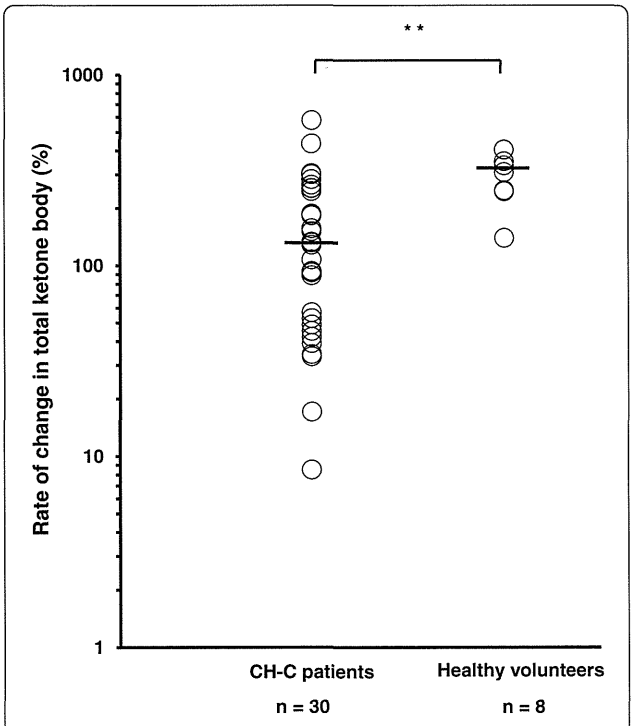

Figure 2 Total ketone body concentration during fasting in patients with chronic hepatitis C and healthy volunteers. The rate of change in total ketone body concentration between 12 h and 15 h after fasting was significantly lower in patients than in healthy volunteers (** denotes P <0.01). The line represents a median value. Log transformation was performed. Student's t test.

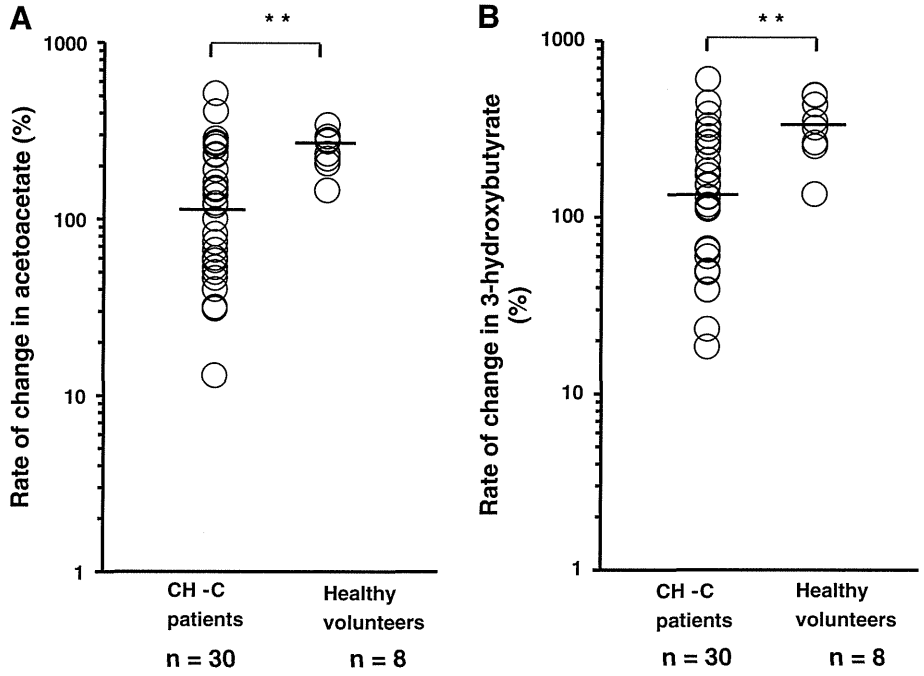

Figure 3 Ketone body fraction concentration during fasting in patients with chronic hepatitis C and healthy volunteers. The rates of change in both acetoacetate **(A)** and 3-hydroxybutyrate **(B)** between 12 h and 15 h after fasting were significantly lower in patients than in healthy volunteers (** denotes P <0.01). The line represents a median value. Log transformation was performed. Student's t test.

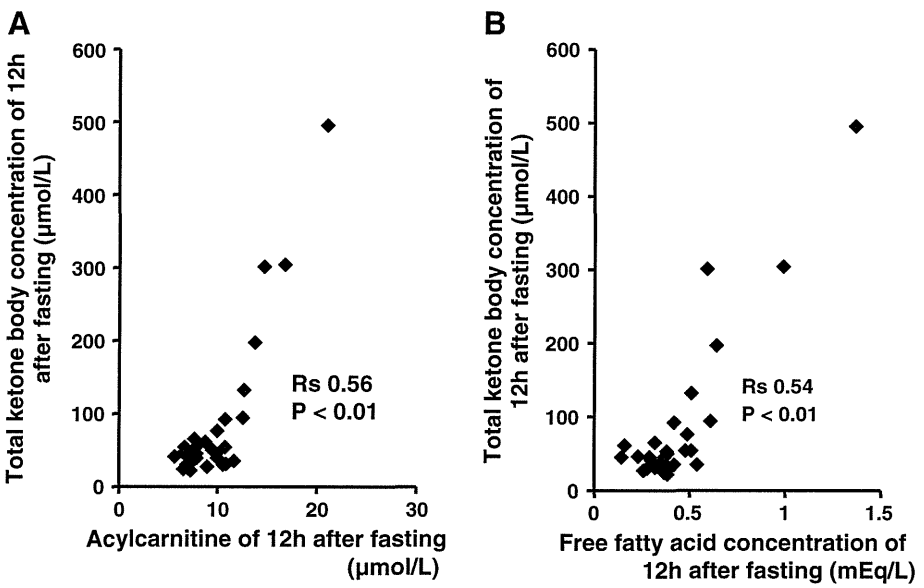

Figure 4 Ketone body concentration are related with acylcarnitine and free fatty acid in patients with chronic hepatitis C. There is a significant positive correlation between the concentration of total ketone body and the levels of acylcarnitine (rs 0.56, P <0.01),**(A)**, as well as free fatty acids (rs 0.54, P <0.01), **(B)**.

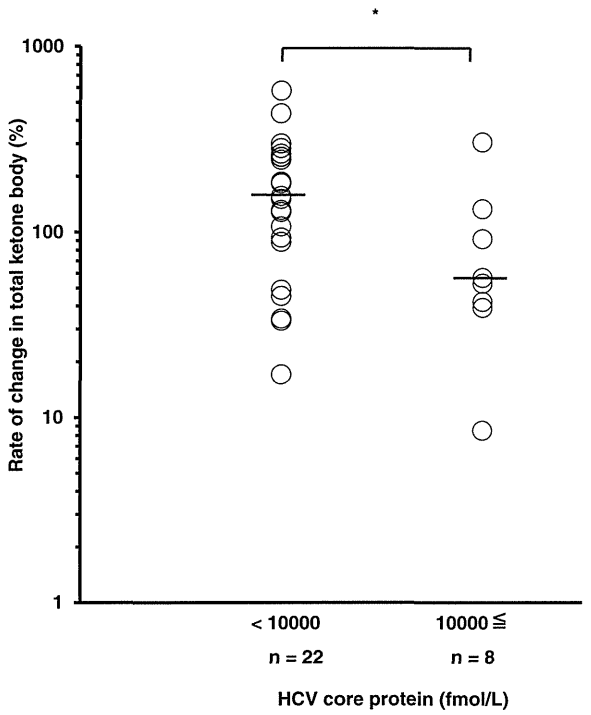

Figure 5 HCV core protein and the change in total ketone body concentration during fasting. The rate of change in total ketone body concentration between 12 h and 15 h after fasting in patients with a serum HCV core protein level of 10000 fmol/L or higher was significantly lower than that in patients with a level of less than 10000 fmol/L (* denotes P <0.05). The line represents a median value. Log transformation was performed. Student's t test.

supposed to be a mechanism of hepatic steatosis observed in the state of HCV infection.

However, there is no previous study which investigated whether mitochondrial β-oxidation is impaired in patients with CH-C *in vivo*. In the present study, therefore, we focused on the mechanism of ketogenesis in humans by investigating ketogenic capacity during fasting. The rate of change in total ketone body concentration between 12 h and 15 h after the start of fasting was significantly lower in CH-C patients than in healthy volunteers, while the rate of change in free fatty acids concentration was similar in both groups. Therefore there is a possibility that steps from acetyl-CoA to ketone bodies are impaired in patients with CH-C. In addition, Hoppel et al. reported that acylcarnitine increased during fasting and ketone bodies correlated with short-chain acylcarnitines. It is speculated that the increase in short-chain acylcarnitines may be a by-product of fatty acid β-oxidation [16]. In our patients, the level of acylcarnitine was significantly lower in CH-C patients than in healthy volunteers. Thus, these support that mitochondrial β-oxidation is impaired in patients with CH-C. Further studies are needed to assess which step is involved in the impairment of ketone bodies formation in HCV infection.

During starvation, ketone bodies increase in the body under conditions of normal mitochondrial β-oxidation. Since insulin secretion decreases during fasting, synthesis of triglyceride from acyl CoA is suppressed. Therefore, acyl CoA is β-oxidized to acetyl CoA in mitochondria. Oxaloacetate is used for gluconeogenesis during fasting. Under this condition, acetyl CoA cannot conjugate oxaloacetate, and the tricarboxylic acid (TCA) cycle is inhibited. Inhibition of the TCA cycle also occurs through consumption of nicotinamide adenine dinucleotide (NAD^+) and the production of reduced nicotinamide adenine dinucleotide (NADH) via β-oxidation. Consequently, acetyl CoA shifts towards ketogenesis. Acetyl CoA enters the TCA cycle and is used as fuel in muscle. Thus, the liver is the only organ that produces ketone bodies and secretes them into blood. In individuals with impaired hepatic mitochondrial β-oxidation, it is expected that ketogenesis would not be adequate. This is a reason why measurement of blood ketone body concentration in a fasting state facilitates assessment of mitochondrial β-oxidation *in vivo* [5].

In the present study, the rate of change in total ketone body concentration in patients with a serum level of

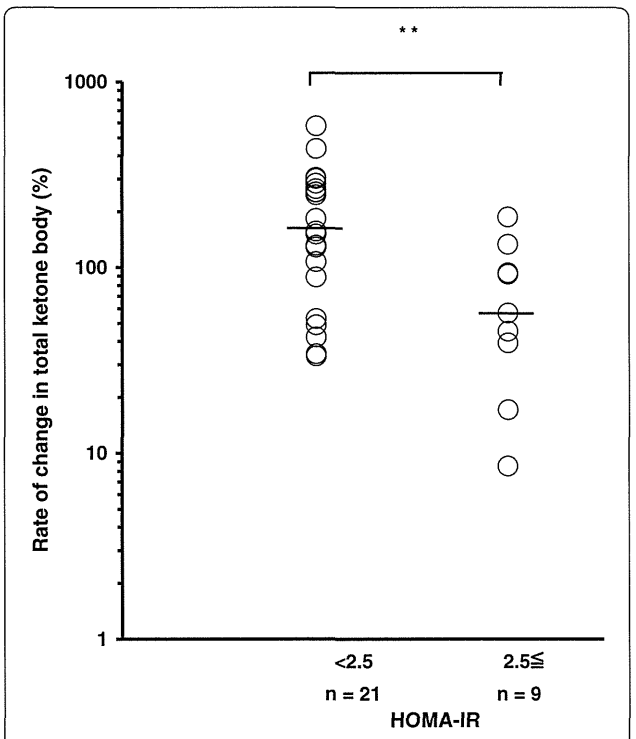

Figure 6 Insulin resistance and the change in total ketone body concentration during fasting. The rate of change in total ketone body concentration in patients with a higher HOMA-IR value (2.5 or greater) was significantly lower than that in patients with a value of less than 2.5 (** denotes P <0.01). The line represents a median value. Log transformation was performed. Student's t test.

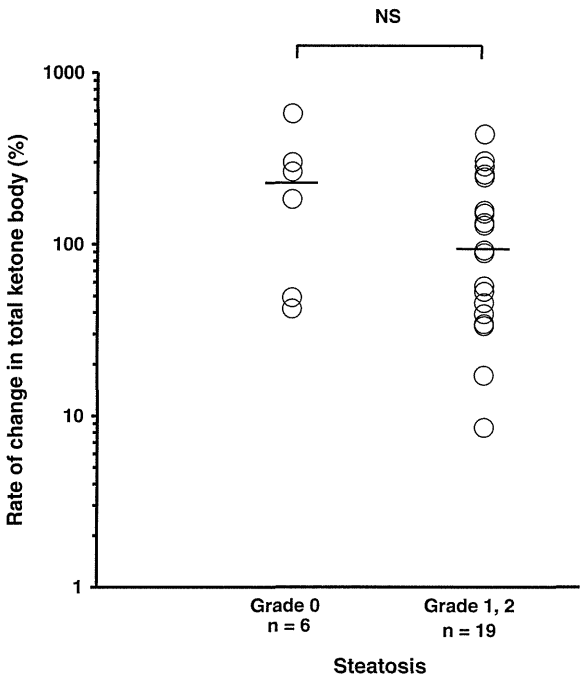

Figure 7 Hepatic steatosis and the change in total ketone body concentration during fasting. The patients with steatosis had a relatively low rate of change in total ketone body concentration between 12 h and 15 h after fasting in comparison with those without steatosis, although it was not significant. The line represents a median value. Log transformation was performed. Student's t test.

HCV core protein of 10,000 fmol/L or higher was significantly lower than in patients with a level of less than 10,000 fmol/L, showing that patients with a higher level of serum HCV core protein had lower ketogenic capacity. HCV core protein induces hepatic steatosis with disappearance of the double structure of mitochondrial membranes in HCV core transgenic mice [2]. HCV core protein is largely associated with mitochondrial dysfunction [17]. Moreover, recent studies have reported that HCV core protein downregulates the expression of PPAR-α, which is abundant in hepatocytes and is an important factor in the regulation of mitochondrial β-oxidation [15,18]. Our data suggest an impairment of mitochondrial β-oxidation by HCV infection.

Although no significant relationship between fatty acid oxidation and the grade of steatosis was demonstrated in this study (Figure 7), this issue would be worth investigating in a larger cohort of patients. HCV infection induces mitochondrial dysfunction as a result of oxidative stress, which is closely related to liver inflammation and hepatocarcinogenesis [19]. Oxidative stress is associated with impairment of fatty acid oxidation, and thus impaired ketogenesis seems to represent the increased oxidative stress in CH-C patients.

Insulin resistance in patients with CH-C has been reported [20]. At this study, insulin resistance, HOMA-IR >2.5, was observed in 9 of 30 patients. In this study, a significant positive correlation was evident between the concentration of total ketone bodies and that of free fatty acids. However, in some patients with insulin resistance, the concentrations of both free fatty acids and ketone bodies were not so high. The rate of change in the concentrations of total ketone bodies was significantly lower in patients with a higher HOMA-IR value (2.5 or greater) than in those with a value of less than 2.5. Many other factors may influence the level of fatty acid. Further studies are needed to elucidate the mechanism of insulin resistance in CH-C patients.

Our CH-C patients were significantly older than the healthy volunteers. However, we did not observe any significant correlation between the age of our subjects and the rate of change in total ketone body concentration within the age range investigated (data not shown). Elderly people in good health have a similar capacity to produce ketones to middle-aged or young adults [21].

Conclusions

The results of our study suggest that mitochondrial β-oxidation is impaired, possibly due to HCV infection. Further studies are needed to elucidate the detailed pathophysiology of impaired fatty acid metabolism in CH-C and its clinical significance.

Abbreviations

CH-C: Chronic hepatitis C; HCV: Hepatitis C virus; HOMA-IR: Homeostasis model assessment of insulin resistance; CTLN2: Adult-onset type 2 citrullinemia; NAFLD: Non-alcoholic fatty liver disease; ALT: Alanine aminotransferase; BMI: Body mass index; SD: Standard deviation; PPAR: Peroxisome proliferator-activated receptor; TCA: Tricarboxylic acid; NAD: Nicotinamide adenine dinucleotide; NADH: Reduced nicotinamide adenine dinucleotide; SREBP: Sterol regulatory element-binding protein.

Competing interests

No financial interests to disclosure that related to this study.

Authors' contributions

SC, MK, KT, TK, IR, HH, OK and NY contributed to data collection and data analysis. SC, ST, WH and UY contributed to data interpretation and manuscript writing. KS contributed to the design and conduct of the study. All authors read and approved the final manuscript.

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Serum Prolactin Levels and Prolactin mRNA Expression in Peripheral Blood Mononuclear Cells in Hepatitis C Virus Infection

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Prolactin is not only a pituitary hormone but an immunoregulatory hormone secreted from lymphocytes. Prolactin induction in relation to hepatitis C virus (HCV) infection has not been elucidated. The serum levels of prolactin were examined in 232 HCV-infected subjects positive for anti-HCV antibody and 65 healthy controls negative for it, who were recruited in the cohort study. The *prolactin* mRNAs were measured in peripheral blood mononuclear cells (PBMCs) of eleven healthy volunteers including five men and six women before and after stimulation by HCV in vitro. The serum level of prolactin and *prolactin* mRNA in PBMCs were measured by chemiluminescence immunoassay and real-time PCR, respectively. The serum levels of prolactin were significantly higher in the HCV-infected subjects (median: 7.5, IQR: 5.7–10.9 ng/ml) than in the controls (median: 5.6, IQR: 4.4–8.3 ng/ml) ($P < 0.01$). They were significantly higher in HCV-infected males (median: 8.0, IQR: 5.9–11.8 ng/ml) than in the controls (median: 4.8, IQR: 4.2–5.9 ng/ml) ($P < 0.001$), however, the difference was not significant between HCV-infected females (median: 7.3, IQR: 5.6–10.5 ng/ml) and the controls (median: 6.4, IQR: 5.3–9.8 ng/ml). The mRNA expression of *prolactin* was induced in PBMCs of all males, but it was induced in PBMCs of the two of six females examined in vitro. These results suggest that the serum level of prolactin is higher in HCV-infected males than in healthy males, and that HCV infection induces the mRNA expression of *prolactin* in PBMCs that is more apparent in male than in females. ***J. Med. Virol. 85:1199–1205, 2013.*** © 2013 Wiley Periodicals, Inc.

KEY WORDS: prolactin; HCV; immunity; pituitary hormone; cohort

INTRODUCTION

Hepatitis C virus (HCV) is a human pathogen that is a major threat to public health. About 170 million people are estimated to be infected worldwide with a potential risk of progression to cirrhosis and hepatocellular carcinoma [Kiyosawa et al., 1990; Cohen, 1999]. HCV-specific humoral and cellular immune responses are detectable in most infected individuals in both early and chronic phase of infection [Di Bisceglie, 2000].

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Prolactin is an anterior pituitary hormone that functions in lactation and pregnancy. Prolactin has been also shown to be an extra-pituitary hormone produced and secreted by immune-mediated cells, and is involved in maintaining the function of the immune-system [Gala, 1991; Leite De Moraes et al., 1995; Ben-Jonathan et al., 1996; Matera, 1996]. In addition, prolactin receptors are expressed on many immune-mediated cells, including T cells, B cells, macrophages, and natural killer (NK) cells [Pellegrini et al., 1992; Dardenne et al., 1994; Leite De Moraes et al., 1995]. Prolactin acts as a cytokine to support the growth and activity of many immune-mediated cells in both a paracrine and an autocrine manner [Sabharwal et al., 1992; Dardenne et al., 1994; Leite De Moraes et al., 1995; Matera, 1996; Yu-Lee, 1997]. Prolactin signaling through the receptor induces the expression of a number of genes involved in immune cell function, and thereby enhances NK cell function, activates interferon regulatory factor-1 (IRF-1) and interacts with or generates interleukin-2 (IL-2) and interferon-gamma (IFN-γ) [Matera, 1997]. In the T cells, IRF-1 is transcribed by the prolactin-signaling with an activation of Janus kinase 2, and it subsequently forms a homocomplex and translocates into the nucleus with the signal transducer and activator of transcription 1 (STAT1) by tyrosine phosphlyration. IRF-1 facilitates IFN-γ transcription, and leads to proliferation and activation of T cells [Yu-Lee et al., 1998; Yu-Lee, 2002]. Thus prolactin is capable of modulating the immune reaction through its signaling.

As prolactin plays a significant role in the regulation of cellular immune responses, it might have an etiological role in infectious diseases including HCV infection. In fact, it has been reported that the serum levels of prolactin increase during the course of several infectious diseases such as blastomycosis [Arora et al., 1979] and biharzial hepatosplenomegaly [Abdel Rahman et al., 1989]. Studies on human immunodeficiency virus (HIV) infection have shown that chronically HIV-infected men have higher serum prolactin levels than healthy men, and that approximately 20% of HIV-infected men have higher serum prolactin levels than the reference level [Croxson et al., 1989; Graef et al., 1994; Montero et al., 2001; Parra et al., 2001; Collazos et al., 2002; Leanos-Miranda and Contreras-Hernandez, 2002]. Recent studies have shown an association of higher serum prolactin levels in patients with chronic HCV infection compared with healthy controls [Durazzo et al., 2006; Hofny et al., 2011]. Sousa et al. [2011] have also demonstrated that hyperprolatinemia was found in 10.1% of hepatitis C patients examined. Although hyperprolatinemia was not associated with autoimmunity in HCV carriers [Sousa et al., 2011], it may be involved in extra-hepatic manifestation in aspect of reproduction in men because the abnormality of hormonal parameters such as serum levels of estradiol as well as that of prolactin have a possibility in impairment of the spermatic function [Durazzo et al., 2006; Hofny et al., 2011]. The association of prolactin with HCV infection is worth for further investigation in larger samples of subjects.

In this study, the levels of serum prolactin were analyzed using the samples collected from the residents recruited in the HCV cohort study [Ishibashi et al., 1996; Watanabe et al., 2003; Saito et al., 2004]. The quantitative assay for *prolactin* mRNA expression was established, and the mRNA expression of *prolactin* was investigated in peripheral blood mononuclear cells (PBMCs) of healthy subjects experimentally stimulated by HCV in vitro. In this article, the association of prolactin induction with HCV infection is reported.

METHODS

Subjects and Serum Samples for Measuring Prolactin Levels

Subjects were recruited from the residents living in an HCV-endemic area where we have continued HCV-related cohort study since 1990 [Ishibashi et al., 1996; Watanabe et al., 2003; Saito et al., 2004]. The study was approved by the institutional ethics committee, and written informed consent was obtained from all the subjects examined. A large-scale search of serologic testing for HCV infection in 675 adult individuals (286 men and 389 women) was conducted previously in this area [Saito et al., 2004]. Briefly, the survey revealed that 277/675 (41.0%) of subjects were positive for anti-HCV antibody. After excluding subjects with a history of antiviral therapy using interferon, or those positive for hepatitis B surface antigen, 238 (35.3%) subjects (87 men and 151 women) were positive for anti-HCV antibody. Of these, 232 subjects (83 men and 149 women, median age: 68.0 year, interquartile range: 63.0–73.0 year) whose samples were kept well under −80°C were assayed for serum prolactin levels. Serum prolactin levels were also measured in sera of age-matched, 65 healthy subjects (26 men and 39 women, median age: 66.0 year, interquartile range: 59.5–72.0 year) negative for anti-HCV antibody with normal blood biochemistry parameters living in the same area. In the present study, subjects with the factors known to influence the serum prolactin level, that is, having a history of renal failure or medication with dopaminergic or antidoperminergic drugs, steroids or protease inhibitors, were excluded.

Laboratory Measurements

Anti-HCV antibody and serum HCV RNA status were examined by an enzyme immunoassay kit (HCV EIAII Abbott; Abbott Japan, Tokyo) and a qualitative HCV RNA detection kit (Amplicor HCV v2.0; Roche Diagnostics, Tokyo, Japan), respectively. HCV typing was carried out by a PCR-based genotyping assay using type-specific primers, as described previously

[Watanabe et al., 2003]. Hepatitis B surface antigen was examined using an enzyme immunoassay kit (HBsAg Dainapac; Dainabot, Tokyo, Japan). The serum prolactin level was measured using a chemiluminescence's immunoassay kit (Architect prolactin; Dainabot, Tokyo, Japan). The upper limit of the reference prolactin level was based on the levels of the Japanese general population provided by the Japanese laboratory, SRL Inc., Tokyo, Japan; the reference levels of male and female were 12.7 ng/ml and 30.5 ng/ml, respectively.

Measurement of Prolactin mRNA Expression in PBMCs Stimulated by Infectious HCV Particles

Preparation of HCV viral stock. To generate HCV viral particles, the HCV replicon system that consists of pFL-J6/JFH-1 and Huh7.5 cell line was utilized [Lindenbach et al., 2005]. Briefly, 10 μg of transcribed full-length FL-J6/JFH-1 RNA was electroporated into 1×10^7 Huh7.5 cells under the condition described by Zhong et al. [2005]. Cells were plated in complete DMEM (Dulbecco's modified eagle medium; Invitrogen) containing 10% heat-inactivated fetal bovine serum (Invitrogen) and 5% non-essential amino acid (Sigma–Aldrich, Tokyo, Japan) for overnight incubation. Then the transfected cells were transferred to complete DMEM in T75 flask and cultured in a humiliated 5% CO_2 atmosphere at 37°C. Cells were passages every 3 days; HCV-RNA and HCV core protein presented in their corresponding supernatants were monitored by the real-time PCR, and radio immunoassay, respectively. Supernatant collected at day 21 after HCV-RNA transfection, containing the highest level of HCV-RNA (5,100 KIU/ml) and HCV core protein (80,000 fmol/L), was aliquoted for storage at −80°C.

PBMC separation and culture. Fresh PBMCs were separated from whole blood drawn from 11 healthy donors including five men and six women by density gradient centrifugation on Ficoll-Paque PLUS as describe above. PBMCs were washed with PBS twice, and suspended in RPMI-1640 (Invitrogen) supplemented with 2 mM L-glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin, 10 mM Hepes, and 10% heat-inactivated FBS at a concentration of 2.0×10^6/ml. PBMCs were subsequently incubated with the above collected FL-J6/JFH-1 supernatant at a concentration of 500 KIU/ml of HCV RNA and cultured in Falcon polystyrene 6-well plates at 37°C. PBMCs cultured with a control medium without FL-J6/JFH-1 supernatant were used as controls.

Detection of prolactin mRNA in PBMCs stimulated by HCV in vitro. To assess *prolactin* mRNA expression in PBMCs from the healthy donors after stimulation by HCV in vitro, the above PBMCs were harvested at 36 hr after culturing with or without FL-J6/JFH-1 supernatant containing HCV, and then washed three times with PBS. The total RNA was extracted from PBMCs by using RNeasy Plus Mini Kit (QIAGEN, Tokyo, Japan) according to the manufacturer's instructions. To avoid amplification of contaminating genomic DNA, total RNA was treated with 10 units of RNase-free DNase (QIAGEN) for 30 min, and stored at −80°C until analysis. The integrity and purity of total RNA was verified spectrophotometrically. Expression of *prolactin* mRNA was analyzed using a TaqMan real-time PCR method (LightCycler) according to the manufacturer's recommendations (Roche Diagnostics). Briefly, the cDNA was synthesized from 600 ng of total RNA by using a commercial SupserScript First-Strand Synthesis Kit (Invitrogen Japan, Tokyo). For *prolactin* mRNA quantification, 2 μl of cDNA sample was subjected to 45 rounds of real-time PCR cycling (preincubating at 95°C for 10 min, amplifying at 95°C for 10 sec, 60°C for 30 sec, 72°C for 3 sec, and cooling at 40°C for 30 sec). The real-time PCR was performed in a final volume of 20 μl in a LightCycler 2.0 detection system (Roche) using the TaqMan Gene Expression Assay for human prolactin (Applied Biosystems, Tokyo, Japan). A five-point standard curve (0.02–200 ng of total RNA from Jurkat cells) was used to estimate the initial amounts of *prolactin* mRNA present in each sample. Finally, the relative amounts of *prolactin* mRNA were calculated according to the method described by Bookout AL [Bookout and Mangeldorf, 2003] after normalization to the housekeeping gene, hypoxanthine guanine phosphoribosyltransferase (HGPRT) (Applied Biosystems).

Statistical Analysis

Statistical analyses were performed using Fisher's exact test and Mann–Whitney *U*-test. Differences at values of $P < 0.05$ were considered significant.

RESULTS

Serum Prolactin Levels in Residents Endemic for HCV Infection

Serum prolactin levels were significantly higher in the HCV-infected subjects (median: 7.5 ng/ml, interquartile range: 5.7–10.9 ng/ml) than in the controls (median: 5.6 ng/ml, interquartile range: 4.4–8.3 ng/ml) ($P < 0.01$) (Fig. 1). Serum prolactin levels were significantly higher in the male subjects positive for anti-HCV antibody (median: 8.0 ng/ml, interquartile range: 5.9–11.8 ng/ml) than in the controls (median: 4.8 ng/ml, interquartile range: 4.2–5.9 ng/ml) ($P < 0.001$), but they were not significantly different between the female subjects positive for anti-HCV antibody (median: 7.3 ng/ml, interquartile range: 5.6–10.5 ng/ml) and the controls (median: 6.4 ng/ml, interquartile range: 5.3–9.8 ng/ml) (Fig. 2). The rate of elevated serum prolactin higher than the upper limits of the reference level was significantly higher in the male subjects positive for anti-HCV antibody (15/83, 18.1%) than in the controls (0/26, 0%) ($P < 0.02$), but in female it was not significantly different between the subjects positive for anti-HCV

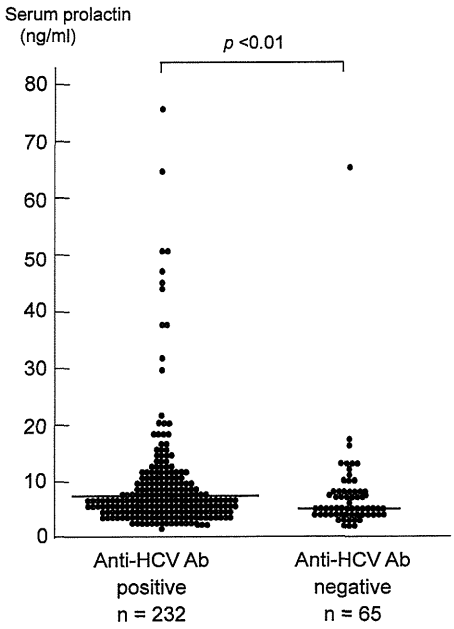

Fig. 1. Serum prolactin levels in residents living in HCV-endemic area. Serum prolactin levels were significantly higher in anti-HCV antibody-positive residents than those negative for it (median: 7.5 ng/ml vs. 5.6 ng/ml, $P < 0.01$). Mann–Whitney U-test.

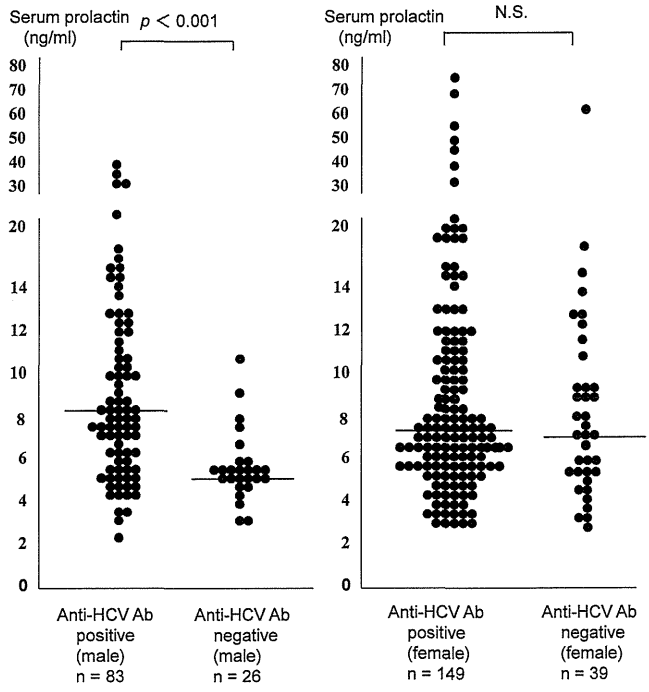

Fig. 2. Serum prolactin levels in male and female residents. Serum prolactin levels were significantly higher in the HCV-infected male than in the controls (median: 8.0 ng/ml vs. 4.8 ng/ml, $P < 0.001$), but they were not significantly different between the HCV-infected female and the controls (median: 7.3 ng/ml vs. 6.4 ng/ml, NS). Mann–Whitney U-test.

antibody (5/149, 3.4%) and the controls (1/39, 2.6%) (Table I).

Serum Prolactin Levels and HCV Genotypes

The 232 residents positive for anti-HCV antibody were classified into those with HCV viremia (184/232, 79%) and those without HCV viremia who were eliminated viremia in the course of infection (48/232, 21%). Thus the 184 subjects of 232 residents in whom the serum HCV RNA was amplified were genotyped. The number of genotype 1 (1b) and genotype 2 (2a/2b) were 77 and 107 (12/95), respectively. There were no subjects infected with genotype 3 or 4. Characteristics of the subjects were shown in Table II. Serum prolactin levels were not significantly different between the HCV genotype 1-infected subjects and genotype 2-infected subjects in both male and female (male: median: 8.2 ng/ml, interquartile range: 5.8–10.4 ng/ml versus median: 6.9 ng/ml, interquartile range: 5.7–11.0 ng/ml, female: median: 7.5 ng/ml, interquartile range: 5.6–10.3 ng/ml versus median: 7.1 ng/ml, interquartile range: 5.6–10.5 ng/ml; genotype 1 versus genotype 2).

Induction of Prolactin mRNA in Human PBMCs in Non-HCV Infected, Healthy Subjects After HCV Stimulation In Vitro

To analyze the induction of *prolactin* mRNA in immune cells, PBMCs were isolated from eleven healthy donors negative for anti-HCV antibody. PBMCs were stimulated with HCV produced in vitro. After incubation for 36 hr with the supernatant cultured with FL-J6/JFH-1 in which HCV RNA and HCV core protein were produced 5,100 KIU/ml and 80,000 fmol/L, respectively, the induction of *prolactin* mRNA in PBMCs was detected in all five male subjects and two of four female subjects before menopause, its mRNA levels being 1.18–4.99× higher than that in PBMCs cultured with the control supernatant without HCV. But, the induction of *prolactin* mRNA in PBMCs was not detected in two females after the menopause (Table III). No correlation between serum prolactin level and *prolactin* mRNA expression in PBMCs was found.

DISCUSSION

Prolactin has a potential role in immunity to various infectious diseases. In this study, higher serum prolactin levels were observed in HCV-infected individuals than in healthy, uninfected individuals. Serum prolactin levels were significantly higher in male subjects positive for anti-HCV antibody than those negative for it and the rate of elevated serum prolactin higher than the upper limits of the reference level in HCV-infected men being 18.1%, although no significant differences were found in females' subjects. Since the anterior pituitary is the

TABLE I. Characteristics of Analyzed Residents Endemic for HCV Infection

	Anti-HCV antibody positive	Anti-HCV antibody negative	Test	*P*
No. of cases	232	65		
Gender				
M/F	83/149	26/39	a	NS
Age (yrs), median (interquartile range)				
Total	68.0 (63.0–73.0)	66.0 (59.5–72.0)	b	NS
Male	68.0 (62.0–72.0)	58.5 (50.8–74.3)	b	NS
Female	68.0 (63.0–73.0)	67.0 (64.0–72.0)	b	NS
ALT (IU/L), median (interquartile range)				
Total	28.0 (19.0–42.3)	16.0 (14.0–21.0)	b	<0.001
Male	31.0 (21.0–59.0)	19.5 (16.0–26.0)	b	<0.001
Female	25.0 (18.0–37.0)	16.0 (14.0–19.0)	b	<0.001
Prevalence of elevated prolactin levels[a] (%)				
Total	20/232 (8.6)	3/65 (4.6)	a	NS
Male	15/83 (18.1)	0/26 (0)	a	<0.02
Female	5/149 (3.4)	1/39 (2.6)	a	NS

a; Fisher's exact test b; Mann–Whitney *U* test NS; no significance.
[a]The upper limits of prolactin are 12.7 ng/ml in male and 30.5 ng/ml in female.

major source of prolactin and its major target organ is the mammary glands, prolactin secretion are largely influenced by the mammary glands. In general, the range of serum prolactin levels is much higher in female than in male, and thus the differences based on the responses to infection would not be discriminated in female subjects. Thus the prolactin studies of many infectious diseases [Croxson et al., 1989; Graef et al., 1994; Montero et al., 2001; Parra et al., 2001; Collazos et al., 2002; Leanos-Miranda and Contreras-Hernandez, 2002] had been selected for the male subjects.

TABLE II. HCV Genotypes and Serum Prolactin Levels in HCV-Infected Subjects

	Genotype 1	Genotype 2	Test	*P*
No. of cases	77	107		
Gender				
M/F	25/52	42/65	a	NS
Age (yrs), median (interquartile range)				
Male	70.0 (65.5–74.0)	67.0 (57.8–74.3)	b	NS
Female	69.5 (63.0–74.0)	68.0 (61.5–72.0)	b	NS
ALT (IU/l), median (interquartile range)				
Male	47.0 (29.5–59.5)	33.0 (23.0–72.5)	b	NS
Female	33.5 (22.0–52.0)	27.0 (18.5–35.5)	b	<0.05
Prolactin (ng/ml), median (interquartile range)				
Male	8.2 (5.8–10.4)	6.9 (5.7–11.0)	b	NS
Female	7.5 (5.6–10.3)	7.1 (5.6–10.5)	b	NS

a, Fisher's exact test; b, Mann–Whitney *U* test NS; not significant.

TABLE III. Prolactin mRNA Induction in PBMCs of Healthy Subjects After Stimulation by HCV In Vitro

				Plolactin mRNA		
Volunteer no.	Gender (M/F)[a]	ALT (IU/L)	Anti-HCV antibody	HCV stimulation[b]	Control[b]	Ratio
1	M	12	—	0.87	0.36	2.38
2	M	6	—	0.12	0.02	4.99
3	M	30	—	0.01	0.00	2.51
4	M	18	—	0.03	0.01	2.53
5	M	30	—	0.08	0.07	1.18
6	F1	17	—	0.57	0.42	1.37
7	F1	13	—	0.15	0.11	1.30
8	F1	17	—	0.05	0.05	0.88
9	F1	28	—	0.15	0.59	0.25
10	F2	30	—	0.06	0.18	0.32
11	F2	20	—	0.03	0.05	0.63

[a]M, male, F1, female before menopause, F2, female after menopause.
[b]Prolactin mRNA/HGPRT mRNA (ratio). The ratio is rounded to two decimal places.

Recently, some studies showed that hyperprolactinemia was found in patients with chronic hepatitis C as a part of abnormality of hormone parameters [Durazzo et al., 2006; Hofny et al., 2011] or with an association of hyperferritinemia [Sousa et al., 2011]. The etiological importance of prolactin in the immune response to HCV infection has not been elucidated. Prolactin is known to be produced and secreted from extra-pituitary sources such as human PBMCs and human T and B lymphocytes, and such cells have prolactin receptors on their surface [Pellegrini et al., 1992; Sabharwal et al., 1992; Dardenne et al., 1994; Ben-Jonathan et al., 1996; Matera, 1997]. Thus an association of prolactin with HCV immunity has been postulated. To date, there is still no evidence that prolactin is produced and secreted by lymphoid cells in patients with HCV infection. This study showed for the first time that the induction of *prolactin* mRNA occurred in the cells stimulated by HCV in vitro. Also higher levels of serum prolactin found in HCV-infected male subjects have suggested an association of prolactin induction with HCV infection. To ascertain these findings, further studies are needed to investigate the synthesis and secretion of prolactin by immune-mediated cells from HCV-infected subjects.

Components of the HCV-specific immune response have a wide variety, and one of the most important in relation to chronic hepatitis is considered to be cytotoxic T cell (CTL)-mediated immunity with a cytokine profile induced by a Th1-dominated T helper cell response [Mizukoshi and Rehermann, 2001]. Prolactin signaling through its receptor on lymphoid cells induces the expression of a number of genes involved in cellular immune responses associated with CTL proliferation and activation [Matera, 1997]. Prolactin has been shown to induce IL-2 receptors and simultaneously promote IL-2 release in rat lymphocytes [Clevenger et al., 1990a,b; Viselli et al., 1991] and to enhance the release of IFN-γ in human peripheral whole blood after stimulation with either phytohemagglutinin or lipopolysaccharide [Brand et al., 2001; Breidthardt et al., 2002]. Prolactin acts also as an antagonist of transforming growth factor (TGF)-beta [Richards et al., 1998], which is produced by NK cells and has an inhibitory effect on lymphocytes to suppress Th1-associated cell-mediated immunity [Mizukoshi and Rehermann, 2001]. The role of prolactin in the immune response to HCV infection is unknown. Several clinical studies on HIV infection have shown that HIV-infected men with secondary infections show higher serum prolactin levels than asymptomatic HIV carriers, and that the two groups differ in their lymphocyte phenotype distribution [Montero et al., 2001; Parra et al., 2001]. Prolactin induction in patients with secondary infection of HIV carriers may represent an adaptive immune mechanism of the infected host to stimulate higher possible cellular immune response [Parra et al., 2004]. Prolactin induction in case of HCV infection may represent such a mechanism of the HCV-infected host to induce an effective immune response to HCV.

A study has shown that infection with HCV genotype 3 was associated with hyperplolactinemia [Sousa et al., 2011]. There were no correlations between serum prolactin levels and infection with HCV genotype 1 or 2. Since there is few patients infected with HCV genotype 3 or 4 in Japan, there are limitations to this study. A further study is needed to make clear an association of serum prolactin levels and HCV genotypes worldwide.

HCV infection shows a variety of clinical courses in infected individuals. Both serum prolactin levels and *prolactin* mRNA in PBMCs stimulated by HCV in vitro showed various expression levels. The host genetic factors such as gender, age, hormonal secretion might relate to the difference in prolactin levels among individuals. In addition, the several genetic variations of the genes related to the prolactin signaling, including STAT5A, TGF-beta and its superfamily that influence the downstream part of the IL-2 induction cascade have been shown in HCV-infected subjects [Saito et al., 2004; Kimura et al., 2006]. Further studies are needed to elucidate how prolactin is involved in the clinical course of HCV infection.

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＜症例報告＞

アポ型ALTによりALTが異常低値を示したC型慢性肝炎に対して抗ウイルス療法を行った1例

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阿蘇　里佳　西瀬　雄子　渡辺　久剛　上野　義之

要旨：症例は65歳の男性．近医にてHCV抗体陽性を指摘され，精査加療目的に当院へ入院となった．初診時よりalanine aminotransferase（ALT）値は2 U/Lと極度に低値であったが，肝組織所見はA1/F2で慢性肝炎であった．ビタミンB6の補酵素であるピリドキサルリン酸（PALP）添加にてALTを測定したところ32 U/Lであり，アポ型のALTを呈していると考えられた．本症例に対し，ゲノタイプ2a型低ウイルス量であるためペグインターフェロン（Peg-IFN）α2a製剤を投与したところ，投与開始後1週目にはHCV RNAは検出感度以下となり，12週間の投与により著効となった．ALTにはアポ型，ホロ型があり本邦における測定系ではアポ型は測定されない．本症例においてALTが低値を呈した原因は，ALTがアポ型を呈していたためと考えられた．アポ型ALTが優位で，ALTが極度に低値を示していたが，Peg-IFNα2a投与により著効となったC型慢性肝炎の1例を報告した．

索引用語：alanine aminotransferase　アポ型ALT　C型慢性肝炎
pyridoxal phosphate　ペグインターフェロンα2a

はじめに

C型慢性肝炎の活動性を示す一つの指標として，alanine aminotransferase（ALT）値が用いられる．ALTが基準値以内の患者に対しての治療方針として，厚生労働省難治性肝炎治療研究班によるガイドラインでは，ALT値30 U/L以下，血小板15万/μL以上であれば2-4カ月ごとに血清ALT値をフォローし，ALT異常を呈した時点で抗ウイルス療法を考慮することが，推奨されている[1]．ALTは肝炎の炎症程度を表す有用な指標の一つであり，その上限値は施設によって異なるが，下限値についての検討は少ない．時に，ALTが下限値以下の症例を経験するが，測定法の影響を受けている可能性がある．今回我々はALTが2 U/Lと極度に低値で経過をみていた稀なC型慢性肝炎に対して，ペグインターフェロン（Peg-IFN）α2a製剤を投与し著効となった症例を経験したので報告する．

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症　例

患者：65歳男性．

主訴：インターフェロン導入目的．

既往歴：58歳より糖尿病で経過観察（内服なし）．

生活歴：輸血歴なし．

飲酒歴：機会飲酒．

現病歴：1998年よりHCV抗体陽性を指摘されていたが，1年1回の採血検査で経過観察されていた．2008年9月，当科外来紹介，精査加療目的に2009年3月に入院となった．

入院時検査成績（Table 1）：HCV抗体は陽性，ゲノタイプ2a型であり，HCV RNAは4.3 LogIU/mLで，血小板は12.5万/μLであった．alanine aminotransferase（ALT）は2 U/Lと極度に低値を示していた．腹部CT検査では，肝形態に異常を認めず，肝硬変の所見も認めなかった（Fig. 1）．肝生検では，門脈域の線維化と炎症細胞の浸潤を認め，A1/F2の肝組織所見であった（Fig. 2）．

入院後経過（Fig. 3）：ALTは2 U/Lと極度に低値であったが，肝生検により慢性肝炎の所見を認めたため，Peg-IFNα2a週1回180 μgを投与開始した．治療

Table 1　Laboratory data on admission

TP	8.1 g/dL	TCho	160 mg/dL
Alb	4.5 g/dL	TG	103 mg/dL
T.Bil	0.9 mg/dL	FBS	150 mg/dL
AST	31 U/L	HbA1c	7.2 %
ALT	2 U/L		
LDH	217 U/L	AFP	3.2 ng/mL
ALP	100 U/L	PIVKA II	10 mAU/mL
γGTP	78 U/L		
WBC	4.61×10^3 /μL	HBsAg	(−)
(Neu	2.39×10^3 /μL)	HCV Ab	(+)
Hb	14.6 g/dl	HCV genotype	2a
Plt	12.5×10^4 /μL	HCV RNA	4.3 LogIU/mL
PT	119 %		
BUN	14 mg/dL	ICGR15	7 %
Crea	0.79 mg/dL		
Na	140 mEq/L	Pyridoxamine	<0.2 ng/mL (<0.6)
K	3.9 mEq/L	Pyridoxal	13.4 ng/mL (6.0-40.0)
Cl	104 mEq/L	Pyridoxine	<3.0 ng/mL (<3.0)

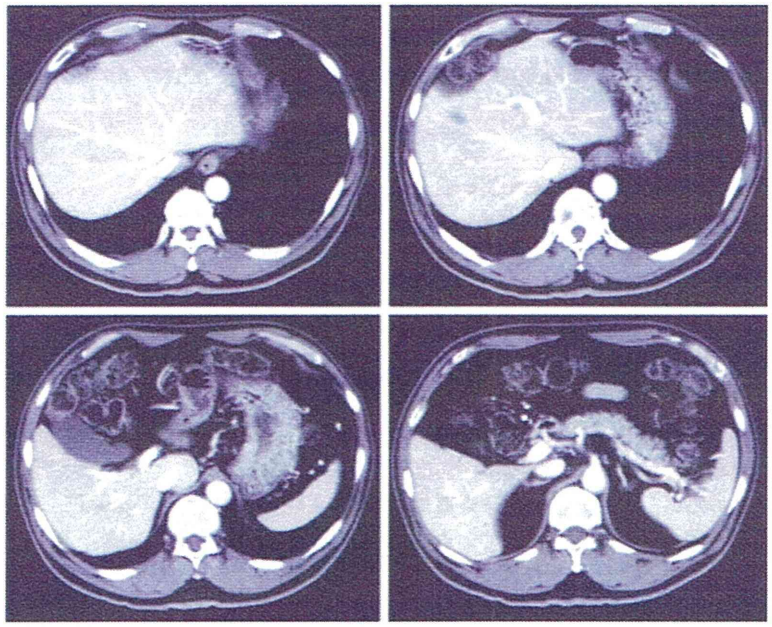

Fig. 1　abdominal CT

開始後の1週目にはHCV RNAは検出感度以下となった．治療開始後12週の時点で，食欲不振，うつ症状が出現し，本人の希望にて治療を中止となった．その後もHCV RNAは検出感度以下が24週間以上持続し，著効となった．ALT低値を呈した原因として，ビタミンB6欠乏症を考え，ビタミンB6の測定を行ったが，ピ

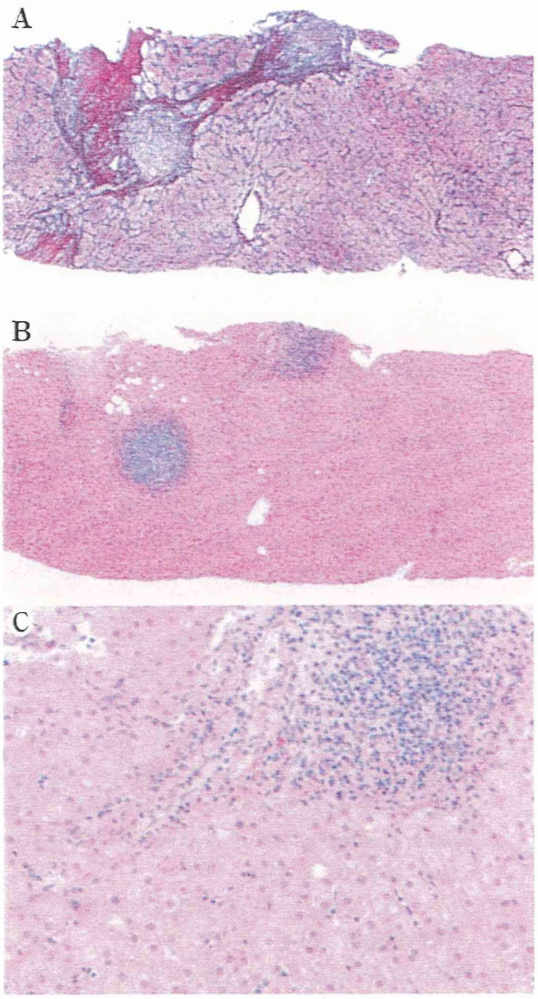

Fig. 2 Liver biopsy specimen shows A1F2
(A) Gitter staining×40 (B) HE staining×40 (C) HE staining×100

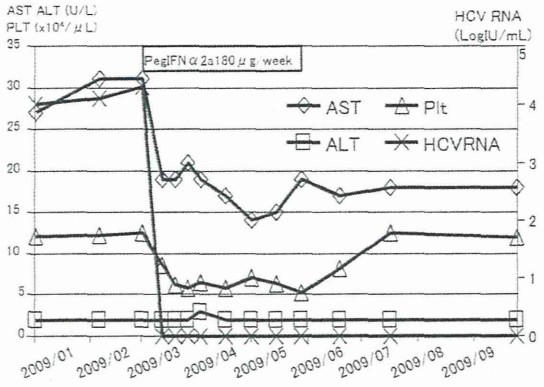

Fig. 3 Clinical course

リドキサミン，ピリドキサール，ピリドキシンはいずれも正常範囲内であった(Table 1)．また，日本モニター株式会社（東京，日本）製測定試薬を用いて，ビタミンB6の補酵素であるピリドキサルリン酸（PALP）添加(27 mM/L)にて，血清を再測定したところ，asparate aminotransferase（AST）は58 U/L（PALP非添加で31 U/L），ALTは32 U/L（PALP非添加で2 U/L）であり，本症例のAST，ALTはアポ型を呈していたため，これらは通常の測定法では低値を示したものと考えられた．

考　察

ASTやALTは，その活性発現にビタミンB6の補酵素であるPALPを必要とする酵素である[2)3)]．AST，ALTには血中においてはPALPと結合しているホロ型と結合していないアポ型が存在する．PALP添加測定試薬系ではホロ型とアポ型の両者が同時に測定されるが，無添加試薬系ではホロ型のみが測定される．国際臨床化学連合（IFCC）勧告法はアポ型をホロ化するために測定系へのPALP添加を推奨しているが，本邦で主に用いられている日本臨床化学会(JCSS)勧告法ではPALP無添加の試薬系であり，アポ型は測定されない反応系である[4)5)]．一般にアポ型酵素の血清中割合は10%程度とされている[6)]が，ビタミンB6欠乏症，人工透析，アルコール性肝障害などではその割合が増加することが知られており，その臨床的意義については不明な点が多い[6)7)]．既報では健常者190例中15例(7.9%)に解離がみられ[3)]，心筋梗塞や腎移植においても解離することが報告されている[8)9)]．

ビタミンB6欠乏では，アポ型が増加し，ALTが低値となるが，本症例においては血清中のビタミンB6の測定を行ったところ，正常範囲内であった．本症例ではアポ型を呈したAST，ALTの割合が多かったため，通常の測定方法では低値を呈したと考えられた．*in vitro*でのPALP添加でALTは上昇しており，生体内では結合しないALTが*in vitro*で結合するためと考えられたが，その機序は不明である．免疫グロブリン結合ASTの症例報告もあり[10)]，免疫グロブリンの結合によって立体障害が生じ，PALP結合部位に変化が生じて解離を生じる可能性が示唆されている．本症例でアポ型を呈した理由は不明であるが，ALTがアポ型を呈する理

由として，AST，ALTのPALPの結合部位や遺伝要因による可能性もあり，今後，検討の余地があると思われた．

ALTが基準値以内でも慢性肝炎を呈している例は多く[11)~13)]，血小板が低下している症例に対してはインターフェロンによる抗ウイルス治療が推奨されている．また，ALTは測定法で値が異なることがあり，実際より低値となることがあり，注意を要する．本症例も前医ではALTが基準値以内の症例として経過をみられていたが，肝組織検査では慢性肝炎(A1/F2)の所見であった．血小板は15万/μL以下に低下しており，HCVゲノタイプ2a型低ウイルス量で抗ウイルス効果が期待できる症例であったため，Peg-IFNα2a製剤を使用し，最終的に著効となった．各施設間において，ALT値の基準値の上限には格差があるが，その下限についての報告はない．稀ではあるが，本症例のようにALTが2U/Lと極度に低値を示す症例もみられ，その原因としてALTがアポ型を呈していることが考えられる．そのような症例に対してはPALP添加による測定で実測値が測定され得ることがあるため，ALTが極度に低値を示す症例にはPALP添加による測定により，アポ型のみのALTの存在を確認してみる必要があると考えられた．

結　語

ルーチンの血液検査法にて極度にALTが低値を示したが，組織学的に慢性肝炎を確認し抗ウイルス療法が奏功したC型慢性肝炎の1例を報告した．ALT値の上昇がPALP添加により確認でき，ALTが極度に低値を示した原因としてアポ型を呈するためと考えられた．

謝辞：本症例報告に際し，ご指導を終始頂きました，兵庫県立西宮病院院長，河田純男先生に感謝いたします．本研究の一部は，厚生労働科学研究費補助金の助成を受けた．

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本論文内容に関連する著者の利益相反：なし

B型肝炎の自然予後（無治療住民検診での長期予後）

渡辺久剛* 上野義之*

索引用語：B型肝炎，自然予後，住民コホート，HBs抗原自然消失，肝癌

1 はじめに

B型肝炎ウイルス(HBV)感染症は，非感染例に比べ明らかに肝発癌リスクが高く[1]，わが国では肝癌リスクの第2位，アジア全体では第1位を占めている．日本赤十字社の初回献血者から推定した，本邦におけるHBV感染者数は，Tanakaらの報告によれば15～65歳において967,753名(95％ CI：806,760～1,128,745)，内訳は男性571,210名(95％ CI：479,267～663,152)，女性396,543名(95％ CI：327,494～465,593)と見積もられている[2]．しかしながらHBVによる肝病態進展や肝発癌機序についてはいまだ不明な点が多く，その解明にはまず，本邦におけるB型肝炎の自然予後がどのようなものかを質の高いコホートで解析・検証する必要がある．これまで，キャリアにおける肝発癌関連因子としていくつかのウイルス側，宿主側因子などが報告されているものの，これらはcross-sectional studyあるいは病院患者コホートなどの限られた集団で検討されたものがほとんどである．

HCV感染と異なり，無症候性キャリア(ASC)が多く潜在するHBV感染は，無治療で経過を追った場合の肝発癌の頻度やHBs抗原自然消失，非感染者との生命予後に差異があるかどうかなど，感染に伴う自然予後の把握が難しい．

そこで本稿では，主に住民を対象としたコホート研究でこれまで明らかにされているB型肝炎の自然予後について，国内外の論文をもとに紹介したい．

2 アジアにおけるB型肝炎自然予後

Kusakabe，Mizokamiらは住民コホートを用いて，本邦におけるHBV単独感染者のHCC発生と関連するリスク因子を前向きコホートで解析している[3]．このコホートは，多目的コホート研究(JPHC研究[4,5])の一環で，全国6保健所管内に住む40～69歳の男女19,393人を平成17年まで(平均観察12.7年)に追跡した調査結果に基づくものであり，期間中110例の肝発癌を認めた．このうち13例がHBV単独感染例(図1)，78例がHCV単

Hisayoshi WATANABE et al：Long-term clinical outcomes in patients with hepatitis B: natural history population-based cohort studies
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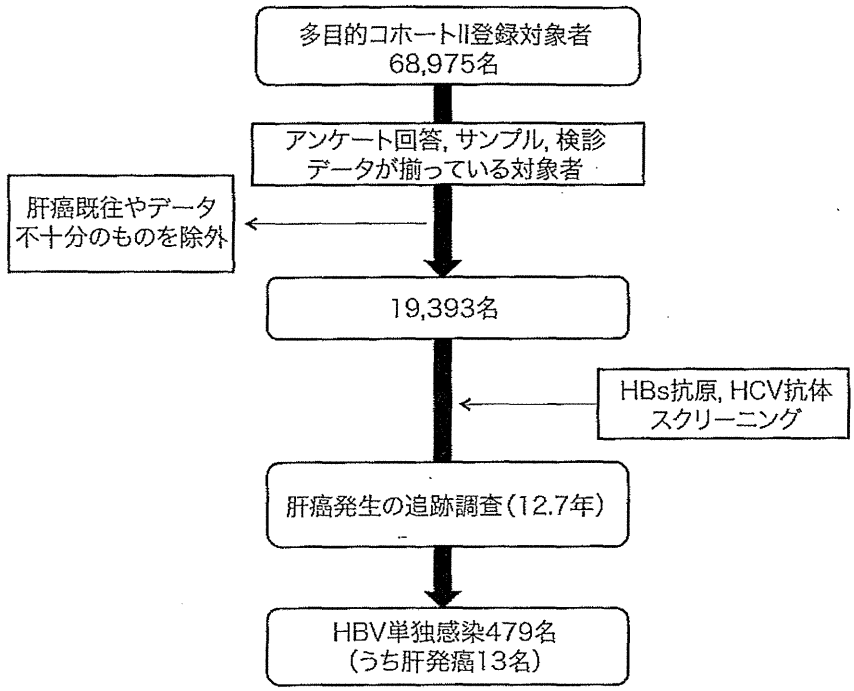

図1　コホート概要(文献3より引用改変)

表1　発癌例と非発癌例の比較(文献3より引用改変)

	HCC (n=13)	Non-HCC (n=466)	P
年齢	58.8±6.3	55.1±8.5	NS
男性	11 (85%)	209 (45%)	<0.005
BMI	22.3±2.9	23.4±3.0	NS
アルコール多量摂取	0	36 (8%) 92 (20%)	NS
喫煙	7 (54%)		<0.005
ALT (IU/L)	44.7±30.0	23.8±20.4	<0.005
γ-GTP (IU/L)	31.7±16.2	23.2±25.2	NS
HBe抗原陽性	3 (23%)	14 (3%)	<0.005
HBcr抗原 (kU/mL)	39,276±121,639	6,486±47,987	<0.005
HBcr抗原陽性	7 (54%)	99 (21%)	<0.005
HBV DNA (log copies/mL)	6.1	4.1	<0.005
HBV DNA ≧ 5 log copies	6 (46%)	39 (8%)	<0.005
Genotype B	4 (31%)	264 (57%)	NS
Genotype C	9 (69%)	202 (43%)	NS
C1653T	6 (46%)	116/421 (28%)	NS
T1753V	6 (46%)	78/421 (19%)	<0.005
A1762T/G1764A	11 (87%)	142/421 (34%)	<0.005
G1896A	11 (87%)	348/421 (83%)	NS

独感染例，2例がHBV・HCV共感染例，17例が非B非C例であった．HBV単独感染者における肝癌(HCC)例，非肝癌(Non-HCC)例の背景を比較すると，男性，喫煙者の割合，ALT値，HBe抗原陽性，HBcr抗原陽性，HBV DNA値がHCC群において有意に高く，

表2　HBV，HCV感染状況による肝発癌ハザード比(文献8より一部改変)

感染状態	対象	人年	HCC例	ハザード比(95% CI)
HBs抗原 (-) / HCV抗体 (-)	5,744	53.504	16	1.0 (reference)
HBs抗原 (+) / HCV抗体 (-)	335	2,981	15	17.1 (8.4～34.8)
HBs抗原 (-) / HCV抗体 (+)	360	3,731	12	10.4 (4.9～22.1)
HBs抗原 (+) / HCV抗体 (+)	14	133	3	115.0 (32.5～407.3)

表3　HBs抗原陰性化例の臨床背景の比較(文献9より一部改変)

	HBs抗原陰性化あり (n=20)	HBs抗原陰性化なし (n=81)	P
年齢(歳)	56	50	0.038
男性	8 (40%)	36 (44%)	>0.2
肝硬変合併	4 (20%)	15 (18%)	1.0
ALT (IU/L)	26	35	0.057
HBV genotype (A:B:C:不明)	0: 2: 18: 0	3: 7: 69: 2	>0.2
HBe抗原	3 (15%)	35 (43%)	0.02
HBs抗原 (Log IU/mL)	1.7	3.3	<0.001
HBcr抗原 (Log U/mL)	3.0	4.7	<0.001
HBV DNA (log copies/mL)	3.0	5.7	<0.001

core promotor二重変異(A1762T/G1764A)の頻度も高かった(表1).

さらにCox比例ハザードモデルにて肝発癌リスク因子を解析すると，core promotor二重変異のみが独立したHCCリスク因子であった(ハザード比7.05，95% CI 1.03-48.12，$P=0.046$)．患者コホートを用いたCore promotor二重変異とHCC発生リスクに関してはこれまでいくつか報告されてきたが[6,7]，この研究は本邦における住民ベースのHBV感染者のHCC発生リスクを調べた初めての大規模前向きコホート研究である．住民コホートという性格上，ほとんどの対象者はHBV健常キャリアと推定されるが，サンプル数をさらに増やした前向き研究が期待される．

韓国ではHBV高浸淫地域におけるHBV/HCV共感染例のHCCリスクについて報告がある[8]．6,694人を平均9.4年追跡し(63,170人年)，50例の発癌を認めている．HBV単独感染によるHCCハザード比は17.1 (95% CI: 7.3-24.4)，HCV単独感染によるHCCハザード比は10.4 (95% CI: 4.9-22.1)であり，HBV/HCV共感染によるHCCハザード比は115.0 (95% CI: 32.5-407.3)であった(表2).

一方，住民コホートではないものの，無治療のHBVキャリアの自然史についてHBs抗原量の長期経過の観点から報告がある[9]．Matsumotoらによると，1999年～2009年まで101例のHBVキャリアのHBs抗原量を追跡したところ，20例でHBs抗原が陰性化し(2.1%)，その陰性化は高齢と低ウイルス複製状態と関連していた(表3).

さらに韓国のAhnらは，病院コホートではあるが無治療のHBVキャリア432名のHBs抗原自然消失に関し，組織学的比較も加えて報告している[10]．19.6カ月の観察の間，9.5%にあたる49名でHBs抗原が自然消失した．

表4　Cox比例ハザードモデルによる肝硬変進展に影響する因子の解析(文献11より一部改変)

因子	30歳未満			30歳以上		
	リスク比	95% CI	P	リスク比	95% CI	P
男性/女性	0	—	0.972	0	0.000～3.993	0.969
HBe抗原						
陽性	1	0.028～6.469	0.538	1	0.185～3.250	0.727
陰性	2.36			1.29		
線維化						
Mild - Moderate	1	0.080～1.030	0.053	1	0.296～3.238	0.972
Severe	10.87			1.02		
Genotype						
B	1	0.019～2.883	0.258	1	0.033～0.916	0.039
C	4.24			5.75		

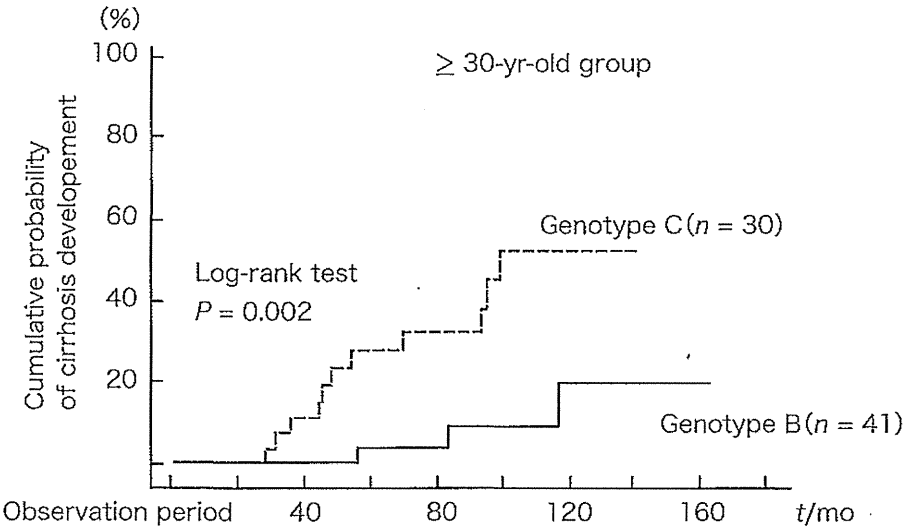

図2　30歳以上のHBV感染者における，genotypeによる肝硬変進展リスク(文献11より引用)

このうち15例においてHBs抗原消失前後で肝生検を施行したところ，血中のHBs抗原が陰性化しているにも関わらず，15例全例で肝組織中にHBV DNAが検出された．また肝組織中の壊死炎症反応は有意に改善されていたが($P<0.0001$)，肝線維化は有意なレベルまで改善されておらず($P=0.072$)，2例ではむしろ悪化していた．また観察期間中，肝発癌はHBs抗原消失49例中5例(10.2%)において認められ，肝硬変合併，周産期の感染，30年以上の長期間の感染歴，がリスク因子であったとされている．このことはHBs抗原消失後も肝線維化進展や肝発癌のリスクがあることを示すものであり，HBs抗原消失後のマネージメントに関する診療ガイドライン策定の必要性をうかがわせるものである．

HBV genotypeが肝硬変の進展にどのように影響するのか，genotype B感染が多い沖縄県からgenotype Cとの比較検討が報告されている[11]．それによると，B型慢性肝炎121例において，30歳未満では有意なLCへの進展予測因子は認めなかったが，30歳以上になるとgenotype Cであることが有意な予測因子となっていた(表4，図2)．特にHBe抗原陽性例では予後不良であることから，抗ウイルス治療の適応を考えるうえで示唆する所