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HBV and HCV infection in Japanese dental care workers

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Abstract. Protective measures against occupational exposure to the hepatitis B virus (HBV) and hepatitis C virus (HCV) must be taken in order to prevent infection in dental care workers. To determine the best way to protect these workers, our study examined viral hepatitis infection in dental care workers in regions with a high prevalence of HCV infections in Japan. In total, 141 dental care workers (including dentists, dental hygienists and dental assistants) were enrolled. After a questionnaire to elicit demographic information was administered by an oral surgeon, hepatitis B surface antigen (HBsAg), antibody to HBs (anti-HBs), antibody to hepatitis B core antigen (anti-HBc) and antibody to HCV (anti-HCV) were measured. When necessary, HBeAg, anti-HBe, levels of HBV DNA, anti-HBc IgM and HCV RNA in serum were measured. Of the dental care workers included, 68 (48.2%) had been immunized with a HBV vaccine. Only 9 wore a new pair of gloves for each new patient being treated, 36 changed to a new pair only after the old gloves were torn and 24 did not wear any gloves at all. No one was positive for HBsAg or anti-HCV, though 73 (51.8%) and 17 (12.1%) workers were respectively positive for anti-HBs and anti-HBc. The positive rate of anti-HBc varied directly with worker age and experience. Of the 68 workers immunized with HBV vaccine, 51 (75%) were positive for anti-HBs. Of the 63 workers who were not so immunized, 17 (27%) were positive for anti-HBs and 15 of these were also positive for anti-HBc. Immunized workers were more protected against HBV infection than non-immunized workers, indicating that HBV vaccine was a useful measure for protection against the infection. The anti-HBc positive rate was significantly higher among dental care workers than general blood donors, suggesting that frequency of exposure to HBV was greater in dental care workers. HBV vaccination should be made compulsory for all dental care workers who handle sharp instruments.

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Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; anti-HBs, antibody to HBsAg; anti-HBc, antibody to hepatitis B core antigen; HCV, hepatitis C virus; anti-HCV, anti-bodies to HCV; HCC, hepatocellular carcinoma; CLEIA, chemiluminescent enzyme immunoassay

Key words: dentists, hepatitis B virus, hepatitis C virus, vaccine

Introduction

It is important to protect dental care workers (who perform invasive procedures daily) from nosocomial, blood-transmissible infections of the hepatitis B virus (HBV) and hepatitis C virus (HCV). There are ~1.5 million persistent HBV carriers and 2 million persistent HCV carriers in Japan. These carriers may develop hepatocellular carcinoma (HCC) decades later. The incidence of HCC continues to increase in Japan and ~80 and 10% of HCC are due to HCV and HBV, respectively (1). Treatment methods for hepatitis C and hepatitis B are now well established, continue to improve annually and their effects are dramatic.

The worldwide HBV infection rate is higher in dentists than in the general population: 6 times higher in the USA, 4 times higher in Germany and 2.5 times higher in Japan. The incidence of HBV infection among dentists is 10.8% in Brazil (2), 9% in the USA (3) and 7% in Germany (4). Among medical care workers, dentists have the highest incidence of HBV infection and this incidence increases with the length of clinical experience of the dentist (5,6). An investigation conducted in 1978 in Japan found approximately half of dentists with 5 or more years of clinical experience were infected with HBV or had a history of HBV infection (7). A study of 998 dentists conducted in 17 regions throughout Japan from 1978 to 1982 reported that 37 (3.7%) were hepatitis B surface antigen (HBsAg)-positive and 420 (42.1%) were antibody to HBs (anti-HBs)-positive (8). The results indicated that infection occurred at work without the dentists' knowledge. Thus dental care workers should be advised to receive a hepatitis B vaccine and it should be confirmed if they have acquired immunity to HBV.

What is the HCV infection rate in dentists in Japan? The anti-HCV-positive rate was 2.6% (10/382) according to the seroepidemiological survey of Shinozaki *et al* who used frozen-preserved serum obtained from dentists between 1986 and 1994 (9). However, the status of HCV infection was unclear, as the mean age of subject dentists and other information were not recorded. In New York city, the positive rate of anti-HCV was clearly higher among oral surgeons (9.3%) and other dentists (1.75%) than blood donors (0.14%) (10). The finding shows that morbidity in dentists differs by specialties.

In recent years, the infection rate in dentists in Japan remains unclear. The last estimates were made in the 1980s and

1990s, before more sensitive tests became available. Viral levels have never been measured (7-9). The status of hepatitis viral infection in dental care workers in the northern part of Kyushu, where the infection rates are the highest in Japan should be determined in order to assess the extent to which further health measures are needed to protect and maintain the health of dental care workers.

The present study screened for the presence of HBV and HCV infections in dental care workers in the Fukuoka prefecture (northern Kyushu). Since viral hepatitis is treatable, this investigation could contribute to the health maintenance of dental care workers.

Patients and methods

Patients. Participants included 141 dentists belonging to the X Dental Association in the Fukuoka prefecture and dental care workers (dental hygienists, assistants, mechanics and clerks) employed at dental clinics. Each member was notified by mail about the study before the examination. The examination was performed on 2 days (September 22 and 27, 2007).

Methods. Each participant gave informed consent and had a blood sample taken. An oral surgery specialist interviewed the subjects. Items of inquiry included gender, age, occupation, years employed as a dental care worker, disposable glove use, history of jaundice, history of blood transfusion, clinical history of liver diseases, family history of liver disease and hepatitis B vaccination status.

Viral markers of hepatitis were measured by chemiluminescent enzyme immunoassay (CLEIA) including HBsAg, anti-HBs and anti-HBc and by solid phase RIA including anti-HCV. When the serum was HBsAg-positive, HBeAg (CLEIA), anti-HBe (CLEIA), HBV DNA level (PCR method) and HBV genotype (PCR method) were assayed; when the serum was anti-HBc-positive, the anti-HBc IgM and HBV DNA level were assayed; and when the serum was anti-HCV-positive, RT-PCR was carried out to determine quantitative HCV RNA and HCV genotype.

Results were mailed to each participant. Ethical guidelines for the research were observed closely in order to protect participant confidentiality.

Results

There were 141 (43 males and 98 females) participants. Table I shows 43 were in their 20s, 35 in their 30s, 36 in their 40s, 17 in their 50s, 7 in their 60s, 2 in their 70s and 1 in his 80s. There were 42 dentists, 35 dental hygienists, 41 dental assistants, 8 dental mechanics and 15 clerks. Six subjects had a clinical history of liver disease that was unrelated to HBV or HCV infection.

As for hepatitis B vaccination, 68 (48.2%) were and 63 (44.7%) were not vaccinated. Dentists were the largest vaccinated group (39.7%, 27/68) and dental assistants were the largest unvaccinated group (34.9%, 22/63).

Regarding disposable glove use, only 9 people reported use of new gloves with every new patient. The highest number of people (36/141) said that they changed gloves only when the old pair of gloves were torn. Twenty-four workers did not wear gloves.

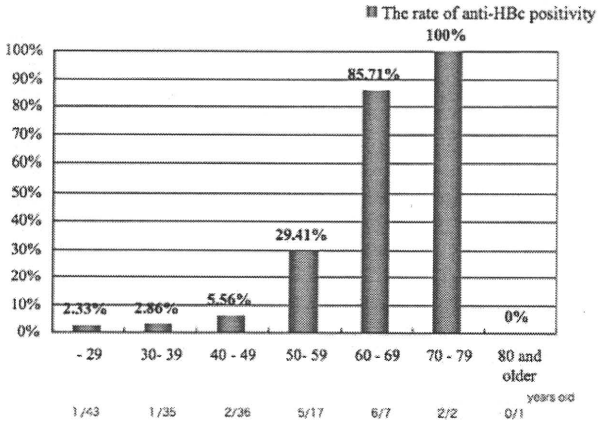

Figure 1. The rate of anti-HBc in 141 subjects classified according to age brackets. The rate of anti-HBc positivity increased with increased age.

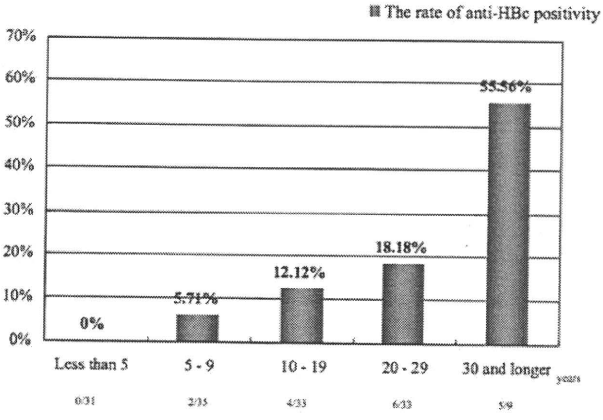

Figure 2. The rate of anti-HBc in 141 subjects classified according to years of experience in dental care. The rate of anti-HBc positivity increased with the number of years of dental care experience.

In hematological tests, no subjects were HBsAg-positive or anti-HCV-positive (Table II). However, 73 (51.8%) subjects were anti-HBs-positive and 17 (12.1%) were anti-HBc-positive. The rates of anti-HBc positivity increased with age: 85.7% of subjects in their 60s and 100% of subjects in their 70s (Fig. 1). The rate of anti-HBc positivity increased with the number of years of dental care experience (Fig. 2). As Table II shows, anti-HBs turned positive, indicating vaccine effectiveness, in 75% (51/68) of the vaccinated group and 27% (17/63) of the unvaccinated group. Fifteen of these 17 were anti-HBc-positive, indicating that these 15 were infected with HBV in the past.

Most (52.9%) of the 17 anti-HBc-positive subjects were dentists (Table III). The largest proportion of the anti-HBc-positive subjects were in their 60s (35.3%) and had 20 years of experience working in dentistry. Sixteen of the HBc-positive subjects (94.1%) were anti-HBs-positive. However, no HBV DNA was detected in the blood.

Table I. Background factors of 141 subjects classified by hepatitis B vaccination status.

	Total	Vaccination yes		Vaccination no		During vaccination (or Drop-out)		Unknown	
	(n)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
		68	48.2	63	44.7	4	2.8	6	4.3
Sex									
Male	43	25	36.8	15	23.8	2	50.0	1	16.7
Female	98	43	63.2	48	76.2	2	50.0	5	83.3
Age									
29 years old	43	18	26.5	19	30.2	2	50.0	4	66.7
30-39	35	20	29.4	13	20.6	0	0.0	2	33.3
40-49	36	24	35.3	12	19.0	0	0.0	0	0.0
50-59	17	4	5.9	11	17.5	2	50.0	0	0.0
60-69	7	2	2.9	5	7.9	0	0.0	0	0.0
70-79	2	0	0.0	2	3.2	0	0.0	0	0.0
80 and older	1	0	0.0	1	1.6	0	0.0	0	0.0
Type of occupation									
Dentist	42	27	39.7	12	19.0	2	50.0	1	16.7
Dental hygienist	35	19	27.9	14	22.2	0	0.0	2	33.3
Dental assistant	41	15	22.1	22	34.9	2	50.0	2	33.3
Dental mechanic	8	4	5.9	4	6.3	0	0.0	0	0.0
Clerk	15	3	4.4	11	17.5	0	0.0	1	16.7
Years engaged in dental care									
<5 years	31	10	14.7	16	25.4	1	25.0	4	66.7
5-9	35	18	26.5	14	22.2	1	25.0	2	33.3
10-19	33	21	30.9	12	19.0	0	0.0	0	0.0
20-29	33	17	25.0	15	23.8	1	25.0	0	0.0
30 and longer	9	2	2.9	6	9.5	1	25.0	0	0.0
How to equip oneself with disposable gloves (Plural answers were given)									
Wear new pair with every new patient	9	6	8.8	3	4.8	0	0.0	0	0.0
Wear new pair with every 2 to 3 patients	31	19	27.9	9	14.3	1	25.0	2	33.3
Wear new pair when old one is torn	36	18	26.5	15	23.8	1	25.0	2	33.3
Wear new pair about twice a day	7	5	7.4	2	3.2	0	0.0	0	0.0

Table I. Continued.

	Total (n)	Vaccination yes (n)	(%)	Vaccination no (n)	(%)	During vaccination (or Drop-out) (n)	(%)	Unknown (n)	(%)
	68	48.2	63	44.7	4	2.8	6	4.3	
Wear when invasive treatment is performed	30	14	20.6	15	23.8	0	0.0	1	16.7
Wear when infected patients are treated	29	8	11.8	19	30.2	1	25.0	1	16.7
Wear when instrument is washed	2	0	0.0	2	3.2	0	0.0	0	0.0
Do not use	24	10	15	12	19	1	25.0	1	17
History of jaundice									
Yes	1	1	1.5	0	0.0	0	0.0	0	0.0
No	133	66	97.1	57	90.5	4	100.0	6	100.0
Unknown	7	1	1.5	6	9.5	0	0.0	0	0.0
History of blood transfusion									
Yes	5	1	1.5	4	6.3	0	0.0	0	0.0
No	133	66	97.1	57	90.5	4	100.0	6	100.0
Unknown	3	1	1.5	2	3.2	0	0.0	0	0.0
Clinical history of liver diseases									
Yes	6[a]	5	7.4	1	1.6	0	0.0	0	0.0
No	135	63	92.6	62	98.4	4	100.0	6	100.0
Unknown	0	0	0.0	0	0.0	0	0.0	0	0.0
Family history of liver diseases									
Yes	9	4	5.9	4	6.3	1	25.0	0	0.0
No	124	63	92.6	54	85.7	3	75.0	4	66.7
Unknown	8	1	1.5	5	7.9	0	0.0	2	33.3

[a]Fatty liver (n=3), acute hepatitis A (n=1), primary biliary cirrhosis (n=1) and hepatitis (unknown cause).

Table II. Hepatitis virus markers classified by hepatitis B vaccination status.

Hepatitis B virus markers	Total	Vaccination yes (n)	(%)	Vaccination no (n)	(%)	During vaccination (or Drop-out) (n)	(%)	Unknown (n)	(%)
		68	48.2	63	44.7	4	2.8	6	4.3
HBsAg									
Positive (+)	0	0	0.0	0	0.0	0	0.0	0	0.0
Negative (-)	141	68	0.0	63	0.0	4	0.0	6	0.0
Anti-HBs									
Positive (+)	73	51	75.0	17	27.0	2	50.0	3	50.0
Negative (-)	68	17	25.0	46	73.0	2	50.0	3	50.0
Anti-HBc									
Positive (+)	17	1	1.5	16	25.4	0	0.0	0	0.0
Negative (-)	124	67	98.5	47	74.6	4	100.0	6	100.0
Anti-HBs positive (+)									
Anti-HBc positive (+)	16	1	1.5	15	23.8	0	0.0	0	0.0
Anti-HBc negative (-)	57	50	73.5	2	3.2	2	50.0	3	50.0
Anti-HBs negative (-)									
Anti-HBc positive (+)	1	0	0.0	1	1.6	0	0.0	0	0.0
Anti-HBc negative (-)	67	17	25.0	45	71.4	2	50.0	3	50.0
Anti-HCV									
Positive (+)	0	0	0.0	0	0.0	0	0.0	0	0.0
Negative (-)	141	68	0.0	63	0.0	4	0.0	6	0.0

Table III. Breakdown of results classified by anti-HBc (+) and anti-HBc (-).

	Subjects with anti-HBc (+) 17		Subjects with anti-HBc (-) 124	
	(n)	(%)	(n)	(%)
Hepatitis B vaccine				
Vaccination yes	1	5.9	67	54.0
Vaccination no	16	94.1	47	37.9
During vaccination (or Drop-out)	0	0.0	4	3.2
Unknown	0	0.0	6	4.8
Sex				
Male	9	52.9	34	27.4
Female	8	47.1	90	72.6
Age				
29 years old	1	5.9	42	33.9
30-39	1	5.9	34	27.4
40-49	2	11.8	34	27.4
50-59	5	29.4	12	9.7
60-69	6	35.3	1	0.8
70-79	2	11.8	0	0.0
80 and older	0	0.0	1	0.8
Type of occupation				
Dentist	9	52.9	33	26.6
Dental hygienist	2	11.8	33	26.6
Dental assistant	2	11.8	39	31.5
Dental mechanic	0	0.0	8	6.5
Clerk	4	23.5	11	8.9
Years engaged in dental care				
<5 years	0	0.0	31	25.0
5-9	2	11.8	33	26.6
10-19	4	23.5	29	23.4
20-29	6	35.3	27	21.8
30 and longer	5	29.4	4	3.2
How to equip oneself with disposable gloves (Plural answers were given)				
Wear new pair with every new patient	1	5.9	8	6.5
Wear new pair with each 2 to 3 patients	1	5.9	30	24.2
Wear new pair when old one is torn	0	0.0	36	29.0
Wear new pair about twice a day	0	0.0	7	5.6
Wear when invasive treatment is performed	7	41.2	23	18.5
Wear when infected patients are treated	9	52.9	20	16.1
Wear when instrument is washed	0	0.0	2	1.6
Do not use	4	23.5	20	16.1
History of jaundice				
Yes	0	0.0	1	0.8
No	17	100.0	116	93.5
Unknown	0	0.0	7	5.6
History of blood transfusion				
Yes	1	5.9	4	3.2
No	15	88.2	118	95.2
Unknown	1	5.9	92	1.6

Table III. Continued.

	Subjects with anti-HBc (+) 17		Subjects with anti-HBc (-) 124	
	(n)	(%)	(n)	(%)
Clinical history of liver diseases				
Yes	1	5.9	5	4.0
No	16	94.1	119	96.0
Unknown	0	0.0	0	0.0
Family history of liver diseases				
Yes	2	11.8	7	5.6
No	15	88.2	109	87.9
Unknown	0	0.0	8	6.5
HBsAg				
Positive (+)	0	0.0	0	0.0
Negative (-)	17	100.0	124	100.0
Anti-HBs				
Positive (+)	16	94.1	57	46.0
Negative (-)	1	5.9	67	54.0
Anti-IgM-HBc				
Positive (+)	0	0.0	-	-
Negative (-)	17	100.0	-	-
HBV DNA quantitative measurement				
≥2.6 log/ml	0	0.0	-	-
<2.6 log/ml	17	100.0	-	-

Discussion

HBV infection is transmitted mostly through blood and body fluid as a result of bites, administration of blood preparations, sexual activities and mother-infant transmission. The principal route of HCV infection is through blood. Medical care workers are always at risk of infection as they are exposed to contaminated fluids from needle sticks and infected blood droplets. Hepatitis B immune globulin (HBIG) has been used since 1981 and hepatitis B vaccination since 1985 (whole virus) and 1988 (recombinant) to prevent infection.

Dental care workers are often exposed to blood because of stomatorrhagia and the use of sharp instruments (11). Meticulous measures should be taken to protect against the spraying of saliva, which contains blood inside the examination room (12,13). Our previous study reported that saliva from HCV carriers contained HCV RNA before and after scaling of dental calculus (14). HCV RNA was detected in exudates from gingival crevicular fluid and on materials used for making dental impressions, a work bench, an air turbine dental handpiece, holders, suction units, forceps, dental mirrors and cutting bar (15-17). HCV RNA was still detectable on the surface of dental instruments several days after the HCV carriers received treatment (18). Although their risk of infection is high, dental care workers are obligated to prevent cross infection (i.e., from dental care workers to patients and patients to patients). Although there are no documented cases of HCV transmission from dentists to patients, there is one case of the transmission of HBV by an oral surgeon (19).

Whether the disease is contracted depends on the levels of the virus in the blood, source of contamination, route of contact and blood volume transfused (20). The rate of acquiring HBV infection through HBV-contaminated needles is high [12% (21) to 60% (22) in unvaccinated persons]. Wounds caused by needles that are contaminated with HBsAg- and HBeAg-positive blood are associated with a 22-31% risk of developing hepatitis B and a 37-62% probability of establishing HBV infection (23). Wounds caused by needles contaminated with HBsAg-positive and HBeAg-negative blood are associated with a 1-6% risk of developing hepatitis B and a 23-37% probability of establishing HBV infection (21). However, infection can be prevented by HB vaccination and the administration of HBIG after these accidents occur.

Accidental prick with a needle contaminated with HCV-positive blood caused HCV infection in ~1.4 (24) to 10% (25) of cases. The probability of infection due to contaminated needle sticks is lower for HCV than HBV. However, the high risk of developing HCC through horizontal infection of HCV is a concern to often-exposed dentists. Feldman and Schiff found that hepatitis morbidity was 6.7% in dentists and 21% in oral surgeons in the State of Florida, USA (26). Although the risk of hepatitis among dentists is high, a long-term cohort study by Tanaka *et al* reported that liver cancer risk was no higher in Japanese dentists than in the general population (27).

In the present investigation, 51 of the 68 recipients of the HBV vaccine were anti-HBs-positive, indicating that 75% of vaccinated subjects developed an antibody to HBV infection. Of the 63 unvaccinated subjects, 16 (25.4%) were anti-HBc-positive and had no clinical history of HBV-related liver diseases, suggesting that they had been transiently and inapparently infected with HBV in the past. Only 1 (1.5%) of the 68 vaccinated subjects was anti-HBc-positive, indicating the protection rate against HBV infection was higher in vaccinated than unvaccinated subjects and that vaccination was a useful protective measure.

The Japanese Red Cross introduced the Hemagglutination Inhibition Test (HI) in 1989 for the screening of anti-HBc (28) and the Nucleic Acid Amplification Test (NAT) in 1999 for the screening of HBV, HCV and HIV in blood that was HBsAg-, anti-HBc-, anti-HCV- and anti-HIV-negative with ALT values <61 IU/l, dramatically increasing the safety of blood transfusion (29).

In Fukuoka and Kitakyushu Red Cross, 3,647 (1.1%) of 323,799 blood donors screened between April 2003 and October 2004 were anti-HBc-positive. Of these 3,647, a total of 445 were HBsAg-positive (30). In the remaining 3,202 anti-HBc-positive, HBsAg-negative donors, the rates of seroconversion to anti-HBc increased with age (0.10, 0.23, 0.57, 1.38, 2.10 and 2.29%, respectively, in age groups 16-19, 20-29, 30-39, 40-49, 50-59 and 60-69).

Seroconversion to anti-HBc occurred at a significantly higher rate in dental care workers (12.1%) than blood donors ($p<0.05$).

Anti-HBc is a marker of latent hepatitis B (31,32). In previous years, it has been reported that HBV infection was transmitted through a liver transplanted from an anti-HBc-positive donor (32). HBV DNA has been detected in the serum of patients recovered from acute hepatitis B (33). Infection of latent HBV has been associated with the onset of HCV-related HCC (34,35). Therefore, from the standpoint of health safety, the prevalence of latent HBV infection among dental care workers must be acknowledged.

Of the 63 unvaccinated subjects, only 4.8% changed gloves to a new pair for each new patient and 19% never wore gloves. Since dental care workers have a high risk of exposure to the hepatitis virus, a compulsory vaccination for the hepatitis B virus is desirable for all dental care workers. In Japan, hepatitis B vaccination is voluntary. However, from the standpoint of effectiveness and safety and to reduce infection risk, it is important to vaccinate these workers.

Regrettably, no hepatitis C vaccine or immunoglobulin has been developed to prevent HCV infection. Although no persistent carriers of HBV and HCV were detected in the present investigation, the rate of infection is higher in the western portion of Japan, especially in the Saga and Fukuoka prefectures, than eastern Japan. Therefore, further precautions must be taken.

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Branched-chain amino acid-enriched supplementation improves insulin resistance in patients with chronic liver disease

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Abstract. Increased insulin resistance is a therapeutic target in patients with chronic liver disease. Branched-chain amino acids (BCAA) have been reported to improve insulin resistance in *in vivo* experiments. Thus, we investigated the effects of BCAA on insulin resistance in patients with chronic liver disease. Twelve patients with chronic liver disease were enrolled. Each patient was given one sachet of a BCAA-enriched supplement after breakfast and another at bedtime. The effects of the BCAA-enriched supplementation on insulin resistance were examined 30, 60 and 90 days after administration by the homeostasis model assessment method for insulin resistance (HOMA-IR) and for ß cell function (HOMA-%B). The HOMA-IR and HOMA-%B values were elevated at baseline, however, these parameters showed no significant changes after administration of the BCAA-enriched supplement in the overall patient population. By stratification via gender, patients in the male group showed a significantly greater elevation in the HOMA-IR value compared to the female patients at baseline. After the administration, the HOMA-IR and HOMA-%B values were significantly decreased only in the male group (9.4±4.8 vs. 2.4±0.7, 657±345 vs. 126±36, respectively; $P<0.05$). We found that there was a gender difference in chronic viral liver disease-related insulin resistance. Moreover, a BCAA-enriched supplement improved insulin resistance and ß cell function in male patients with chronic viral liver disease. Thus, a BCAA-enriched supplement may be a useful therapeutic agent for decreasing insulin resistance in male patients with chronic viral liver disease.

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Introduction

The liver is one of the major target organs of insulin and chronic liver disease is associated with insulin resistance (1-3). Increased insulin resistance is related to the progression of hepatic fibrosis (4), development of hepatocellular carcinoma (HCC) (5,6) and reduction in long-term survival (6,7). Thus, the increase in insulin resistance is an important therapeutic target in patients at any stage of chronic liver disease.

Insulin resistance is treated by dietary modification, physical activity and/or drugs (8). However, a sufficient energy intake is required for patients with liver cirrhosis and dietary restrictions may lead to a decrease in liver function (9,10). Although physical activity is not restricted in compensated cirrhotic patients (11), such individuals often complain of fatigue, thus adequate exercise is not always possible. Biguanides and thiazolidinediones are insulin sensitizing agents and are currently utilized for the reduction of insulin resistance (12,13). However, it is not always possible to use these drugs in cirrhotic patients due to adverse effects, including lactic acidosis, fluid retention and severe hepatotoxicity (14,15).

Decreases in serum branched-chain amino acids (BCAA) levels are often seen in patients with chronic liver disease and lead to a decline of detoxified ammonia and albumin production. Therefore, BCAA are used for the treatment of hepatic encephalopathy and hypoalbuminemia (16,17). Previously, BCAA have been reported to modulate insulin signaling in an *in vivo* study. BCAA cause glucose up-take in the skeletal muscle, adipocytes and hepatocytes in rodents and in a rat model of liver cirrhosis (18-21). In addition, BCAA are known to up-regulate the mammalian target of rapamycin (mTOR), which cross-talks with intracellular insulin signaling (22,23). Taken together, these previous studies imply that BCAA improve glucose metabolism through the reduction of insulin resistance. In this study, we examined the effects of BCAA on insulin resistance in patients with chronic viral liver disease.

Materials and methods

A prospective, consecutive-patient entry study was conducted. Eligibility criteria were chronic viral liver disease with

Table I. Contents of one sachet of BCAA-enriched supplement (Aminofeel®).

Substance	Amount
BCAA	3200.0 mg
Valine	800.0 mg
Leucine	1600.0 mg
Isoleucine	800.0 mg
Calcium	22.1 mg
Magnesium	12.6 mg
Zinc	5.0 mg
Copper	0.2 mg
Selenium	49.6 μg
Chromium	14.4 μg
Pantothenic acid calcium	6.8 mg
Vitamin A	315.0 μg
Vitamin B1	2.4 mg
Vitamin B2	2.6 mg
Vitamin B6	2.4 mg
Vitamin B12	10.0 μg
Folic acid	0.2 mg
Vitamin C	40.0 mg
Vitamin D3	3.0 μg
Vitamin E	6.4 mg
Vitamin K2	29.6 μg
Niacin	12.0 mg

Table II. Characteristics of patients.

	Normal range	All patients
n		12
Age	n/a	64.3±2.4
BMI (kg/m^2)	18.5-25.0	24.3±0.6
Fat%BW (%)	20-27	32.8±2.4
Muscle%BW (%)	40-50	36.0±1.6
Visceral fat area (cm^2)	<100	118.2±14.9
Hemoglobin (g/dl)	14.0-18.0	13.6±0.4
White blood cell (μl)	4000-9000	4358±414
Platelet count (x10^4/μl)	13-36	15.3±1.6
AST (U/l)	12-33	43.1±5.6
ALT (U/l)	8-42	39.8±7.3
LDH (U/l)	119-229	190.8±13.0
γ-GTP (U/l)	10-47	49.7±8.3
Total protein (g/dl)	6.7-8.3	7.7±0.2
Albumin (g/dl)	4.0-5.0	3.8±0.1
BTR	4.4-10.1	5.1±0.4
BCAAs (μmol/l)	344-713	435.6±27.5
Tyrosine (μmol/l)	51-98	90.1±7.9
Cholinesterase (U/l)	214-466	155.8±16.1
Total bilirubin (mg/dl)	0.3-1.5	0.9±0.1
Total cholesterol (mg/dl)	128.0-220.0	178.5±9.1
Fasting glucose (mg/dl)	80.0-109.0	104.5±6.4
HbA1c (%)	4.3-5.8	5.5±0.2
IRI (μU/ml)	5.0-20.0	22.8±9.7
HOMA-IR	<5.4	5.5±2.1
HOMA-%B	>156.5	326.4±159.1
Zinc (μg/dl)	80-130	82.9±6.1

The values are expressed as mean ± standard error. BMI, body mass index; BW, body weight; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; γ-GTP, γ-glutamyl transpeptidase; BTR, BCAAs tyrosine ratio; BCAAs, branched-chain amino acids; HbA1c, hemoglobinA1c; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment method for insulin resistance; HOMA-%B, homeostasis model assessment method for ß cell function; n/a, not applicable.

sufficient food intake and serum albumin concentration >3.5 g/dl and <4.0 g/dl. Patients with hepatic encephalopathy, ascites, HCC or renal failure were excluded. A total of 12 patients with HCV-related chronic liver disease (n=11), or HBV-related chronic liver disease (n=1) were enrolled in this study from August 2006 to June 2007 at Kurume University Hospital. The diagnosis of liver disease was based on clinical, serological, imaging and/or histological evidence. The patients were treated as outpatients and no therapeutic interventions such as changes in eating habits and physical activity were made in the patients' life-style after entering the study. Informed consent for participation in the study was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a prior approval by the Ethics Committee of the Kurume University School of Medicine. None of the subjects were institutionalized.

Study design. Each patient was given one sachet of a BCAA-enriched supplement (Aminofeel®, Seikatsu Bunkasya Co. Inc, Chiba, Japan) after breakfast and another at bedtime. The contents of this supplement are summarized in Table I. Using blood biochemical tests, effects on liver function including glucose metabolism, derived from the administration of the BCAA-enriched supplement were examined at 30, 60 and 90 days.

Measurements of body composition and visceral fat area. Body fat and skeletal muscle were evaluated by an eight-polar direct segmental multifrequency-bioelectrical impedance analyzer (DSM-BIA; InBody 3.2, Biospace, Tokyo, Japan) before and after 90 days of administration of the BCAA-enriched supplement and were expressed as fat%body weight (fat%BW) and muscle%body weight (muscle%BW), respectively. Visceral fat area was measured by a DSM-BIA

Table III. Effects of BCAA-enriched supplementation on body composition, protein, lipid and glucose metabolism.

	Before	Administration of BCAA-enriched supplement		
		30 days	60 days	90 days
BMI	24.3±0.6	n/a	n/a	24.2±0.8
Fat%BW	32.8±2.4	n/a	n/a	32.6±2.4
Muscle%BW	36.0±1.6	n/a	n/a	36.1±1.6
Visceral fat area	118.2±14.9	n/a	n/a	110.9±6.2
BCAAs (μmol/l)	435.6±27.5	512.5±45.3	506.6±24.3	527.3±47.1
Tyrosine (μmol/l)	90.1±7.9	84.2±7.0	77.0±7.0[a]	89.1±7.1
BTR	5.1±0.4	6.6±0.8[a]	7.2±0.9[b]	6.4±0.7
Zinc (μg/dl)	82.9±6.1	108.3±6.9[a]	106.8±5.6[a]	103.3±6.9[a]
ALT (U/l)	39.8±7.3	43.8±7.0	42.5±7.5	42.1±6.1
Total protein (g/dl)	7.8±0.2	7.7±0.2	7.6±0.1	7.6±0.2
Albumin (g/dl)	3.8±0.1	3.9±0.1	3.9±0.1[a]	3.9±0.1
Cholinesterase (U/l)	155.8±16.1	154.1±13.8	170.7±18.3	170.1±18.5
Fasting glucose (mg/dl)	104.5±6.4	103.9±6.1	101.8±5.3	102.8±5.4
HbA1c (%)	5.5±0.2	5.5±0.2	5.5±0.2	5.4±0.3
IRI (μU/ml)	22.8±9.7	12.7±1.4	10.8±1.5	13.3±1.9
HOMA-IR	5.5±2.1	3.3±0.4	2.8±0.5	3.5±0.6
HOMA-%B	326.4±6.4	127.5±17.6	112.0±16.8	140.8±28.8
Total cholesterol (mg/dl)	178.5±9.1	172.8±9.4	178.6±10.0	173.1±7.2
Total bilirubin (mg/dl)	0.9±0.1	1.0±0.1	0.9±0.1	0.9±0.1

The values are expressed as mean ± standard error. Statistical comparisons between before and after 30, 60 or 90 days of the administration were performed by Wilcoxon's test. [a]P<0.05 and [b]P<0.01. BMI, body mass index; BW, body weight; BCAAs, branched-chain amino acids; BTR, BCAAs tyrosine ratio; ALT, alanine aminotransferase; HbA1c, hemoglobinA1c; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment method for insulin resistance; HOMA-%B, homeostasis model assessment method for ß cell function; n/a, not applicable.

before and after 90 days of administration of the BCAA-enriched supplement. The accuracy of the DSM-BIA analyzer has been reported (24).

Laboratory determinations. Venous blood samples were obtained in the morning after an overnight fast. Complete blood cell counts and levels of serum aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, γ-glutamyl transpeptidase, total protein, albumin, BCAA, tyrosine, cholinesterase, total bilirubin, total cholesterol, immunoreactive insulin (IRI), zinc, plasma glucose and HbA1c were measured by standard clinical methods (Department of Clinical Laboratory, Kurume University Hospital) as previously described (25,26). BCAA tyrosine ratio (BTR) was calculated as BCAA/tyrosine.

Evaluation for insulin resistance and ß cell function. Insulin resistance and ß cell function were evaluated on the basis of fasting levels of plasma glucose and insulin, according to the homeostasis model assessment (HOMA) method (27). The formulas used for the HOMA model are as follows: Insulin resistance (HOMA-IR) = fasting glucose (mg/dl) x fasting insulin (μU/ml)/405; ß cell function (HOMA-%B) = fasting insulin (μU/ml) x 360/[fasting glucose (mg/dl) - 63].

Statistical analysis. All data are expressed as mean ± standard error. Differences between the two groups were analyzed using the Mann-Whitney U test. Statistical comparisons between before administration of the BCAA-enriched supplement and after 30, 60, or 90 days, were performed by Wilcoxon's test. P-values <0.05 were considered significant.

Results

Patient characteristics. Patient characteristics prior to BCAA-enriched supplementation administration are summarized in Table II. Serum aspartate aminotransferase and γ-glutamyl transpeptidase levels were elevated in comparison to normal limits. Serum albumin and cholinesterase levels were decreased. Although the BMI value and the levels of fasting plasma glucose and HbA1c were within normal limits, the values of fat%BW, visceral fat area, serum IRI, HOMA-IR, and HOMA-%B were elevated.

Effects of BCAA-enriched supplement on body composition, protein, lipid and glucose metabolism. The effects on body composition, protein, lipid and glucose metabolism as a result of administering the BCAA-enriched supplementation are summarized in Table III. There were no significant changes on body composition between before and after 90 days

Table IV. Characteristics of male and female groups.

	Normal range	Male group	Female group	P
n		5	7	
Age	n/a	61.2±3.4	66.4±3.3	0.29
BMI (kg/m^2)	18.5-25.0	24.8±1.1	23.9±0.8	0.29
Fat%BW (%)	20-27	23.2±3.0	35.5±1.6	0.002
Muscle%BW (%)	40-50	42.9±1.7	34.0±0.9	0.002
Visceral Fat Area (cm^2)	>100	111.0±12.9	120.3±18.4	0.66
Hemoglobin (g/dl)	14.0-18.0	14.4±0.7	13.0±0.5	0.17
White blood cell (/μl)	4000-9000	4040±738	4585±508	0.46
Platelet count (x10^4/μl)	13-36	11.8±1.5	17.9±2.0	0.06
AST (U/l)	12-33	43.6±6.5	42.7±8.8	0.46
ALT (U/l)	8-42	51.8±15.7	31.8±4.5	0.46
LDH (U/l)	119-229	177.0±27.4	205.2±10.8	0.56
γ-GTP (U/l)	10-47	50.6±8.1	49.0±13.5	0.68
Total protein (g/dl)	6.7-8.3	7.9±0.3	7.6±0.2	0.46
Albumin (g/dl)	4.0-5.0	3.8±0.1	3.8±0.1	0.57
BTR	4.4-10.1	4.6±0.6	5.5±0.5	0.29
BCAAs (μmol/l)	344-713	509.1±38.7	383.1±23.7	0.03
Tyrosine (μmol/l)	51-98	115.0±38.7	72.3±4.8	0.02
Cholinesterase (U/l)	214-466	171.6±36.4	144.6±11.5	0.80
Total bilirubin (mg/dl)	0.3-1.5	1.0±0.3	0.8±0.1	0.80
Total cholesterol (mg/dl)	128.0-220.0	160.2±9.9	191.5±12.0	0.10
Fasting glucose (mg/dl)	80.0-109.0	105.2±14.9	104.0±5.0	0.51
HbA1c (%)	4.3-5.8	6.0±0.4	5.2±0.1	0.17
IRI (μU/ml)	5.0-20.0	40.4±22.0	10.2±1.8	0.04
HOMA-IR	<5.4	9.4±4.8	2.7±0.6	0.03
HOMA-%B	>156.5	657.6±345.8	89.8±14.4	0.17
Zinc (μg/dl)	80-130	81.8±10.9	83.7±7.8	0.46

The values are expressed as mean ± standard error. Differences between the two groups were analyzed using the Mann-Whitney U test. BMI, body mass index; BW, body weight; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; γ-GTP, γ-glutamyl transpeptidase; BTR, BCAAs tyrosine ratio; BCAAs, branched-chain amino acids; HbA1c, hemoglobinA1c; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment method for insulin resistance; HOMA-%B, homeostasis model assessment method for ß cell function; n/a, not applicable.

of the administration of the BCAA-enriched supplement. Although serum BCAA levels were not significantly increased, the BTR value was significantly increased 30 and 60 days after administration of the BCAA-enriched supplement. The serum zinc level was significantly increased 30, 60 and 90 days after the administration and the serum albumin level was also significantly increased 60 days after the administration. There were no significant changes in fasting plasma glucose, HbA1c, IRI levels, HOMA-IR or HOMA-%B values after administration of the BCAA-enriched supplement.

Characteristics of the male and female groups. Characteristics of the male and female groups before administration of the BCAA-enriched supplement are summarized in Table IV. Fat%BW value was significantly higher in the female group than that in the male group. Muscle%BW value was significantly higher in the male group than that in the female group. Serum BCAAs and tyrosine levels were significantly higher in the male group than those in the female group. Although fasting plasma glucose and HbA1c levels were not significantly different between the two groups, serum IRI level and HOMA-IR values were significantly higher in the male group than those in the female group prior to supplement administration.

Effects of BCAA-enriched supplement on body composition, protein and lipid metabolism in the male and female groups. The effects of administering the BCAA-enriched supplement on body composition, protein and lipid metabolisms in the male and female groups are summarized in Tables V and VI, respectively. There were no significant changes on body composition between before and after 90 days of administration of the BCAA-enriched supplement.

Table V. Effects of BCAA-enriched supplementation on protein and lipid metabolism in the male group.

	Before	Administration of BCAA-enriched supplement		
		30 days	60 days	90 days
BMI	24.8±1.1	n/a	n/a	24.4±2.1
Fat%BW	23.2±3.0	n/a	n/a	23.1±3.1
Muscle%BW	42.9±1.7	n/a	n/a	42.8±1.9
Visceral fat area	111.0±12.9	n/a	n/a	109.1±14.1
BCAA (μmol/l)	509.1±38.7	569.4±98.0	488.9±35.4	437.4±44.0
Tyrosine (μmol/l)	115.0±10.0	103.7±6.8	94.2±11.5[a]	102.0±9.8
BTR	4.6±0.6	5.7±1.2	5.5±0.7[a]	4.4±0.5
Zinc (μg/dl)	81.8±10.9	116.0±15.0[a]	97.4±4.0	87.4±6.6
ALT (U/l)	51.8±15.7	55.6±14.6	54.0±16.7	53.2±13.2
Total protein (g/dl)	7.9±0.3	7.7±0.2	7.7±0.2	7.7±0.2
Albumin (g/dl)	3.8±0.1	3.9±0.1	3.9±0.1	4.0±0.1[a]
Cholinesterase (U/l)	171.6±36.4	166.0±31.3	201.8±38.8	203.2±39.0
Total cholesterol (mg/dl)	160.2±9.9	157.2±13.9	162.4±12.7	163.0±11.8
Total bilirubin (mg/dl)	1.0±0.3	1.2±0.3	1.0±0.3	1.1±0.3

The values are expressed as mean ± standard error. Statistical comparisons between before and after 30, 60 or 90 days of the administration were performed by Wilcoxon's test. [a]P<0.05. BMI, body mass index; BW, body weight; BCAAs, branched-chain amino acids; BTR, BCAAs tyrosine ratio; ALT, alanine aminotransferase; n/a, not applicable.

Table VI. Effects of BCAA-enriched supplement on protein and lipid metabolism in the female group.

	Before	Administration of BCAA-enriched supplement		
		30 days	60 days	90 days
BMI	23.9±0.8	n/a	n/a	24.3±1.8
Fat%BW	35.5±1.6	n/a	n/a	35.3±1.4
Muscle%BW	34.0±0.9	n/a	n/a	34.1±1.0
Visceral fat area	120.3±18.4	n/a	n/a	111.4±4.2
BCAAs (μmol/l)	383.1±23.7	471.9±34.8[a]	519.1±34.6[a]	591.6 ± 66.3[a]
Tyrosine (μmol/l)	72.3±4.8	70.3±7.3	66.2±6.0	79.9±8.9
BTR	5.5±5.0	7.3±1.0	8.5±1.3[a]	7.7±9.0
Zinc (μg/dl)	83.7±7.8	102.9±5.7	113.6±8.5	114.6±8.8[a]
ALT (U/l)	31.1±4.5	35.3±5.0	34.3±4.0	34.1±4.9
Total protein (g/dl)	7.6±0.2	7.6±0.2	7.7±0.2	7.7±0.2
Albumin (g/dl)	3.8±0.1	3.9±4.7	3.9±2.4	3.9±0.1
Cholinesterase (U/l)	144.6±11.5	145.6±10.1	148.4±11.5	146.4±11.3
Total cholesterol (mg/dl)	191.6±12.0	183.9±11.5	190.1±13.5	180.3±8.7
Total bilirubin (mg/dl)	0.8±0.1	0.8±0.1	0.8±4.2	0.7±0.1

The values are expressed as mean ± standard error. Statistical comparisons between before and after 30, 60 or 90 days of the administration were performed by Wilcoxon's test. [a]P<0.05. BMI, body mass index; BW, body weight; BCAAs, branched-chain amino acids; BTR, BCAAs tyrosine ratio; ALT, alanine aminotransferase; n/a, not applicable.

Significant increases in serum BTR value and zinc level were seen in the two groups after the supplement administration (Tables V and VI). In addition, the serum BCAA level was significantly increased in the female group (Table VI). The serum albumin level was also significantly increased, though only in the male group (Table V).

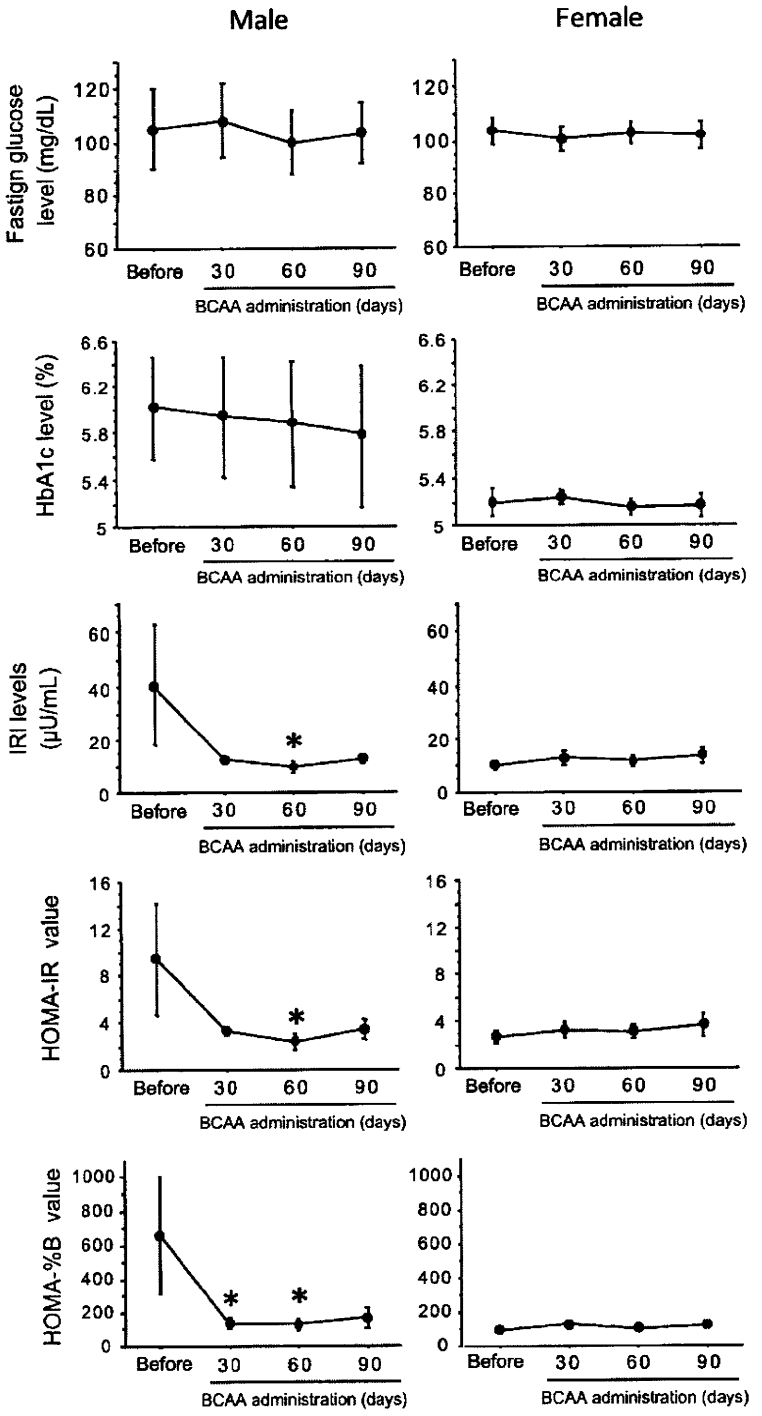

Figure 1. The effects of BCAA-enriched supplementation on glucose metabolism in the male and female groups. Glucose metabolism was evaluated by the fasting glucose level, HbA1c value, serum IRI level, HOMA-IR and HOMA-%B values. The values are expressed as mean ± standard error. Statistical comparisons between before and after 30, 60 or 90 days of the administration was performed by Wilcoxon's test. *P<0.05. HbA1c, hemoglobinA1c; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment method for insulin resistance; HOMA-%B, homeostasis model assessment method for ß cell function.

Effects of BCAA-enriched supplementation on glucose metabolism in the male and female groups. Although there were no significant changes in the fasting plasma glucose and HbA1c levels during the administration of the BCAA-enriched supplement in the two groups, the serum IRI level was significantly decreased in the male group after the supplement administration (Fig. 1). Similarly, a significant decrease was seen in the HOMA-IR and HOMA-%B values in the male group, though not in the female group (Fig. 1).

Discussion

In this study, we demonstrated that there was a gender difference in chronic viral liver disease-related insulin resistance. Moreover, BCAA-enriched supplementation caused decreases in the serum IRI level, the HOMA-IR and HOMA-%B values without changes in body composition including visceral fat, suggesting the direct effects of the BCAA-enriched supplement on glucose metabolism among the male patients with chronic viral liver disease.

Increased insulin resistance is known to exist in pre-cirrhotic patients (2) and is a risk factor for the progression of hepatic fibrosis (4), development of HCC (5,6) and a reduction in long-term survival (6,7). Similar to previous reports (2,6,25,26,28), the enrolled patients in this study exhibited chronic hepatitis with increased insulin resistance. Since BCAA are known to modulate insulin signaling (18-21), we examined the effects of the BCAA-enriched supplementation on insulin resistance. In our study, the serum IRI level and the HOMA-IR and HOMA-%B values were all reduced after the supplement administration. However, the reduction in these parameters was not statistically significant. We previously reported on a gender difference in chronic viral liver disease-related insulin resistance (6). Therefore, we examined the effects of the BCAA-supplement on insulin resistance by stratification via gender.

Male cirrhotic patients show a significantly greater increase in insulin resistance compared to female cirrhotic patients (6). Similarly, increased IRI levels and HOMA-IR values were seen only in the male group in this study. Although the reason for the gender difference in insulin resistance is unclear, one possibility is that tyrosine is involved in the development of insulin resistance. The serum tyrosine level in our study of the male group was significantly increased compared to the female group. A synthetic enzyme of tyrosine, henylalanine hydroxylase, is known to be regulated by testosterone, a sex hormone (29). Tyrosine is the precursor of epinephrine, which causes peripheral and hepatic insulin resistance (30). The serum tyrosine level has a positive correlation with insulin resistance (31). Thus, the gender difference in the tyrosine production pathway and the metabolite of tyrosine may have been responsible for the increased insulin resistance in the male group among our patients.

BCAA-enriched supplementation decreased the serum IRI level and the HOMA-IR and HOMA-%B values without changes in body composition including visceral fat area. These data suggest that the BCAA-enriched supplementation improves insulin resistance and ß cell function. In this study, insulin resistance was significantly reduced 60 days after administration of the BCAA-enriched supplement. It remains unclear, however, how the supplement improved insulin resistance. Since the supplement used in this study contains trace elements such as zinc, chromium and selenium, which are known to decrease insulin sensitivity (32-34), it is reasonable to assume that these trace elements may contribute to improve insulin resistance. Alternatively, changes in the constitution of amino acids may be involved in the improvement in insulin resistance. Moreover, tyrosine is the precursor of epinephrine, which causes peripheral and hepatic insulin resistance (30). In this study, changes in serum tyrosine level and BTR reflected changes in insulin resistance. Insulin resistance was significantly reduced 60 days after the administration of the BCAA-enriched supplement. Likewise, the serum tyrosine level was significantly decreased and BTR was significantly increased 60 days after the administration of the BCAA-enriched supplement. Thus, a decrease in the serum tyrosine level and changes in the constitution of amino acids may contribute to a reduction in insulin resistance. In good agreement with our results, Vlasakova *et al* reported that BTR shows a negative correlation with insulin resistance (31). Although the reason for changes in the constitution of amino acids remains uncertain, a possibility is that BCAA activate mTOR, which in turn, promotes protein synthesis (35). Desai *et al* reported that BCAA cause the incorporation of tyrosine into protein as well as albumin renewal (36). In our study, a significant increase in serum albumin level was seen following the decrease in serum tyrosine level and an increase in BTR. Taken together, these findings suggest that the BCAA-enriched supplementation seems to lead to a dramatic reduction of insulin resistance through both changes in the constitution of amino acids and effects of trace elements.

A limitation of this study was the small sample size. However, leucine is reported to improve insulin resistance in *in vivo* experiments (37). In human subjects, two cases which showed BCAA improved insulin resistance were recently reported (38). These previous reports suggest the effects of the BCAA-enriched supplement on glucose metabolism. In order to confirm the significance of the BCAA-enriched supplement on insulin resistance in patients with LC, a large-scale multicenter clinical study is required.

This study revealed that the BCAA-enriched supplementation improved insulin resistance and ß cell function in male patients with chronic viral liver disease. Thus, the BCAA-enriched supplementation may be one of the useful therapeutic agents for insulin resistance in male patients with chronic viral liver disease.

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Graves' ophthalmopathy and tongue cancer complicated by peg-interferon α-2b and ribavirin therapy for chronic hepatitis C: A case report and review of the literature

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Abstract. Hepatitis C virus (HCV) infection induces not only chronic liver disease, but also extrahepatic manifestations such as thyroid disease and oral cancer. Thyroid dysfunction is also a complication known to be associated with interferon (IFN) therapy for HCV infection. We report on a 69-year-old Japanese man who developed Graves' ophthalmopathy and tongue cancer (malignant transformation of leukoplakia) while receiving peg-interferon (Peg-IFN) α-2b and ribavirin (RBV) treatment for chronic hepatitis C. This patient had no history of thyroid disease before the combination therapy, but did have bilateral leukoplakia of the tongue. The leukoplakia lesions did not change until 20 weeks after the start of the combination therapy, and ophthalmopathy was not diagnosed until 47 weeks later. As ophthalmopathy is considered to be a severe adverse event induced by Peg-IFN α-2b plus RBV, therapy was discontinued after 47 weeks. The patient received a partial glossectomy to remove the malignant neoplasm as well as extraocular muscle surgery for the ophthalmopathy, and was treated with an antithyroid agent and steroids. In conclusion, it is necessary to clinically examine organs other than the liver in patients with HCV infection.

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Abbreviations: HCV, hepatitis C virus; IFN, interferon; Peg-IFN, peg-interferon; RBV, ribavirin; HCC, hepatocellular carcinoma; MRI, magnetic resonance imaging; HBsAg, hepatitis B surface antigen; TSH, thyroid stimulating hormone; FT_3, free tri-iodothyronine; FT_4, free thyroxine; TPOAb, thyroid peroxidase antibodies; TgAb, thyroglobulin antibodies; anti-HCV, HCV antibody; RBC, red blood cell; Hb, hemoglobin; WBC, white blood cell; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; LDH, lactate dehydrogenase; TRAb, TSH receptor antibody; TSAb, thyroid stimulating antibody; hTRAb, human TSH receptor antibody

Key words: hepatitis C virus, peg-interferon, ribavirin, Graves' ophthalmopathy, extrahepatic manifestation, oral squamous cell carcinoma

Introduction

Hepatitis C virus (HCV) frequently causes persistent infection, which leads to chronic liver disease and hepatocellular carcinoma (HCC). HCV-related HCC represents 80% of all HCC cases in Japan (1) and primary liver cancer, 95% of which is HCC-related, ranks third in men and fifth in women as the cause of death from malignant neoplasms. Interferon (IFN) α monotherapy for chronic hepatitis C infection leads to a sustained virological response in only 10-15% of HCV-infected patients (2,3). A substantial improvement in response of approximately 2-fold over IFN monotherapy was noted using the combination of IFN α plus ribavirin (RBV) (4,5). Recently, a combination treatment of peg-interferon (Peg-IFN) plus RBV has been adopted as standard care for patients with chronic hepatitis C, as it is associated with significant improvements in the rate of sustained virological response (50%) as compared to IFN α plus RBV or Peg-IFN α alone (6).

HCV infection has also been associated with extrahepatic manifestations and immune-mediated phenomena (7), including mixed cryoglobulinemia (8), thyroid disease (9), Sjögren's syndrome (10), porphyria cutanea tarda (11), lichen planus (12), oral cancer (13,14) and type 2 diabetes mellitus (15). The incidence of HCV infection in oral squamous cell carcinoma in Japanese patients has been reported as being 16.7-24.0% (13,14).

The side effects of IFN therapy for HCV have been well documented (16,17). Flu-like symptoms such as fever, chills, muscle ache, nausea, vomiting and fatigue are common side effects of treatment. Depression and related symptoms, such as anxiety, irritability, insomnia and mental confusion, are not rare and, in patients with a previous history, may be significant. Withdrawal rates in IFN-based combination studies due to side effects have ranged from 6 to 7% (5). Various side effects have been reported in patients treated with this cytokine, including the appearance or exacerbation

of underlying autoimmune diseases and the development of a variety of organ- and non-organ-specific autoantibodies (18). Auto-immune thyroid disease is a common side effect of IFN treatment of viral hepatitis C, affecting 2-19% of IFN-treated patients (19). We previously reported the case of a patient with chronic hepatitis C who developed worsening lichen planus lesions during treatment with IFN plus RBV (20), and the case of a patient who developed oral cancer after IFN therapy (21).

We now describe a patient with chronic hepatitis C infection who developed hyperthyroidism, Graves' ophthalmopathy and malignant transformation of tongue leukoplakia during combination therapy with Peg-IFN α-2b and RBV. This patient was treated successfully.

Case report

A 67-year-old Japanese man, diagnosed in 1998 with chronic hepatitis C, consulted the Digestive Disease Center of Kurume University on June 6, 2003 for treatment of his chronic liver disease. The patient had received a right upper lobectomy for lung tuberculosis at the age of 23 (in 1958), and had been administered blood transfusions of 600 ml during the procedure. Hypertension was noted at the age of 67, and antihypertensive treatment was started at 68. Hemangioma of the right middle finger was diagnosed at 69. For 20 years, he smoked 50 cigarettes a day, though he had not smoked for the last 30 years. His alcohol consumption was 500 ml of beer daily for 49 years. His family history was non-contributory.

Periodic blood tests and abdominal ultrasound exams were conducted by a hepatologist at Kurume University. As the patient's aminotransferase levels were in the normal range, he was monitored regularly for chronic hepatitis C. On July 30, 2004, at the age of 69, his aminotransferase levels were found to be elevated and a liver biopsy revealed chronic active hepatitis, diagnosed as F2A2 according to the new Inuyama classification (22). As of June 14, 2005, for a period of 1-3 months, the patient was treated by a family doctor with a combination of peg-IFN α-2b (Peg-Intron®; Schering-Plough, Kenilworth, NJ, USA) (40-100 μg/week) plus RBV (Rebetol®; Schering-Plough) (300-800 mg/day). During this time, he was examined by a hepatologist once.

At the start of Peg-IFN α-2b plus RBV therapy, laboratory data regarding hepatitis virus markers indicated that the patient was negative for hepatitis B surface antigen (HBsAg), but positive for HCV antibody (anti-HCV) and HCV RNA. Both free thyroxine (FT_4) and thyroid stimulating hormone (TSH) levels before Peg-IFN plus RBA therapy were within normal ranges (Table I).

In March 2006, while undergoing Peg-IFN plus RBV therapy, the patient experienced double vision. He did not consult a family doctor or a hepatologist, but was examined by an ophthalmologist, and then by a neurosurgeon who prescribed magnetic resonance imaging (MRI) followed by a neurological examination at Kurume University Hospital on May 9, 2006. Thyroid function tests on February 10, 2006 revealed suppressed TSH at 0.016 μIU/ml (normal value 0.21-3.85) and elevated free tri-iodothyronine (FT_3) at 4.1 pg/ml (normal value 1.9-3.5), but the hepatologist did not diagnose thyroid disease. Over the next 3 months, thyroid function tests revealed hyperthyroidism of autoimmune etiology, indicated by the following laboratory values from a test taken on May 16, 2006: TSH, 0.007 μIU/ml (normal value 0.21-3.85); FT_3, 4.6 pg/ml (normal value 1.9-3.5); FT_4, 1.58 ng dl (normal value 0.88-1.56); positive thyroid peroxidase antibodies (TPOAb), 92.2 IU/ml (normal value <5); thyroglobulin

Table I. Laboratory data of patient with hepatitis C virus infection at the time of admission for Peg-IFN and RBV therapy.

Laboratory assay	Value	Unit	Standard value
RBC	483	$x10^4/mm^3$	430-570
Hb	16.0	g/dl	14.0-18.0
Ht	46.9	%	40.0-52.0
WBC	63	$x10^4/mm^3$	40-90
Plt	17.4	$x10^4/mm^3$	13.0-36.0
AST	32	U/l	13-33
ALT	32	U/l	8-42
LDH	170	U/l	119-229
ALP	209	U/l	115-359
γ-GTP	90	U/l	10-47
TP	7.21	g/dl	6.70-8.30
Alb	4.11	g/dl	4.00-5.00
ChE	160	IU/l	107-233
TC	140	mg/dl	128-256
TB	1.14	mg/dl	0.00-1.50
DB	0.12	mg/dl	0.00-0.60
BUN	15.3	mg/dl	8.0-22.0
Crea	0.72	mg/dl	0.60-1.10
Na	139	mEq/l	138-146
K	4.0	mEq/l	3.6-4.9
Cl	104	mEq/l	99-109
Fe	190	μg/dl	80-170
UIBC	68	μg/dl	180-274
Ferritin	167.7	ng/ml	23.0-183.0
CRP	0.04	mg/dl	0.00-0.40
IgA	225	mg/dl	103-409
IgM	65	mg/dl	40-221
IgG	1856	mg/dl	918-1742
FT_4	1.24	ng/dl	0.88-1.56
TSH	2.970	μIU/ml	0.210-3.850
AFP (L3)	3.3	ng/dl	0.0-8.7
HbA1c	5.1	%	4.3-5.8
HBsAg	Negative		
Anti-HBc	Negative		
Anti-HCV	Positive		
HCV RNA level	2400	KIU/ml	
HCV genotype	1b		

June 14, 2005.