whereupon the IFN monotherapy was discontinued. He stayed negative for HCV RNA until the last observation 25 weeks after the withdrawal of IFN monotherapy.

Figure 2 depicts the clinical course of chimp No. 224 who was inoculated with 8.4×10^6 copies of HCV of genotype 1b. HCV RNA was detected in his serum at week 1. HCV RNA stayed positive through 20 weeks, and he was considered to have developed persistent infection. IFN-α 6 MU was given daily for 7 days since the 21st week. Because HCV RNA was positive at the next examination, IFN monotherapy was given again during the 23rd week.

However, HCV RNA did not disappear from the serum after 2 courses of IFN monotherapy. At 36 weeks when HCV RNA was confirmed to be present in the serum, he received a combination therapy with IFN-α 3 MU, daily for 2 weeks and then 3 times a week for 14 weeks, along with oral ribavirin 600 mg daily in 2 divided doses. HCV RNA decreased 1 week after the institution of combination therapy, and became undetectable the next week; the loss of HCV RNA continued throughout the following 15 weeks on treatment. He was confirmed negative for serum HCV RNA at tests performed 4, 12 and 24 weeks, respectively, after the completion of combined IFN and ribavirin. Transaminase levels increased moderately 6 weeks after the initiation of combination therapy, but thereafter they returned to normal through the observation till 24 weeks after the completion of therapy. Chimp No. 224 did not respond to HCV infection by raising anti-HCV, and remained seronegative throughout 76 weeks since he received inoculation.

The biggest problem with HCV infection in human beings is its strong propensity to persist in up to 85% of individuals who contract it, although chances of persistence depend on sex, age and route of transmission [4, 5]. We have reported that HCV replicates very rapidly in chimpanzees inoculated with it at a doubling time of 6.3–8.6 h [7]; it is much shorter than that of HBV estimated at 1.9–3.4 days [8]. Such a fast replication velocity of HCV might contribute toward a high persistence rate after the primary infection; cellular immune responses to clear HCV may not be able to catch up with exponentially increasing population and rapidly evolving HCV quasispecies.

The sustained virological response to pegyrated-IFN combined with ribavirin in patients with chronic hepatitis C remains insufficient; it is achieved in merely one half of the patients infected with HCV genotype 1 in a high viral load [9]. This stands in sharp contrast to the excellent efficacy of IFN on patients with acute prolonged hepatitis C [10]. Hence, we started treating 2 chimpanzees in whom acute infection with HCV had prolonged after they were experimentally transmitted with HCV [6, 7]. The preacute serum from one of them (chimp 210) served for illustrating the early dynamics of HCV infection, and provided blood centers with the standards of HCV RNA, containing defined copy numbers per milliliter, for calibrating nucleic acid amplification test (NAT).

Chimp 210 cleared HCV infection after he had received IFN-α 6 MU daily for 1 week (fig. 1). Chimp 224 failed to clear HCV after 2 courses of the IFN monotherapy. Thereafter, he responded to IFN 3 MU daily for 2 weeks followed by 3 times a week for 14 weeks in combination with oral ribavirin 600 mg daily. The virological response with loss of HCV RNA from the serum was achieved during treatment, and sustained 24 weeks after the completion of combination therapy (fig. 2). They both had kept HCV for 29 and 36 weeks before treatment, respectively, exceeding 6 months for the clinical definition of persistent infection. There remains a possibility, however, that chimp 210 may have been clearing HCV naturally without therapeutic intervention, in view of his remarkable response to a short-term IFN monotherapy. Chimp 210 was infected with HCV of genotype 1b and 2a, and chimp 224 with HCV of genotypes 1b. HCV of genotype 2a might have disappeared earlier than HCV of genotype 1b in chimp 210, in view of different sensitivity to IFN of these 2 HCV genotypes in clinical trials [11, 12].

We have shown that acute prolonged HCV infection can be cured in chimps if they receive IFN alone or combined with ribavirin soon enough after they have been infected, as in the treatment of acute hepatitis C in patients [10]. Hopefully, the efficacy of IFN with or without ribavirin would be extended in additional chimps with acute prolonged HCV infection after they have completed transmission studies. Furthermore, such treatments would need to be considered in many chimps who have acquired persistent HCV infection after experimental transmission during the long past.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Predictive value of tumor markers for hepatocarcinogenesis in patients with hepatitis C virus

Takashi Kumada · Hidenori Toyoda · Seiki Kiriyama · Makoto Tanikawa · Yasuhiro Hisanaga · Akira Kanamori · Toshifumi Tada · Junko Tanaka · Hiroshi Yoshizawa

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Abstract
Background Increases in tumor markers are sometimes seen in patients with chronic liver disease without hepatocellular carcinoma (HCC). The aim of this study was to determine the relationship between the levels of three tumor markers [alpha-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3%), and des-γ-carboxy prothrombin (DCP)] and hepatic carcinogenesis to identify hepatitis C virus (HCV) carriers at high risk for cancer development.
Methods A total of 623 consecutive HCV carriers with follow-up periods of >3 years were included. The average integration values were calculated from biochemical tests, and tumor markers, including AFP, AFP-L3%, and DCP, and factors associated with the cumulative incidence of HCC were analyzed.
Results HCC developed in 120 (19.3%) of the 623 patients. Age >65 years [adjusted relative risk, 2.303 (95% confidence interval, 1.551–3.418), $P < 0.001$], low platelet count [3.086 (1.997–4.768), $P < 0.001$], high aspartate aminotransferase value [3.001 (1.373–6.562), $P < 0.001$], high AFP level [≥10, <20 ng/mL: 2.814 (1.686–4.697), $P < 0.001$; ≥20 ng/mL: 3.405 (2.087–5.557), $P < 0.001$] compared to <10 ng/mL, and high AFP-L3% level [≥5, <10%: 2.494 (1.291–4.816), $P = 0.007$; ≥10%: 3.555 (1.609–7.858), $P < 0.001$] compared to <5% were significantly associated with an increased incidence of HCC on multivariate analysis.
Conclusions Increased AFP or AFP-L3% levels were significantly associated with an increased incidence of HCC. Among HCV carriers, patients with ≥10 ng/mL AFP or patients with ≥5% AFP-L3% are at very high risk for the development of HCC even if AFP is less than 20 ng/mL or AFP-L3% is less than 10%, which are the most commonly reported cutoff values.

Keywords Alpha-fetoprotein (AFP) · *Lens culinaris* agglutinin-reactive fraction of AFP · Hepatic regeneration · Necroinflammatory activity · Hepatocarcinogenesis

Introduction

Serum alpha-fetoprotein (AFP) is a widely used marker for hepatocellular carcinoma (HCC) [1]. However, serum AFP levels are increased in patients with liver diseases other than HCC, including viral hepatitis [2–4], with a prevalence of 10–42% [2, 5–7]. Increases in AFP are a marker of hepatic regeneration following hepatocyte destruction in viral hepatitis [8]. However, the pathogenesis and clinical significance of this phenomenon remain unclear.

The *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3%) and des-γ-carboxy prothrombin (DCP) are also markers for HCC [9–12]. Available data suggest that these tumor markers are more highly specific for HCC than AFP alone [9]. However, there are no reports examining the prognostic value of these markers in hepatocarcinogenesis.

T. Kumada (✉) · H. Toyoda · S. Kiriyama · M. Tanikawa · Y. Hisanaga · A. Kanamori · T. Tada
Department of Gastroenterology,
Ogaki Municipal Hospital, 4-86,
Minaminokawa-cho, Ogaki, Gifu 503-8052, Japan
e-mail: hosp3@omh.ogaki.gifu.jp

J. Tanaka · H. Yoshizawa
Department of Epidemiology,
Infectious Disease Control and Prevention,
Graduate School of Biomedical Sciences,
Hiroshima University, Hiroshima, Japan

Results of biochemical tests, including tumor markers, can fluctuate for a given patient and can vary between different patients, and repeated measurements over time may provide a more accurate picture of disease development or progression. The arithmetic mean value is often used to assess biochemical parameters over time, but this value can be greatly affected by the interval between measurements such that a short period of very high values can inappropriately skew the mean. We have previously argued that the average integration value is more meaningful than the arithmetic mean value for the purposes of monitoring disease progression [13, 14].

The aim of this study was to determine the relationship between three tumor markers (AFP, AFP-L3%, and DCP) to better identify hepatitis C virus (HCV) carriers at high risk for the development of HCC. Of note, we used the average integration values of these parameters in our analysis.

Patients, materials, and methods

Patient selection

A total of 1623 consecutive patients positive for anti-HCV antibody visiting the Department of Gastroenterology at Ogaki Municipal Hospital during the period January 1995 to December 1997 were considered for enrollment. The present study cohort included the following criteria for enrollment: (1) positive for anti-HCV antibody by second- or third-generation enzyme-linked immunosorbent assay and detectable HCV RNA for at least 6 months; (2) no evidence of positivity for hepatitis B surface antigen; (3) exclusion of other causes of chronic liver disease (i.e., alcohol consumption lower than 80 g/day, no history of hepatotoxic drug use, and negative tests for autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, and Wilson's disease); (4) follow-up period greater than 3 years; (5) measurement of AFP, AFP-L3%, and DCP at least every 6 months; (6) no evidence of HCC for at least 3 years from the start of the observation periods; and (7) interferon (IFN) therapy completed greater than 3 years before the detection of HCC in patients who received IFN therapy. A total of 623 patients fulfilled these criteria (Fig. 1).

Fibrosis was histologically evaluated in 187 of the 623 patients and staged according to Desmet et al. [15] as follows: F0, no fibrosis; F1, mild fibrosis; F2, moderate fibrosis; F3, severe fibrosis; and F4, cirrhosis. The remaining 436 patients were evaluated by ultrasound (US) findings and biochemical tests. The diagnosis of cirrhosis was made according to typical US findings, e.g., superficial nodularity, a coarse parenchymal echo pattern, and signs of portal hypertension (splenomegaly >120 mm, dilated portal vein diameter >12 mm, patent collateral veins, or ascites) [16–18]. In this study patients who did not satisfy these criteria were classified as having chronic hepatitis. Four hundred and sixty-three patients were diagnosed with chronic hepatitis and 160 patients with cirrhosis.

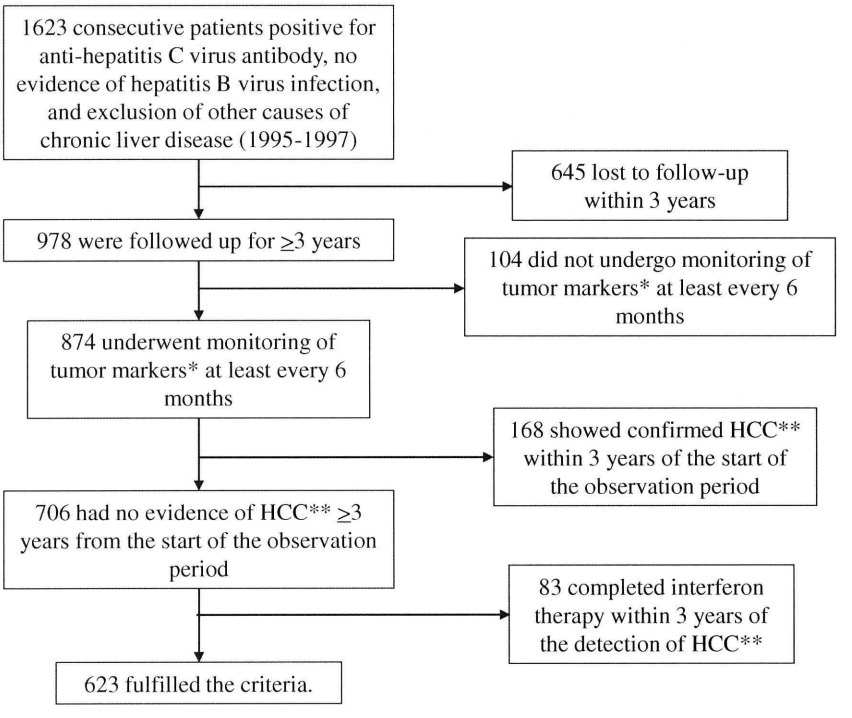

Fig. 1 Schematic flowchart of enrolled patients. *Serum alpha-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3%), and des-γ-carboxy prothrombin (DCP). **Hepatocellular carcinoma (*HCC*)

All patients were followed up at our hospital at least twice a year. During each follow-up examination, platelet count, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (γ-GTP), total bilirubin, cholinesterase, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), albumin, total cholesterol, AFP, AFP-L3%, and DCP were measured. Platelet count and ALT, AST, γ-GTP, total bilirubin, cholinesterase, ALP, LDH, albumin, total cholesterol, AFP, AFP-L3%, and DCP values were expressed as average integration values [13, 14]. Briefly, using ALT as an example, the area of a trapezoid is calculated by multiplying the sum of two ALT values by one-half of the interval between the measurements. This value is then divided by the observation period to obtain the average integration value, and this technique provides a better representation of values over time when there are extremes of high and low values [14, 16]. In patients who developed HCC during the observation period, AFP, AFP-L3%, and DCP values obtained at least 1 year before the diagnosis of HCC were assessed. Serum AFP concentration was determined with a commercially available kit. AFP-L3% was measured by lectin-affinity electrophoresis and antibody-affinity blotting with the AFP Differentiation Kit L (Wako Pure Chemical Industries, Osaka, Japan) [10]. DCP was measured with a DCP reagent (Picolumi PIVKA-II; Eizai, Tokyo, Japan) [11]. Cutoff levels for AFP, AFP-L3%, and DCP were set at 20 ng/mL, 10%, and 40 mAU/mL, respectively, according to previous reports [10–12]. HCV genotype and quantification of HCV RNA (Amplicor 2; Roche Diagnostics, Tokyo, Japan) were determined in 513 cases. All patients underwent imaging modalities (US, computed tomography [CT], or magnetic resonance imaging [MRI]), every 3 months in patients with cirrhosis and every 6 months in patients with chronic hepatitis.

The diagnoses of HCC were confirmed by histologic examination of resected hepatic tumors or US-guided needle biopsy specimens. When biopsy of the tumor was contraindicated, the HCC diagnosis was made using clinical criteria and imaging findings obtained from B-mode US, CT angiography, or MRI [19, 20]. HCC was histologically diagnosed in 46 patients, and in the remaining 74 patients, the diagnosis was made based on clinical criteria [19, 20]. All tumors were 3 cm or less in maximum diameter, and there were 3 nodules or less on diagnosis.

One hundred eighty-nine patients received IFN therapy. Patients were classified into three groups according to the type of response to IFN therapy: sustained virologic response (SVR), defined as the absence of serum HCV RNA at 6 months after IFN therapy; the non-SVR group, defined as the presence of serum HCV RNA at 6 months after IFN therapy; and the no IFN therapy group.

Patients were classified into three groups for each of the tumor markers according to the average integration values of AFP, AFP-L3%, and DCP: A1, <10 ng/mL ($n = 452$); A2, ≥10, <20 ng/mL ($n = 80$); and A3, ≥20 ng/L ($n = 91$); L1, <5% ($n = 588$); L2, ≥5, <10% ($n = 18$); and L3, ≥10% ($n = 17$); and D1, <20 mAU/mL ($n = 379$); D2, ≥20, <40 mAU/mL ($n = 170$); and D3, ≥40 mAU/mL ($n = 51$), respectively.

The present study ended on 31 December 2008 or the date of identification of HCC occurrence. The median follow-up period was 9.0 years (range 3.0–13.0 years). The total number of blood examinations was 25.721, and the median number of blood examinations was 23 (range 6–105) per subject.

Statistical analysis

Statistical analysis was performed with the Statistical Program for Social Science (SPSS ver.17.0 for Windows; SPSS Japan, Tokyo, Japan). Continuous variables are shown as medians (ranges). The Mann–Whitney *U*-test was used for continuous variables, and Fisher's exact test was used for categorical variables. Actuarial analysis of the cumulative incidence of hepatocarcinogenesis was performed by the Kaplan–Meier method, and differences were tested by the log-rank test. The Bonferroni correction was performed for multiple comparisons. The Cox proportional hazards model and forward selection method were used to estimate the relative risk of HCC development associated with age (≤65 or >65 years), sex (female or male), body mass index (BMI ≤25.0 or >25.0 kg/m^2), HCV genotype (type 1 or type 2), viral concentration (≤100 or >100 KIU/mL), platelet count (<12.0 × 10^4/mm^3 or ≥12.0 × 10^4/mm^3), ALT (≤35 or >35 IU/mL), AST (≤40 or >40 IU/mL), total bilirubin (≤1.2 or >1.2 mg/dL), γ-GTP (≤56 or >56 IU/mL), ALP (≤338 or >338 IU/mL), cholinesterase (<431 or ≥431 IU/mL), LDH (≤250 or >250 IU/mL), albumin (<3.5 or ≥3.5 g/dL), total cholesterol (<130 or ≥130 mg/dL), cirrhosis (presence or absence), and IFN treatment (no therapy, non-SVR, or SVR) for univariate and multivariate analyses. We used the lower or upper limit of the reference values at our institute as cutoff values for platelet count, ALT, AST, total bilirubin, γ-GTP, ALP, cholinesterase, LDH, albumin, and total cholesterol levels. Statistical significance was set at $P < 0.05$.

The study protocol was approved by the Ethics Committee at Ogaki Municipal Hospital in January 2009 and the study was performed in compliance with the Helsinki Declaration. Informed consent was obtained from each patient for analyzing patient records and images.

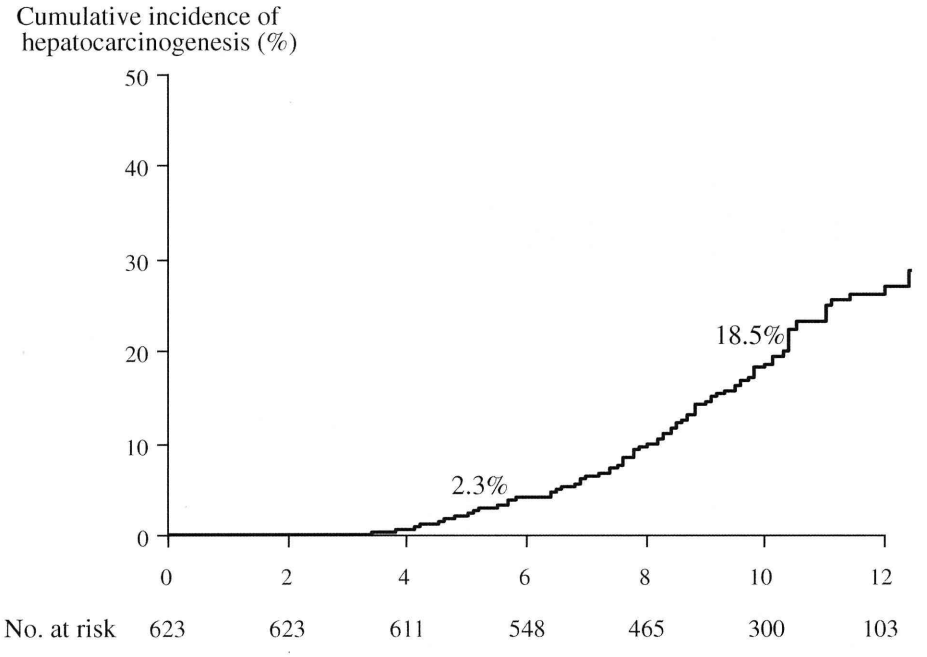

Fig. 2 Overall cumulative incidence rate of HCC

Table 1 Patient characteristics

Age (years)	61 (26–84)
Sex (F/M)	265/358
BMI (kg/m^2)	22.5 (12.0–34.9)
HCV genotype (type 1/type 2)	356/157
Viral concentration (KIU/mL)	270 (0.5–6300)
AFP (ng/mL)	4.8 (0.8–341.5)
AFP-L3 (%)	0.1 (0.0–32.5)
DCP (mAU/mL)	18.1 (8.5–99.6)
Platelets ($\times 10^4$/mm^3)	14.8 (3.0–33.9)
ALT (IU/L)	46.4 (10.1–340.4)
AST (IU/L)	48.5 (13.3–168.9)
γ-GTP (IU/L)	37.6 (9.9–2207)
Total bilirubin (mg/dL)	0.6 (0.2–2.7)
ALP (IU/L)	276.4 (86.8–845.5)
Cholinesterase (IU/L)	242.9 (38.8–545.30)
LDH (IU/L)	196.4 (118.4–650.1)
Albumin (g/dL)	4.0 (2.4–4.9)
Total cholesterol (mg/dL)	155.8 (77.9–264.1)
Fibrosis (F0/F1/F2/F3/F4)[a]	32/73/56/24/2
Cirrhosis (present/absent)	160/463
IFN therapy (none/non-SVR/SVR)	434/146/43

Continuous variables are quoted as medians (ranges)

BMI body mass index, *HCV* hepatitis C virus, *AFP* alpha-fetoprotein, *AFP-L3Lens culinaris* agglutinin-reactive fraction of AFP, *DCP* des-γ-carboxy prothrombin, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *GTP* gamma glutamyl transpeptidase, *ALP* alkaline phosphatase, *LDH* lactate dehydrogenase, *IFN* interferon, *SVR* sustained virologic response

[a] Staging of chronic hepatitis according to Desmet et al. [15]

Results

HCC developed in 120 (19.3%) of the 623 patients. The 5- and 10-year cumulative incidences of HCC were 2.3 and 18.5%, respectively (Fig. 2). Demographic and medical data for the 623 patients are summarized in Table 1.

Factors associated with the incidence of hepatic carcinogenesis on univariate analysis

Factors associated with the incidence of HCC are listed in Table 2. Age ≥65 years, high AFP level, high AFP-L3% level, high DCP level, low platelet count, high ALT level, high AST level, high LDH level, high ALP level, low cholinesterase level, low albumin level, presence of cirrhosis, and response to IFN therapy were significantly associated with the development of HCC on univariate analysis.

The 5-, 7-, and 10-year cumulative incidences of HCC were 1.1, 2.1, and 7.5% in group A1; 2.6, 9.6, and 42.1% in group A2; and 6.6, 18.3, and 50.0% in group A3, respectively, and the cumulative incidence of HCC differed significantly between groups A1 and A2 and groups A1 and A3 (Fig. 3). The 5-, 7-, and 10-year cumulative incidences of HCC were 1.4, 4.6, and 15.6% in group L1; 19.6, 39.7, and 73.6% in group L2; and 12.5, 25.0, and 56.7% in group L3, respectively, and the cumulative incidence of HCC differed significantly between groups L1 and L2 and groups L1 and L3 (Fig. 4). The 5-, 7-, and 10-year cumulative incidences of HCC were 0.5, 4.6, and

Table 2 Factors associated with hepatocarcinogenesis (univariate analysis)

	Crude hazard ratio (95% CI)	*P*
Age (years)		
≤65	1	
>65	2.318 (1.580–3.400)	<0.001
AFP (ng/mL)		
A1; <10	1	
A2; ≥10, <20	6.061 (3.768–9.750)	<0.001
A3; ≥20	8.985 (5.874–13.744)	<0.001
AFP-L3 (%)		
L1; <5	1	
L2; ≥5, <10	8.032 (4.388–14.700)	<0.001
L3; ≥10	3.781 (1.838–7.778)	<0.001
DCP (mAU/mL)		
D1; <20	1	
D2; ≥20, <40	1.209 (0.788–1.855)	0.385
D3; ≥40	4.535 (2.840–7.241)	<0.001
Platelets ($\times 10^4$/mm^3)		
≥12.0	1	
<12.0	5.887 (3.982–8.702)	<0.001
ALT (IU/L)		
≤35	1	
>35	2.632 (1.574–4.400)	<0.001
AST (IU/L)		
≤40	1	
>40	8.120 (4.115–16.024)	<0.001
LDH (IU/L)		
≤250	1	
>250	1.970 (1.249–3.106)	<0.001
ALP (IU/L)		
≤338	1	
>338	2.509 (1.724–3.650)	<0.001
Cholinesterase (IU/L)		
>431	1	
≤431	3.288 (2.209–4.893)	<0.001
Albumin (g/dL)		
≥3.5	1	
<3.5	3.948 (2.635–5.917)	<0.001
Cirrhosis		
Absent	1	
Present	3.474 (2.413–5.002)	<0.001
IFN therapy		
No therapy	1	
Non-SVR	0.312 (0.180–0.539)	<0.001
SVR	0.215 (0.075–0.620)	0.004

Continuous variables are quoted as medians (ranges)

CI confidence interval, *AFP* alpha-fetoprotein, *AFP-L3Lens culinaris* agglutinin-reactive fraction of AFP, *DCP* des-γ-carboxy prothrombin, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *LDH* lactate dehydrogenase, *ALP* alkaline phosphatase, *IFN* interferon, *SVR* sustained virologic response

14.8% in group D1; 1.8, 4.3, and 16.3% in group D2; and 10.0, 25.0, and 48.2% in group D3, respectively, and the cumulative incidence of HCC differed significantly between groups D1 and D3 and groups D2 and D3 (Fig. 5).

Factors associated with the incidence of hepatic carcinogenesis on multivariate analysis

Factors associated with the incidence of HCC as analyzed by the Cox proportional hazards model and the forward selection method are listed in Table 3. Age >65 years, low platelet count, high AST level, high AFP level, and high AFP-L3% level were significantly associated with the incidence of HCC. Factors associated with the incidence of HCC were analyzed in patients with chronic hepatitis and cirrhosis (Table 4). High age, low platelet count, high AST level, and high AFP level were significantly associated with the incidence of HCC in chronic hepatitis, and male sex, high age, low platelet count, high AFP level, and high AFP-L3% level were significantly associated with the incidence of HCC in cirrhosis. Factors associated with the incidence of HCC were analyzed in patients with and without IFN treatment (Table 5). Male sex, low platelet count, low cholinesterase level, and high AFP level were significantly associated with the incidence of HCC in patients with IFN therapy and male sex, high age, low platelet count, high AFP level, and high AFP-L3% level were significantly associated with the incidence of HCC in patients without IFN therapy.

Discussion

Advances in US, CT, and MRI have allowed for the more frequent and earlier detection of small HCC tumors less than 2 cm in diameter during the routine follow-up of patients with chronic liver disease [21–23]. However, the performance and resolution of the imaging device, the skills of individual operators, and the diagnostic acumen of the interpreting radiologist all affect the early detection of HCC. AFP, AFP-L3%, and DCP levels have been used as prognostic markers rather than diagnostic markers for HCC [9]. However, the detection rate of small HCC tumors with these markers is low; AFP-L3% and DCP have low sensitivity, and AFP has low specificity. Sassa et al. [12] reported detection rates of 22.6 and 48.4% for AFP-L3% and DCP, respectively, in patients with small HCC tumors. It is currently thought that serum markers are useful for follow-up after HCC therapy in patients with high tumor marker levels before treatment [24].

We have previously reported that the average integration value of ALT correlates with the cumulative incidence of hepatocarcinogenesis, even within the normal range [13, 14]. In the present study, the average integration value of AFP was not selected as a factor associated with the

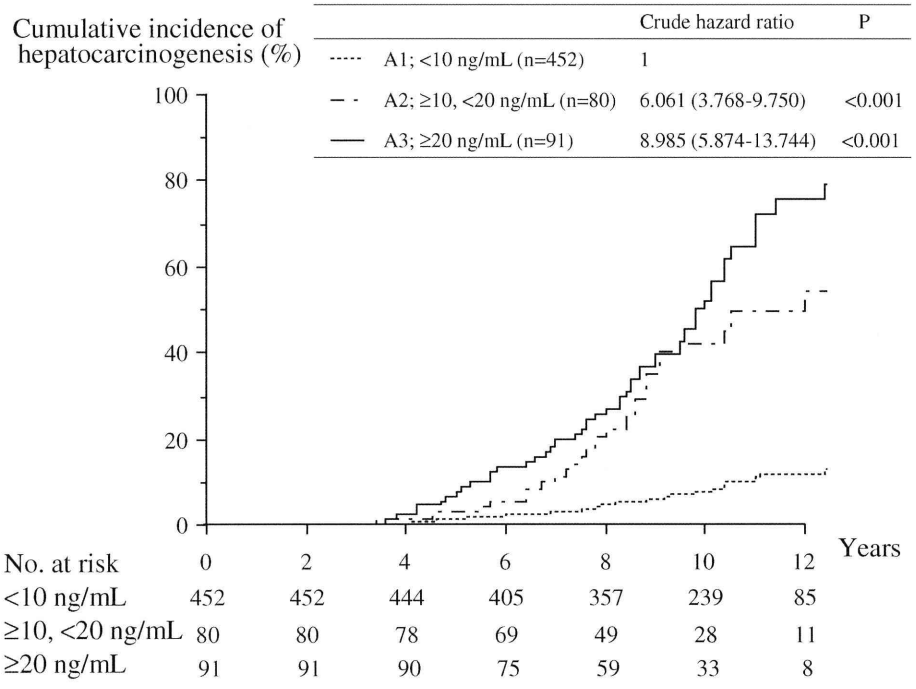

Fig. 3 Incidence of HCC according to the average integration value of AFP. The cumulative incidence of HCC differed significantly between groups A1 (<10 ng/mL) and A2 (≥10, <20 ng/mL) and groups A1 and A3 (≥20 ng/L)

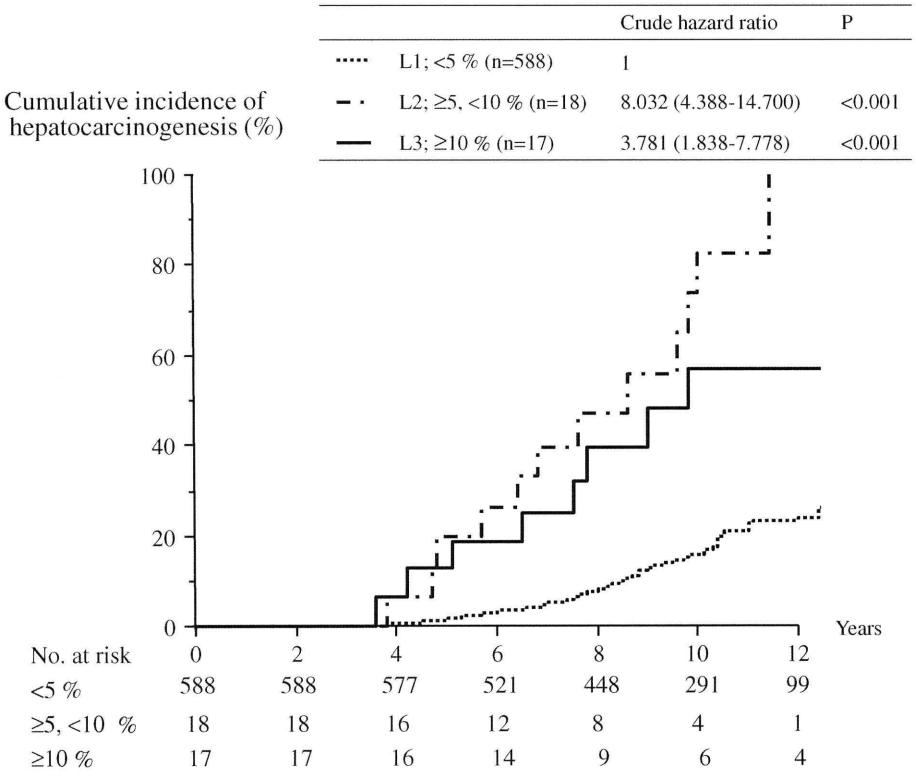

Fig. 4 Incidence of HCC according to the average integration value of AFP-L3%. The cumulative incidence of HCC differed significantly between groups L1 (<5%) and L2 (≥5, <10%) and groups L1 and L3 (≥10%)

incidence of HCC on multivariate analysis. AFP production is thought to be increased in response to injury, possibly due to increased hepatocyte turnover, in patients with HCV who do not have HCC [25]. In contrast, increased ALT levels are correlated with hepatocellular necrosis but not with hepatocyte proliferation. This difference may at least partially explain the absence of correlation between ALT and AFP levels.

The multivariate analysis in our series was carried out to minimize the influence of confounding factors, and 5 factors were selected by the forward selection method. Age >65 years, low platelet count, high AST value, high AFP

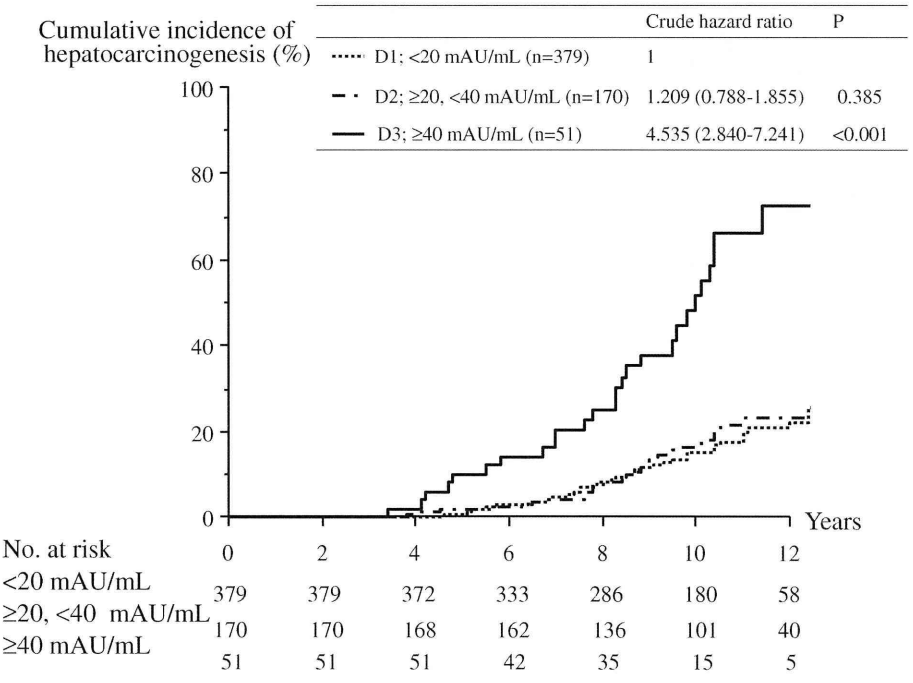

Fig. 5 Incidence of HCC according to the average integration value of DCP. The cumulative incidence of HCC differed significantly between groups D1 (<20 mAU/mL) and D3 (≥40 mAU/mL) and groups D2 (≥20, <40 mAU/mL) and D3

Table 3 Factors associated with hepatocarcinogenesis (multivariate analysis)

	Adjusted hazard ratio (95% CI)	*P*
Age (years)		
≤65	1	
>65	2.303 (1.551–3.418)	<0.001
Platelets ($\times 10^4$/mm^3)		
≥12.0	1	
<12.0	3.086 (1.997–4.768)	<0.001
AST (IU/L)		
≤40	1	
>40	3.001 (1.373–6.562)	0.006
AFP (ng/mL)		
A1; <10	1	
A2; ≥10, <20	2.814 (1.686–4.697)	<0.001
A3; ≥20	3.405 (2.087–5.557)	<0.001
AFP-L3 (%)		
L1; <5	1	
L2; ≥5, <10	2.494 (1.291–4.816)	0.007
L3; ≥10	3.555 (1.609–7.858)	0.002

AST aspartate aminotransferase, *AFP* alpha-fetoprotein, *AFP-L3 Lens culinaris* agglutinin-reactive fraction of AFP

level, and high AFP-L3% level were significantly associated with hepatic carcinogenesis in our multivariate analysis, but serum ALT level was not a risk factor for developing HCC. Ikeda et al. [26] reported that the cumulative incidence of HCC increased significantly in cirrhotic patients with an AFP level ≥10 ng/mL compared to those with an AFP level <10 ng/mL, and the adjusted risk ratio was 15.788 in HCV patients. They speculated that AFP is a marker of disease activity or severity and cellular regeneration, and it acts as a better predictor of HCC with viral etiology of cirrhosis. As an index of hepatic regeneration, the AFP level better represents the risk of hepatic carcinogenesis than an index of liver injury (e.g., ALT level). In addition to AFP, AFP-L3% was identified as a factor predicting the development of HCC, and this is a specific marker for the existence of HCC. Therefore, elevations in AFP-L3% may reflect an occult cancer that is undetectable with current imaging modalities. More intensive surveillance is needed for patients such as those who fulfill the criteria of groups L2 and L3 in our series, although these groups were very small in size. However, similar to other laboratory values, as high AFP-L3% values may be associated with severe liver damage, it is necessary to interpret these values carefully. DCP is well known to be also a specific marker of HCC. DCP is more closely related to tumor size than AFP and AFP-L3% [27]. Therefore, it is thought that these were the reasons that DCP was not selected as a predictive marker for HCC in our multivariate analysis.

Among the other risk factors we identified for the development of HCC, a low platelet count stands out. The platelet count is a useful marker for the diagnosis of cirrhosis [28], and cirrhosis is an established risk factor for HCC in HCV carriers [26, 28–30]. Taken together with our other findings, the low platelet count suggests that HCC develops in patients with progressive or advanced liver disease. We additionally used ultrasound (US) to distinguish cirrhotic patients from non-cirrhotic patients [16–18]. The presence of cirrhosis on US was strongly associated with an increased

Table 4 Factors associated with hepatocarcinogenesis on multivariate analysis in patients with chronic hepatitis and cirrhosis

	Chronic hepatitis ($n = 463$)	Cirrhosis ($n = 160$)
Age (years): ≤65 vs. >65	<0.001	0.008
Gender: female vs. male		<0.001
Platelets ($\times 10^4$/mm^3): ≥12.0 vs. <12	0.001	0.007
AST (IU/L): ≤40 vs. >40	0.043	
AFP (ng/mL): <10 vs. ≥10, <20 vs. ≥20	<0.001	0.003
AFP-L3 (%): <5 vs. ≥5, <10 vs. ≥10		0.017

AST aspartate aminotransferase, *AFP* alpha-fetoprotein, *AFP-L3 Lens culinaris* agglutinin-reactive fraction of AFP

Table 5 Factors associated with hepatocarcinogenesis on multivariate analysis in patients with and without IFN treatment

	With IFN ($n = 189$)	Without IFN ($n = 434$)
Age (years): ≤65 vs. >65		0.001
Gender: female vs. male	0.005	<0.001
Platelets ($\times 10^4$/mm^3): ≥12.0 vs. <12.0	0.047	<0.001
Cholinesterase (IU/L): ≥431 vs. <431	0.007	
AFP (ng/mL): <10 vs. ≥10, <20 vs. ≥20	<0.001	<0.001
AFP-L3 (%): <5 vs. ≥5, <10 vs. ≥10		<0.001

IFN interferon, *AFP* alpha-fetoprotein, *AFP-L3 Lens culinaris* agglutinin-reactive fraction of AFP

incidence of HCC on univariate analysis, but US-determined cirrhosis was not identified as a risk factor on multivariate analysis. Histologic assessment of fibrosis and cirrhosis was obtained in only 187 patients (30.0%), and patients with F4 fibrosis had a higher incidence of HCC in our univariate analysis. However, the population of patients with material available for histologic review was only one-third the size of the entire study population, and this small number may have negatively affected our ability to detect the predictive nature of fibrosis at all levels of severity. In contrast to serum ALT, serum AST levels were significantly associated with the incidence of HCC. AST levels are often abnormal in patients with cirrhosis when ALT values are in the normal range, and the AST/ALT ratio is frequently greater than 1 in cirrhotic patients [31]. Elevated AST activity is a surrogate marker for cirrhosis. Aging is associated with a number of events at the molecular, cellular, and physiological levels that influence carcinogenesis and subsequent cancer growth [32]. It has been hypothesized that an age-associated decrease in DNA repair [33] contributes to the development of HCC.

Recent reports have shown that AFP levels fall following the administration of IFN with or without ribavirin [34, 35]. IFN has been shown to have antiviral, anti-inflammatory, and anticancer activities [36]. One study demonstrated an anticancer effect of IFN when this agent was given following intrahepatic recurrence after HCC resection [37], and in our study, previous treatment with IFN was a factor associated with a reduced incidence of HCC on univariate analysis. The median ages of our patients with and without IFN treatment were 53 years (range 28–71) and 65 years (range 26–84), respectively; the age in those receiving IFN was significantly lower than the age in the group without IFN ($P < 0.0001$). It is thought that age and IFN therapy are confounding factors because IFN therapy has better results in younger patients. Although IFN was not identified as a predictive factor on multivariate analysis, the possibility cannot be denied that IFN may play an important role in modulating AFP levels prior to the onset of HCC.

In conclusion, increased AFP or AFP-L3% levels were significantly associated with an increased incidence of HCC. Among HCV carriers, patients with ≥10 ng/mL AFP or patients with ≥5% AFP-L3% are at very high risk for the development of HCC even if AFP is less than 20 ng/mL or AFP-L3% is less than 10%, which are the most commonly reported cutoff values. Intensive imaging modalities including US, CT, and MRI are recommended every 3–6 months for these patients.

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Conflict of interest There is no conflict of interest to disclose.

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ORIGINAL ARTICLE

Endogenous estrogen may prevent bone loss in postmenopausal hemodialysis patients throughout life

N. Sugiya · A. Nakashima · N. Takasugi · A. Kawai · K. Kiribayashi · J. Tanaka · N. Kohno · N. Yorioka

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Abstract
Summary Postmenopausal hemodialysis patients are at risk of complications related to renal mineral and bone disorder, and postmenopausal osteoporosis. In 112 postmenopausal hemodialysis patients, free estrogen index was positively correlated with bone mineral density (BMD) Z-score and the annual percent change of BMD in multiple regression analysis. Endogenous estrogen may prevent bone loss in postmenopausal hemodialysis patients throughout life.

Introduction Women on dialysis are not only at risk of developing mineral and bone disorder, but also suffer from postmenopausal osteoporosis. We assessed the effect of sex hormones on bone metabolism in postmenopausal hemodialysis patients.
Methods We enrolled 112 postmenopausal hemodialysis patients with a mean age of 68.4±10.4 years. We measured the serum levels of estradiol, testosterone, sex hormone-binding globulin (SHBG), and intact parathyroid hormone (intact-PTH), as well as bone metabolism parameters and radial bone mineral density (BMD). The free estrogen index (FEI) was calculated from the estradiol and SHBG values. After conventional dialysis was performed for 12 months, BMD was measured again and the annual percent change was calculated. Estradiol and SHBG were also measured in 25 postmenopausal women without chronic kidney disease.
Results Estradiol levels were higher in the hemodialysis patients than in the postmenopausal women without chronic kidney disease. In patients with relatively normal bone turnover (intact-PTH: from 150 to 300 pg/ml), the FEI showed a positive correlation with the BMD Z-score. The annual percent change of BMD showed a positive correlation with the FEI according to multiple regression analysis.
Conclusions Endogenous estrogen may prevent bone loss in postmenopausal hemodialysis patients throughout life.

Keywords Bone metabolism markers · Bone mineral density · Estradiol · Hemodialysis · Parathyroid hormone · Post menopausal osteoporosis

N. Sugiya · N. Kohno
Department of Molecular and Internal Medicine,
Graduate School of Biomedical Sciences,
Hiroshima University,
Minami-ku, Hiroshima, Japan

A. Nakashima (✉) · N. Yorioka
Department of Advanced Nephrology,
Graduate School of Biomedical Sciences, Hiroshima University,
1-2-3 Kasumi Minami-Ku,
Hiroshima City 734-8551, Japan
e-mail: ayumu@hiroshima-u.ac.jp

A. Nakashima · J. Tanaka
Department of Epidemiology,
Infectious Disease Control and Prevention,
Graduate School of Biomedical Sciences, Hiroshima University,
Minami-ku, Hiroshima, Japan

N. Takasugi
Department of Internal Medicine, Hakuai Clinic,
Kure, Hiroshima, Japan

A. Kawai
Department of Internal Medicine, Chuonaika Clinic,
Kure, Hiroshima, Japan

K. Kiribayashi
Department of Internal Medicine, Clear Yakeyama Clinic,
Kure, Hiroshima, Japan

Introduction

A significant decrease of the bone mineral density (BMD) has been reported in hemodialysis patients compared with

the general population and this decline of BMD becomes more marked as the duration of dialysis lengthens [1]. Hemodialysis patients often have secondary hyperparathyroidism due to hyperphosphatemia, impaired vitamin D activation, and hypocalcemia. Hyperparathyroidism not only leads to a low BMD and high fracture rate, but also impairs the health-related quality of life [2].

Estrogen is a sex hormone that is known to inhibit bone resorption [3]. Estrogen deficiency stimulates the proliferation and differentiation of osteoclast precursors, and activates mature osteoclasts [3, 4]. In addition, estrogen activates osteoblasts both directly and indirectly via growth factors such as insulin-like growth factor (IGF) I and II [5]. For these reasons, estrogen deficiency after menopause leads to a decrease of BMD. Postmenopausal women with an undetectable serum estradiol level have a higher risk of fracture than women with a serum estradiol level ≥5 pg/ml [6]. Thus, endogenous estrogen production still has an important influence on bone strength after menopause.

Despite frequent contact with medical care providers, women's health issues may receive less attention in patients on dialysis compared with women in the general population [7]. Women on dialysis are at risk of developing complications related to both mineral and bone disorder and postmenopausal osteoporosis, but there has been little investigation of the relationship between endogenous hormones and bone metabolism. Therefore, we studied the influence of endogenous sex hormones on bone metabolism in postmenopausal Japanese women receiving hemodialysis.

Subjects and methods

Subjects

We enrolled postmenopausal women (all ethnic Japanese) who had been on hemodialysis for over 1 year at Hakuai Clinic (Kure, Japan), Clear Yakeyama Clinic (Kure, Japan), and Chuonaika Clinic (Kure, Japan). We excluded patients who had received hormone replacement therapy, parathyroidectomy, or kidney transplantation. Patients who had undergone limb amputation were also excluded because of difficulty in calculating the body mass index (BMI). Furthermore, we excluded patients who were on steroid therapy. We also enrolled 25 postmenopausal women without chronic kidney disease (serum creatinine: <1.0 mg/dl). The definition of menopause was the same as for the hemodialysis group. None of the subjects had been on estrogen replacement therapy or had undergone oophorectomy.

The definition of menopause according to the World Health Organization is "The permanent cessation of menstruation resulting from loss of ovarian follicular activity." In our study, menopausal status was defined as a history of bilateral oophorectomy or an age ≥55 years without menstruation for over 1 year, because more than 80% of women in the general population are postmenopausal by the age of 55 years [8]. Women younger than 55 years who had been without menstruation for over 1 year or who had received hysterectomy or oophorectomy were considered to be menopausal if they had a follicle-stimulating hormone (FSH) level≥30 mIU/mL. We measured the serum FSH of the 12 patients who were under 55 years old, and excluded one patient with an FSH level <30 mIU/ml. Accordingly, we enrolled a total of 112 patients in this study and they continued conventional hemodialysis for 12 months. During the study period, ten patients were lost to follow-up because of transfer to another hospital or death, so a total of 102 patients could be followed for 12 months.

This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the hospital ethics committees of the participating hospitals. All of the subjects gave informed consent to participation.

Measurement of the radial BMD

We measured the BMD at the distal one-third of the radius by dual-energy X-ray absorptiometry (DOS-600, Aloka, Tokyo). The BMD Z-score was calculated by the following equation: (actual BMD−average BMD for the same age and gender)/standard deviation of the BMD for the same age and gender. After 12 months, we measured the BMD again and calculated the annual percent change as follows: 100× (follow-up BMD−baseline BMD)/baseline BMD.

Biochemical parameters

At the time of measuring the baseline BMD, venous blood samples were collected after an overnight fast for measurement of the serum concentrations of intact parathyroid hormone (intact-PTH), calcium, phosphate, bone-specific alkaline phosphatase (B-ALP), cross-linked N-terminal telopeptide of type I collagen (NTx), tartrate-resistant acid phosphatase (TRAP), estradiol, testosterone, and sex hormone-binding globulin (SHBG). For calcium and phosphate levels, the mean values were determined over a period of 3 months. The adjusted calcium level was calculated by Payne's formula [9].

Estradiol was measured with a DPC estradiol double-antibody kit (Mitsubishikagaku Yatoron, Tokyo, Japan) and all samples were evaluated in duplicate. The DPC estradiol double-antibody kit is a highly sensitive assay with a detection limit of 2.5 pg/ml. Intact-PTH was measured with an intact-PTH kit (Roche Diagnostics, Tokyo, Japan). B-ALP was measured by an immunoassay using microtiter

strips coated with a monoclonal anti-B-ALP antibody (Metra Biosystems, Mountain View, CA, USA), NTx was measured with an Osteomark-NTx serum kit (Ostex International, Seattle, WA, USA), and TRAP was measured with an N-Assay ACP Nittobo kit (Nitto Boseki, Tokyo, Japan). Testosterone was measured with an Immulite-1000 Testosterone kit (Mitsubishikagaku Yatoron), and SHBG was measured with an Immulite-2000 SHBG kit (Mayo Medical Laboratories, Rochester, MN, USA). The free estrogen index (FEI) was calculated from total estradiol and SHBG by the following equation: FEI = estradiol(pg/ml) × 0.367/SHBG(nmol/l). The free androgen index (FAI) was calculated from total testosterone and SHBG by the following equation: FAI = testosterone(ng/ml) × 3.47 × 100/SHBG(nmol/l) [10, 11].

Statistical analysis

All variables were expressed as the mean±SD or median and interquartile range (25th to 75th percentiles), unless otherwise indicated. The patients were divided into two groups according to whether the serum estradiol level was <2.5 pg/ml or ≥2.5 pg/ml. Statistical analysis was performed by the Mann-Whitney U test, or the χ^2 test was used for categorical data.

The following variables were included in univariate and multivariate models: age (1-year intervals), duration of hemodialysis (1-month intervals), diabetes (present/absent), vitamin D therapy (present/absent). In multiple regression analysis, we used log-transformed ($\log_{10}$) values for the following parameters: duration of hemodialysis, intact-PTH, B-ALP, NTx, and TRAP.

Multiple regression analysis with forward elimination was used to evaluate possible independent predictors of the FEI by testing a total of 13 variables (age, duration of hemodialysis, diabetes, BMI, vitamin D therapy, intact-PTH, adjusted calcium, phosphate, B-ALP, NTx, TRAP, FAI, and SHBG).

We examined the relationship between the BMD Z-score and the following factors using Spearman's rank correlation analysis: duration of hemodialysis, diabetes, BMI, vitamin D therapy, dose of oral calcium, dose of sevelamer hydrochloride, intact PTH, adjusted calcium, phosphate, B-ALP, NTx, TRAP, FEI, FAI, and SHBG. Furthermore, multiple regression analysis with forward elimination was used to evaluate possible predictors of the BMD Z-score by testing a total of 15 variables (duration of hemodialysis, diabetes, BMI, vitamin D therapy, dose of oral calcium, dose of sevelamer hydrochloride, intact-PTH, adjusted calcium, phosphate, B-ALP, NTx, TRAP, FEI, FAI, and SHBG). Then we selected a subgroup of patients with relatively normal bone turnover who had intact-PTH levels ranging from 150 to 300 pg/ml. In all of the patients and in this subgroup, Spearman's rank correlation analysis was used to assess the relation between the BMD Z-score and the FEI. The BMD Z-score was used instead of raw BMD data because employing a BMD Z-score adjusted for age and sex made the model much closer to ideal. Finally, we examined the annual percent change of BMD by Spearman's rank correlation analysis and multiple regression analysis with forward elimination. The variables employed were the same as for the cross-sectional analysis of BMD Z-score.

Results

Although age and SHBG did not differ between the postmenopausal hemodialysis patients and postmenopausal women without chronic kidney disease (age: 68.4±10.4 vs. 69.7±8.6, P=0.393; SHBG: 67.4±25.4 vs. 67.7±25.6, P=0.850, respectively), estrogen levels were higher in the hemodialysis patients. Despite using a highly sensitive estradiol kit, 43 out of 112 postmenopausal hemodialysis patients had undetectable estradiol levels versus 22 out of 25 women without chronic kidney disease (38.4% vs. 88.0%, P<0.0001).

We stratified the subjects into two groups based on a serum estradiol level <2.5 pg/ml or ≥2.5 pg/ml. Table 1 shows the clinical and laboratory parameters of these two groups. In the group with a serum estradiol level <2.5 pg/ml, the levels of intact-PTH, B-ALP, and NTx, as well as the BMD, BMD Z-score, and BMD T-score, were all smaller than in the group with a serum estradiol level >2.5 pg/ml, but these differences did not reach significance.

Stepwise multiple regression analysis was performed for all 112 patients to find independent predictors of the FEI (Table 2). As a result, the FEI showed a positive correlation with the FAI, diabetes, and intact-PTH, as well as a negative correlation with SHBG.

Next, we examined the factors that influenced the BMD Z-score by Spearman's rank correlation analysis. There was a negative correlation with the duration of hemodialysis, vitamin D therapy, B-ALP, and NTx. When stepwise multiple regression analysis was performed in all 112 patients to find independent predictors of the BMD Z-score, there was a negative correlation with duration of dialysis and B-ALP, as well as a positive correlation with the FEI (Table 3).

Secondary hyperparathyroidism is strongly associated with a decrease of the BMD [12], so we also investigated correlations in our patients with relatively normal bone turnover who had intact-PTH levels in the range from 150 to 300 pg/ml. In this subgroup (n=32), the FEI showed a positive correlation with the BMD Z-score (r=0.658, P< 0.001), despite showing no correlation with the Z-score (r=

Table 1 Clinical and laboratory parameters of the two estradiol groups

	Estradiol <2.5pg/ml	Estradiol ≥2.5pg/ml	*P* value
Number	43	69	
Age (years)	69.7±9.3	67.6±11.0	0.330
Duration of hemodialysis (months)	66 (39-111)	69 (37-133)	0.609
Diabetes (%)	13 (30.2%)	26 (37.7%)	0.509
BMI (kg/m^2)	21.1±3.5	20.7±3.0	0.809
sBP (mmHg)	137.3±26.1	133.4±19.9	0.209
dBP (mmHg)	69.6±12.3	67.5±12.2	0.114
Vitamin D therapy (%)	17 (39.0%)	36 (52.2%)	0.747
Dose of oral calcium (mg/day)	1825.6±1353.5	1840.6±1511.0	0.940
Dose of sevelamer hydrochloride (mg/day)	476.7±1088.1	789.9±1161.3	0.182
Intact-PTH (pg/dl)	130.8 (57.0-194.0)	145.2 (66.8-289.4)	0.114
Adjusted Calcium (mg/dl)	9.3±0.6	9.4±0.7	0.637
Phosphate (mg/dl)	4.9±1.0	5.2±1.2	0.140
B-ALP (U/l)	31.2 (24.0-40.2)	33.3 (25.1-47.5)	0.189
NTx (nmol BCE/l)	76.2 (59.4-119.0)	100.0 (60.7-175.5)	0.076
TRAP (IU/l)	12.3 (10.8-15.0)	15.1 (10.0-15.1)	0.876
FEI	–	0.072±0.065	–
SHBG (nmol/l)	70.8±31.8	64.1±27.3	0.284
BMD (g/cm^2)	0.439±0.100	0.474±0.111	0.091
BMD-Z score (SD)	−0.504±1.214	−0.232±1.198	0.212
BMD-T score (SD)	−3.861±1.881	−3.270±2.186	0.142

Statistical analysis was performed by the Mann-Whitney *U* test, or the χ^2 test was used for categorical data

BMI body mass index, *sBP* systolic blood pressure, *dBP* diastolic blood pressure, *intact-PTH* intact parathyroid hormone, *B-ALP* bone-specific alkaline phosphatase, *NTx* cross-linked N-terminal telopeptide of type I collagen, *TRAP* tartrate-resistant acid phosphatase, *FEI* free estrogen index, *SHBG* sex hormone-binding globulin, *BMD* bone mineral density

0.136, P=0.155) in all patients (n=112). When we examined factors that influenced the annual percent change of BMD by Spearman's rank correlation analysis, there was a positive correlation with the FEI, while there was a negative correlation with intact-PTH, NTx, and TRAP. Stepwise multiple regression analysis was performed to find independent predictors of the annual percent change of BMD, showing a positive correlation with the FEI, as well as a negative correlation with intact-PTH and NTx (Table 4).

Discussion

Women on dialysis are at risk of suffering from renal mineral and bone disorders as well as postmenopausal osteoporosis. In this study, we investigated the effect of sex hormones on bone metabolism in postmenopausal hemodialysis patients, and we found that estradiol levels were higher in these patients than in women without chronic kidney disease. In patients with relatively normal bone turnover (intact-PTH: 150-300 pg/ml), the FEI had a positive correlation with the BMD Z-score. The annual percent change of BMD showed a positive correlation with the FEI according to multiple regression analysis. These findings suggest that endogenous estrogen prevents bone loss in postmenopausal hemodialysis patients throughout life.

Table 2 Factors with an independent influence on the FEI according to stepwise multiple regression analysis

Factor	β	*F* value	*P* value
FAI	0.207	5.883	0.017
Diabetes	0.187	4.883	0.031
Intact-PTH[a]	0.182	4.623	0.033
SHBG	−0.379	19.407	<0.001

R^2=0.246; P<0.001; the F value was set at 4 in each step

[a] The intact-PTH levels were transformed to log values

In the present study, the FEI demonstrated a positive correlation with intact-PTH (Table 2). Estrogen has been reported to promote PTH secretion [13], while expression of estrogen receptor mRNA has been demonstrated in rat parathyroid tissue and binding of estrogen to the parathyroid glands has been shown by immunohistochemistry [14]. These findings suggest that estrogen might promote PTH secretion in postmenopausal hemodialysis patients as it does in healthy postmenopausal women. During early menopause, a sudden decrease of estrogen leads to high bone turnover, increased bone resorption, and a reduction of PTH. In late menopause, however, low intestinal calcium absorption [15] and low renal calcium handling [16] are primarily responsible for a higher serum PTH level [17]. Therefore, healthy postmenopausal women first show a decrease of the PTH level and then it gradually rises with increasing age. This suggests that there might be no

Table 3 Factors correlated with the BMD Z-score

Factor	Simple		Multiple		
r^2	ρ –	*P* –	β 0.219 (*P*<0.001)	*F*	*P*
Duration of hemodialysis[a]	−0.322	0.001*	−0.289	11.096	0.001
Diabetes	0.104	0.272	–	NA	–
BMI	0.151	0.111	–	NA	–
Vitamin D therapy	−0.243	0.011	−0.179	4.312	0.040
Dose of oral calcium	−0.021	0.825	–	NA	–
Dose of sevelamer hydrochloride	−0.038	0.688	–	NA	–
Intact-PTH[a]	−0.175	0.064	–	NA	–
Adjusted calcium	−0.046	0.963	–	NA	–
Phosphate	0.065	0.495	–	NA	–
B-ALP[a]	−0.225	0.017	−0.214	6.106	0.015
NTx[a]	−0.215	0.023	–	NA	–
TRAP[a]	0.172	0.070	–	NA	–
FEI	0.136	0.155	0.204	5.683	0.019
FAI	0.023	0.809	–	NA	–
SHBG	−0.154	0.106	–	NA	–

BMI body mass index, *intact-PTH* intact parathyroid hormone, *B-ALP* bone-specific alkaline phosphatase, *NTx* cross-linked N-terminal telopeptide of type I collagen, *TRAP* tartrate-resistant acid phosphatase, *FEI* free estrogen index, *FAI* free androgen index, *SHBG* sex hormone-binding globulin, *BMD* bone mineral density, *NA* not accepted as significant.

Multiple: stepwise multiple regression analysis. The *F* value was set at 4.0 in each step

[a] These variables were transformed to log values for stepwise multiple regression analysis

*$P<0.01$, simple: Spearman's rank correlation analysis

correlation between estradiol and PTH in healthy postmenopausal women.

BMD has been reported to show a strong correlation with intact-PTH [12]. Therefore, we investigated the relation between the FEI and BMD Z-score in our patients with relatively normal bone turnover (an intact-PTH level from 150 to 300 pg/ml). We considered that the intact-PTH level would have little influence on the BMD of this subgroup. As a result, we found that the FEI had a positive correlation with the BMD Z-score in this subgroup. Estrogen deficiency was reported to increase the secretion of interleukin-1, interleukin-6, interleukin-11, and tumor necrosis factor-α and -β [18–20], which activate mature osteoclasts indirectly via a primary effect on osteoblasts and by stimulating the proliferation and differentiation of osteoclast precursors [3, 4]. These findings suggest that estrogen could inhibit bone loss in postmenopausal hemodialysis patients.

Table 4 Factors correlated with the annual percent change of BMD

Factor	Simple		Multiple		
r^2	ρ –	*P* –	β 0.183 (*P*<0.001)	*F*	*P*
Age	0.158	0.113	–	NA	–
Duration of hemodialysis[a]	−0.200	0.044	–	NA	–
Diabetes	0.204	0.041	–	NA	–
BMI	0.101	0.308	–	NA	–
Vitamin D therapy	−0.015	0.877	–	NA	–
Dose of oral calcium	0.127	0.202	–	NA	–
Dose of sevelamer hydrochloride	−0.117	0.240	–	NA	–
Intact-PTH[a]	−0.325	0.001*	−0.233	5.109	0.026
Adjusted calcium	0.069	0.485	–	NA	–
Phosphate	0.038	0.702	–	NA	–
B-ALP[a]	−0.197	0.048	–	NA	–
NTx[a]	−0.260	0.009*	−0.204	4.017	0.048
TRAP[a]	−0.270	0.007*	–	NA	–
FEI	0.363	<0.001*	0.271	8.569	0.004
FAI	0.173	0.083	–	NA	–
SHBG	−0.152	0.127	–	NA	–

BMI body mass index, *intact-PTH* intact parathyroid hormone, *B-ALP* bone-specific alkaline phosphatase, *NTx* cross-linked N-terminal telopeptide of type I collagen, *TRAP* tartrate-resistant acid phosphatase, *FEI* free estrogen index, *FAI* free androgen index, *SHBG* sex hormone-binding globulin, *BMD* bone mineral density, *NA* not accepted as significant

Multiple: stepwise multiple regression analysis. The *F* value was set at 4.0 in each step

[a] These variables were transformed to log values for stepwise multiple regression analysis

*$P<0.01$, simple: Spearman's rank correlation analysis

In the present study, the BMD Z-score showed a positive correlation with the FEI and a negative correlation with B-ALP according to multiple regression analysis (Table 3). In addition, the annual percent change of BMD was positively correlated with the FEI according to multiple regression analysis, as well as being negatively correlated with intact-PTH and NTx. The negative correlation between BMD and B-ALP indicates that BMD was lower in patients with a high bone turnover. B-ALP showed a strong correlation with intact-PTH. On the other hand, PTH secretion was promoted by estrogen, even though the FEI showed a positive correlation with both the BMD Z-score and the annual percent change of BMD. Thus, estrogen may have two opposing effects on bone metabolism in postmenopausal hemodialysis patients. Estrogen is well known to directly inhibit bone resorption [3]. In addition, estrogen activates osteoblasts both directly and indirectly via the action of growth factors such as IGF-I and -II [5]. Thus, estrogen is thought to decrease bone resorption and increase bone formation in postmenopausal hemodialysis patients. On the other hand, estrogen has previously been reported to promote PTH secretion [13], and we also showed a positive correlation between the FEI and intact-PTH. An increase of PTH increases bone remodeling. In hemodialysis patients, however, a high PTH level is strongly associated with a decrease of BMD. If estrogen only acted to promote PTH secretion, BMD would decrease. However, our study showed that the FEI was positively correlated with the BMD Z-score and the annual percent change of BMD, so the direct effect of estrogen on bone appears to outweigh its indirect effect via PTH.

In the present study, the FEI showed a positive correlation with the presence of diabetes according to multiple regression analysis (Table 2). Many studies have assessed the relation between estrogen and diabetes, and it has been reported that the plasma estradiol level is positively associated with insulin resistance in postmenopausal women [21]. In addition, higher plasma estradiol levels are prospectively related to an increased risk of type 2 diabetes in postmenopausal women [22]. Furthermore, exposure to estradiol induces an increase of pancreatic β-cell insulin in mice and leads to chronic hyperinsulinemia, while longer exposure to estradiol enhances the risk of type 2 diabetes [23].

Weisinger et al. [24] reported on the correlation between serum estradiol and BMD in women under 50 years old. They showed that persistently amenorrheic younger women on dialysis had a lower trabecular BMD compared with normally menstruating women on dialysis, and they found that lumbar spine BMD was significantly correlated with the total estradiol level in the amenorrheic group. We studied postmenopausal women under 85 years old on hemodialysis and showed that the serum estradiol level had a positive correlation with the BMD-Z score. Accordingly, estradiol seems to influence bone metabolism in postmenopausal women on hemodialysis throughout life. Cummings et al. [6] reported that an undetectable serum estradiol level was a risk factor for fracture in postmenopausal women. Our results indicate that patients with a low FEI have a low BMD and might have a higher risk of fracture. Hemodialysis patients already show an increased risk of fracture compared with healthy persons, so we have to pay close attention to the estradiol level in postmenopausal women on hemodialysis.

There have been conflicting reports about serum estradiol levels in postmenopausal women with end-stage renal disease [25–27]. Tanaka et al. [27] reported that estradiol levels were higher in hemodialysis patients and our findings support their results.

One of the limitations of this study is that we only measured the BMD at the radius. However, Ettinger et al. [28] reported that women with estradiol levels from 10 to 25 pg/ml had a 4.9%, 9.6%, 7.3%, and 6.8% higher BMD of the total hip, calcaneus, proximal radius, and spine, respectively, than women with estradiol levels below 5 pg/ml. According to their report, estradiol prevents both cortical and trabecular bone loss in healthy postmenopausal women.

In healthy postmenopausal women, the risk of breast cancer, pulmonary embolism, coronary artery disease, and cerebrovascular disease is increased by long-term combined estrogen and progesterone therapy [29]. However, treatment with raloxifene (a selective estrogen receptor modulator) seems to be less harmful in women with osteoporosis, and 3 years of raloxifene therapy increases the lumbar spine BMD along with a marked decrease of vertebral fractures [30]. Thus, newer therapeutic regimes for postmenopausal hemodialysis patients are expected to include raloxifene.

In conclusion, this study revealed that the FEI was positively correlated with the BMD Z-score and the annual percent change of BMD in postmenopausal women on hemodialysis. Endogenous estrogen may prevent bone loss in postmenopausal hemodialysis patients throughout life.

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Conflicts of interest None.

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質疑応答

questions and answers

要項

- 質問は「質疑応答係」宛に
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質問送付先

〒101-8718
東京都千代田区神田駿河台2-9　日本医事新報社
質疑応答係
FAX：03-3292-1550

内科

Q C型肝炎ウイルスキャリアの慢性肝炎発症率

C型肝炎ウイルスの無症候性キャリアが慢性C型肝炎を発症する率は，どの程度か．

（京都府　N）

A 観察を続けると高率に慢性肝炎へ進展していく．定期的なフォローアップを心がける

肝臓は「沈黙の臓器」と言われ，肝炎ウイルスに感染していても自覚症状が乏しいため，感染していることが分かった時点ですでに肝疾患が進んだ状態であることも多い[1]．ASTやALTが正常でも，実際には肝組織での炎症がすでに起こっており，肝臓の線維化を認めることがある．そのため，一般的な血液検査が正常であるだけで簡単に無症候性キャリア（asymptomatic carrier；ASC）とすることは，実際の肝疾患の状態と異なり，予後の判断を誤ることがある．

献血を契機にC型肝炎ウイルスに感染していることが判明した供血者（自覚症状はない）を対象に行った前向き研究では，献血後の初診時にすでに52％が慢性肝炎の状態と診断された[1]．特に男性では，慢性肝炎を指摘された者が62.6％と女性に比べ有意に多く，性差が認められた．

ご質問の「無症候性キャリア」を，ここでは一般的に解釈して，血液検査や腹部超音波検査など侵襲性の少ない検査で特に異常が認められない者とする．

筆者らは，インターフェロン（IFN）治療を受けていないC型肝炎ウイルス持続感染者の1年ごとの病態推移を集計し，Markovモデルを用いて自然経過での肝疾患の進行を予測した（図1）[2]．各病態からの年間移行率を求めると，男性の40代ASCの14.3％が1年間で慢性肝炎に移行する．慢性肝炎は年率1.1％が肝硬変に移行する．すると表1に示すよう

表1　ASCからの肝疾患移行率

	年齢					
	40	41	45	50	60	70
男性						
ASC	100.00	85.71	46.27	21.41	7.13	2.62
CH	0.00	14.29	51.99	72.44	69.39	48.38
LC	0.00	0.00	1.31	4.62	12.94	14.62
HCC	0.00	0.00	0.44	1.54	10.55	34.38
女性						
ASC	100.00	83.61	41.35	17.96	6.22	1.85
CH	0.00	16.39	56.85	75.88	78.49	45.37
LC	0.00	0.00	1.80	6.16	10.84	32.79
HCC	0.00	0.00	0.00	0.00	4.45	20.00

*ASC：無症候性キャリア，CH：慢性肝炎，LC：肝硬変，HCC：肝がん

（文献[2]より）

に，40歳男性のASCは5年後に52%が慢性C型肝炎を発症，10年後までASCのままでいられるのは約21%で，慢性肝炎を発症しているのは約72%となる．この時さらに肝硬変への進行は4.6%，肝がんへの進行は1.5%となる．40歳女性では1年後の慢性肝炎の移行確率は男性より高いが，20～30年後の肝がんへの進展率は男性より低い．

以前はトランスアミナーゼの上昇を伴わないASCの場合，特に治療対象とみなされず，通院の必要性も重要視されていない時代があった．しかし，現在ではASCは経過観察中に高率にトランスアミナーゼが変動し始め，慢性肝炎へ移行することが指摘されているため，「通院の必要はありません」と説明できなくなっている．定期的な経過観察が重要であり，早期に治療を開始することも選択肢として考える必要がある．近年は，IFN治療効果が事前に予測できる遺伝子診断もあることから，抗ウイルス療法については専門医に相談し，連携をとりながら肝がんへの進展の阻止へ向けた治療を進めていただけると幸いである．

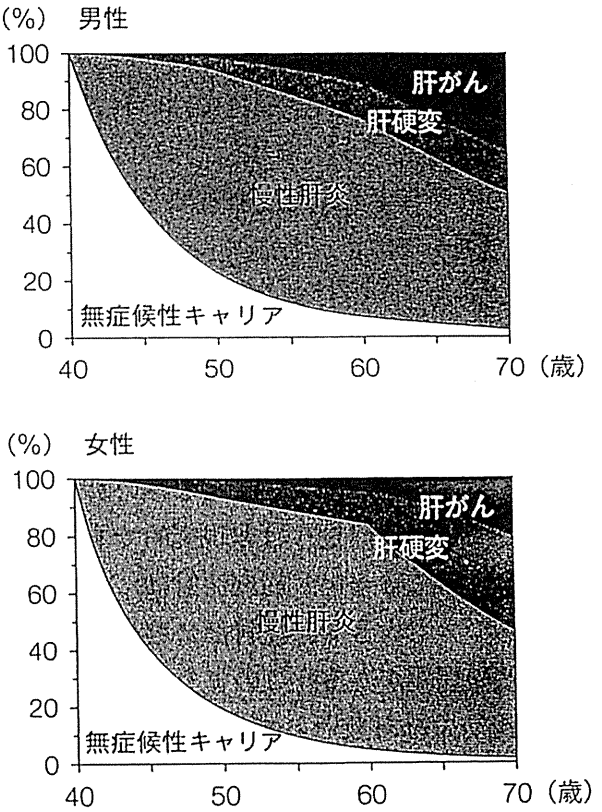

図1　ASCからの肝病態の推移
（無治療の場合，Markovモデルによる推計）

▶文　献

1) Mizui M, et al : Hepatol Res 37 : 994, 2007.

2) Tanaka J, et al : J Med Virol 70 : 378, 2003.

▶回　答

広島大学大学院医歯薬学総合研究科
疫学・疾病制御学　*教授

松尾順子　*田中純子