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.

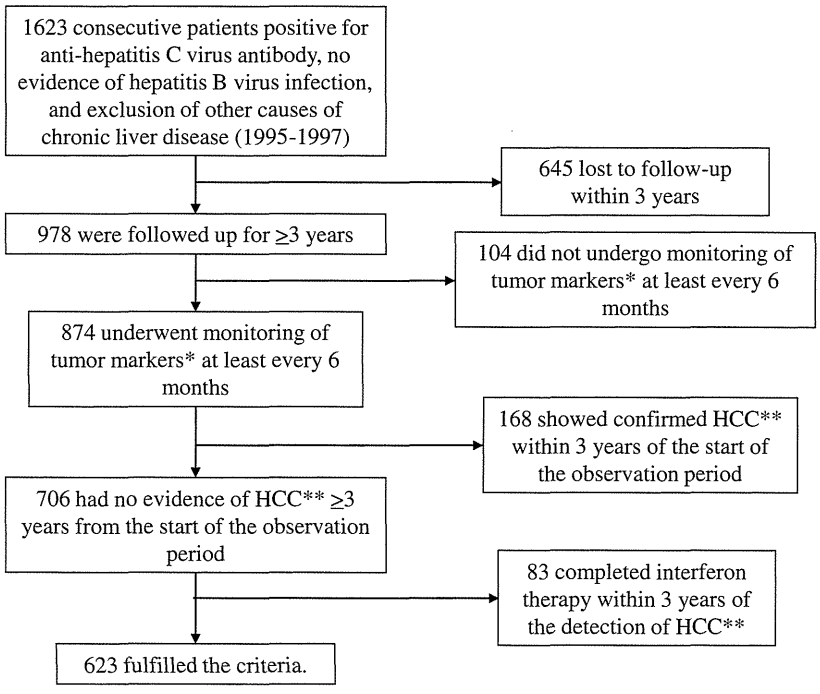

Fig. 1 Schematic flowchart of enrolled patients. *Serum alpha-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3%), and des-γ-carboxy prothrombin (DCP). **Hepatocellular carcinoma (*HCC*)

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.

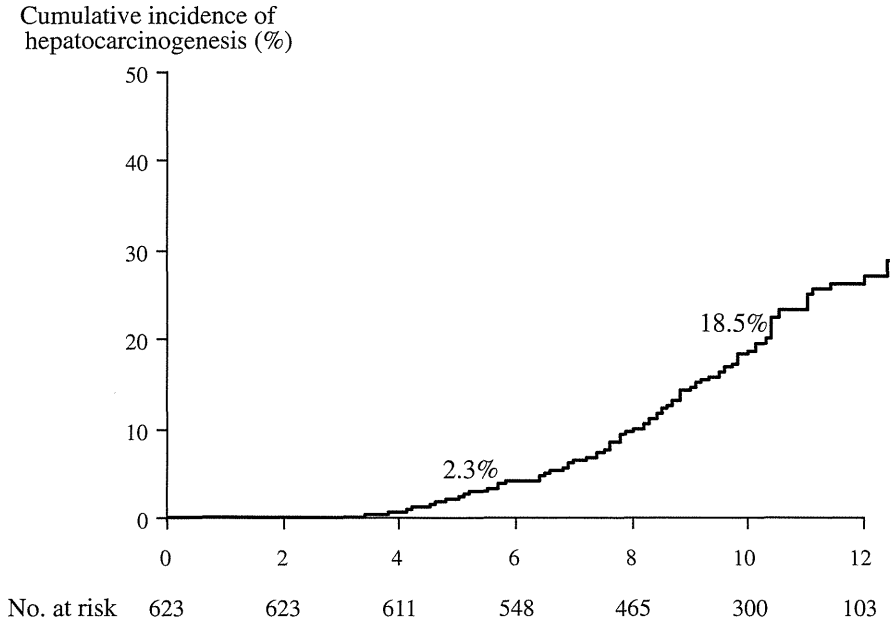

Fig. 2 Overall cumulative incidence rate of HCC

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

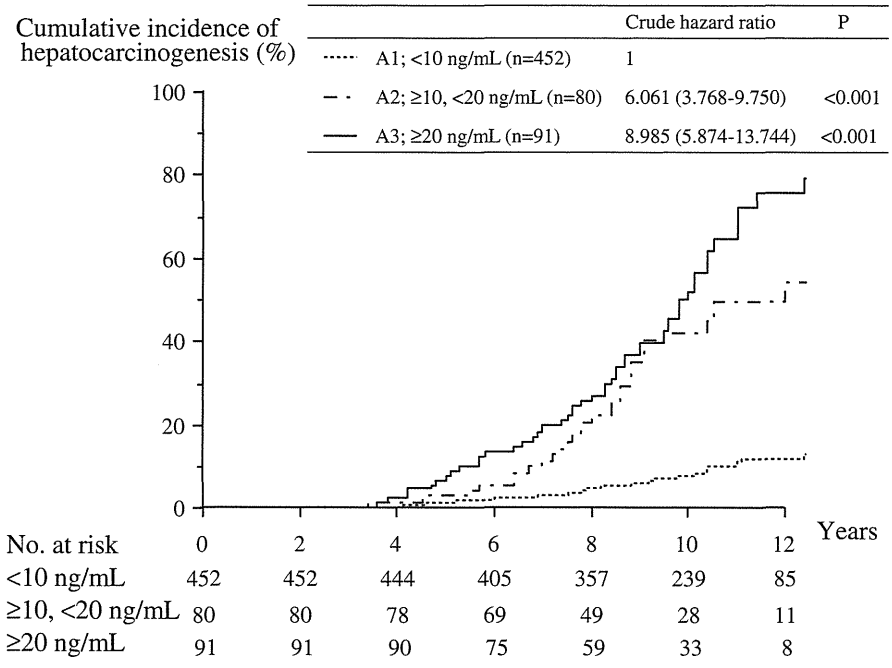

Fig. 3 Incidence of HCC according to the average integration value of AFP. The cumulative incidence of HCC differed significantly between groups A1 (<10 ng/mL) and A2 (≥10, <20 ng/mL) and groups A1 and A3 (≥20 ng/L)

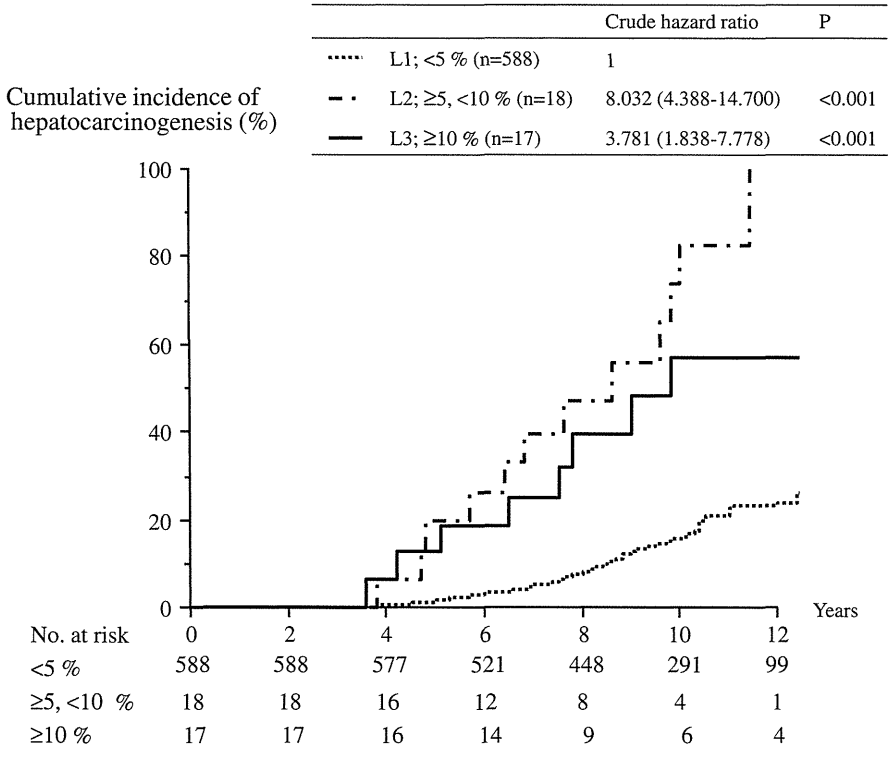

Fig. 4 Incidence of HCC according to the average integration value of AFP-L3%. The cumulative incidence of HCC differed significantly between groups L1 (<5%) and L2 (≥5, <10%) and groups L1 and L3 (≥10%)

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

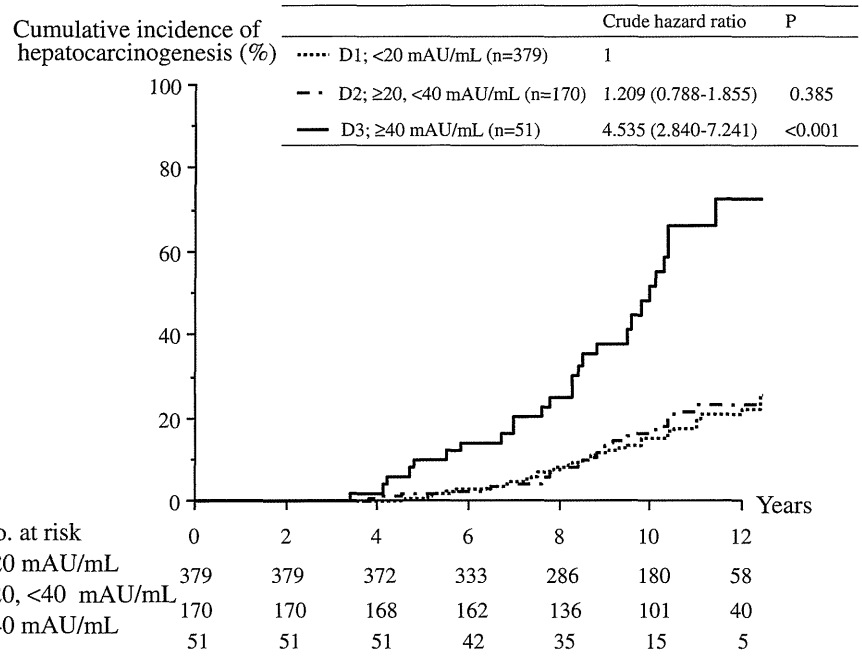

Fig. 5 Incidence of HCC according to the average integration value of DCP. The cumulative incidence of HCC differed significantly between groups D1 (<20 mAU/mL) and D3 (≥40 mAU/mL) and groups D2 (≥20, <40 mAU/mL) and D3

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

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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

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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		
	ρ	*P*	β	*F*	*P*
r^2	–	–	0.219 ($P<0.001$)		
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		
	ρ	*P*	β	*F*	*P*
r^2	–	–	0.183 ($P<0.001$)		
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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新時代のウイルス性肝炎学

—基礎・臨床研究の進歩—

II. C型肝炎

我が国におけるC型肝炎の疫学
—国際比較を含めて—

田中純子　　片山惠子

我が国におけるC型肝炎の疫学—国際比較を含めて—

Hepatitis C virus infection in Japan—epidemiology—

田中純子　片山恵子

Key words：HCVキャリア率，HCVキャリア数，初回供血者，節目検診受診者，キャリア対策

はじめに，および背景

ウイルス肝炎の病因ウイルスとして現在確認されている5種類のうち，最後に見つかったのは，C型肝炎ウイルス(HCV)である．HCVは感染したヒトの血液中に見いだされることから，血液を介して感染し，持続感染を起因として肝発がんとの関連が知られている．

1989年に米国のHoughtonらによりHCV遺伝子の一部がクローニング[1]された後，同年12月には，我が国では世界に先駆けて輸血用血液のスクリーニングとしてC100-3抗体[2]による検査を開始した．その後，PCR(polymerase chain reaction)法によるHCV RNAの検出法の確立や[3,4]，第二世代のHCV関連抗体検出系の開発が進み，HCVに関する診断能力は飛躍的な進歩をとげた．

輸血用血液のスクリーニングとしては，より精度の高い[5]第二世代のHCV抗体測定系(HCV passive hemagglutination: HCV PHA法)が1992年2月から導入され(1993年9月以後，PA法(particle agglutination)も追加導入)，輸血後肝炎の発生率は大幅に減少した[6]．

WHO(World Health Organization)は，HCV抗体検査が輸血用血液のスクリーニングとして導入・普及し始めた1992年以前には，世界中の輸血後肝炎の主な原因はHCVであったこと，米国における輸血後肝炎の90％はHCVによるものであったことを報告[7]している．我が国では，1999年10月から導入された核酸増幅検査(nucleic acid amplification test: NAT)により，輸血に伴うHCV感染はほぼ駆逐されたといえる状況となった[6](なお，2008年以後はCLEIA法によるHCV抗体検査および新NAT検査が行われている)．

このように1990年代はHCV関連抗体の検出系の開発と普及が進み，献血者集団だけでなく地域住民を対象とした肝炎ウイルス検査や調査，また病医院における検査などが広く行われるようになり，徐々に肝炎ウイルス感染状況が明らかになってきたといえる．

2000年代に入ると，検査方法や検査手順が整理されたこと，抗ウイルス療法などの治療法や診断技術が進歩したことなどを背景に，病因論に基づく肝炎肝がん対策として，40歳以上の住民を対象とした肝炎ウイルス検査(節目・節目外検診)が2002年度から5カ年計画で実施された．5年の間に約800万人がC型肝炎ウイルス検査を受けたが，2007年度以後も引き続き保健所での検査や健康増進法による検査など，公費補助を伴った検査が行われている．

本稿では20年にわたるこれらの状況を背景に，供血者集団をはじめとする幾つかの大規模集団を対象として得られた成績をもとに，C型

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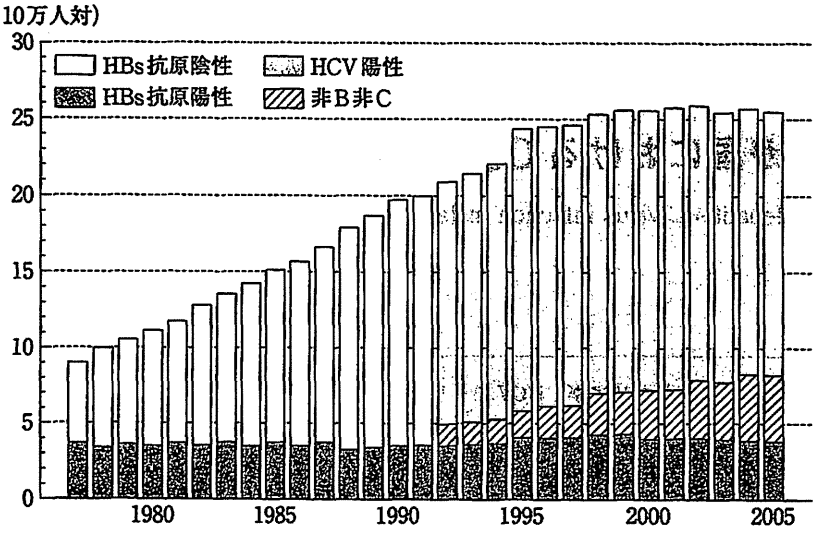

図1 病因別にみた肝がんによる死亡数の経年的推移(1977-2005年)
下記の資料(1977-2005)より試算：2010.5
厚生労働省大臣官房統計情報部：人口動態統計
日本肝癌研究会：全国原発性肝癌追跡調査報告

肝炎の疫学を紹介したい.

1. 肝がん死亡の年次推移とその成因

我が国の悪性新生物'肝'(肝および肝内胆管の悪性新生物，人口動態統計[8]，2008年)による死亡は，肺がん，胃がんについで，第3位(死亡実数33,665人，26.7/人口10万人対)と上位である．1975年までは人口10万人あたり10人前後であった肝がん死亡率は，増加の一途をたどったが，2002年からしばらく横ばいの状態で推移している．男性の肝がん死亡率は，女性の約2倍の高値を示し，女性では2002年以後も微増の状態である.

肝がん死亡の病因別内訳について，日本肝癌研究会による調査成績[9]と人口動態統計資料[8]とを用いて推計したものを図1に示す．人口10万人あたりの肝がん死亡のうち，B型肝炎ウイルス(HBV)の持続感染に起因する肝がん死亡の割合は，1977年から現在に至るまで5以下の一定値を示している．HCV感染の診断が可能となった1992年以後，肝がんの約9割がHBVあるいはHCVの持続感染に起因し，そのうちの80％がHCVによることが明らかとなった．しかし近年特に2000年以後，非B非C型に由来する肝がんの割合が10％を超え更に増加傾向にある．その原因や将来動向については，nonalcoholic steatohepatitis/nonalcoholic fatty liver disease(NASH/NAFLD)との関連性の解明とともに課題といえる.

2. 我が国におけるHCVキャリア率

我が国で2000年以後に得られた大規模集団におけるHCVキャリア率を図2-aに示す．まず，2001年から6年間の日本赤十字血液センター初回供血者3,748,422人の資料から，日本赤十字社の協力のもとに厚労省疫学班として算出した[10,11]HCVキャリア率である．なお，HCV抗体陽性率に70％を乗じた値をHCVキャリア率と読みかえている．次に，2002年度から5カ年計画で実施された'肝炎ウイルス検査'について厚生労働省から公表されている成績のうち，'節目検診'(40-70歳までの5歳刻みの節目の年齢にあたる人を対象とした検診)の成績から得たHCVキャリア率[10]である.

2つの集団のHCVキャリア率の値はほぼ近似しているが，初回供血者集団はその約84％が40歳以下の年齢に偏っている．40歳以下の年齢層は初回供血者集団の，40歳以上の年齢

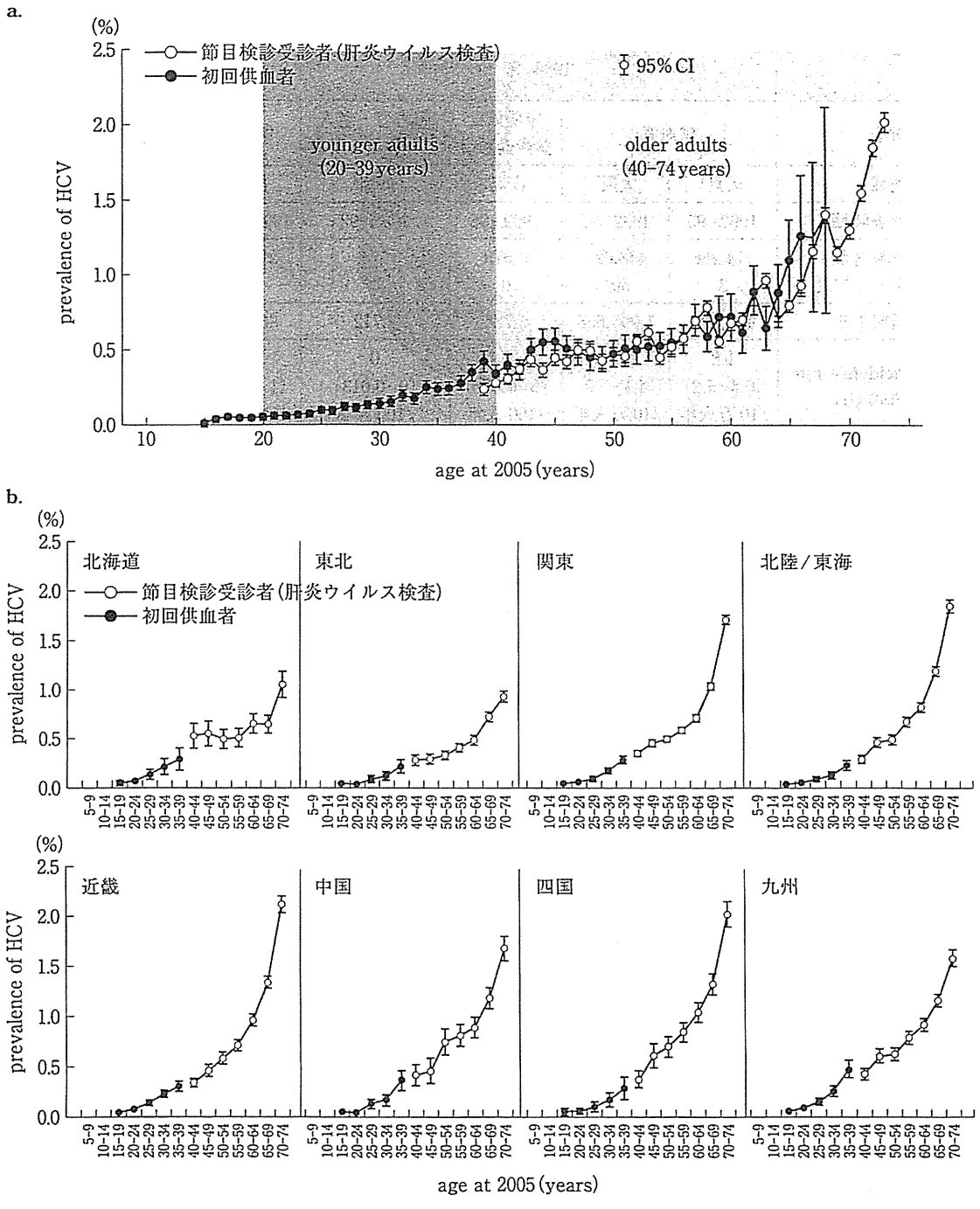

図2–a 初回供血者集団と節目検診受診者集団からみたHCVキャリア率

–b 8地域別・5歳刻みの年齢階級別にみたHCVキャリア率

層については節目検診受診者のHCVキャリア率を用いるのが妥当と考えられる(図2–a).

年齢とHCVキャリア率の関係について全国を8地域に分割して図2–bに示す. HCVキャリア率の高低差は認められるものの, いずれの地域も高年齢集団ではHCVキャリア率が高い値を示し若年齢層では低い値を示す傾向であることがわかる.

表1 各種集団におけるHCV感染の新規発生率

	1988-97				1994-2004	
対象	供血者		定期健康診断受診者	障害者・老人福祉施設入所者	供血者	血液透析患者
地域	広島[a]	大阪[b]	広島[a]	静岡[a]	広島[c]	広島[d]
調査時期	1992-95	1992-97	1992-95	1988-92	1994-2004	1999-2003
対象者数	114,266	448,020	3,079	678	218,953	2,114
キャリア化	3	59*	0	0	16	16
観察人年	168,726	1,095,668	5,786	2,712	861,884	4,893
incidence rate (95%CI)	1.8 (0.4-5.2) /10万人年	5.4* (4.1-7.0) /10万人年	0 (0-0.006) /100人年	0 (0-0.013) /100人年	1.9 (1.1-3.0) /10万人年	3.3 (1.7-4.7) /1000人年

*抗体陽性

[a]J Epidemiol 6: 198-203, 1996. [b]J Epidemiol 8: 292-296, 1998. [c]Intervirology 51: 33-41, 2008. [d]J Med Virol 76: 498-502, 2005.

3. 我が国におけるHCVキャリアの推計数

40歳を境にした前述2つの大規模集団におけるHCVキャリア率をもとに，国勢調査人口(2005年)を用いて8地域別HCVキャリア数の推計を試みた．

その結果，2000年以後の資料をもとにした'感染を知らないまま潜在している'HCVキャリアの推計数は，全国で807,903人(95％信頼区間：68.0-97.4万人，2005年時点)となった[11]．2000年以前(1996-2000年)の初回供血者集団の資料を用いた推計値[12]'15-69歳の年齢集団で88.5万人'と比べ，'感染を知らないまま潜在している'HCVキャリア数は減少していることが明らかとなった．その理由の一つとして，1990年代後半から，行政・医師会などによる啓発活動の普及や感染事例の報道などにより急速にHCV感染の知識が浸透したこと，そのため様々な検査の機会(診療，手術時における肝炎ウイルス検査，各地域における肝炎ウイルス検査など)が増え，結果的に'感染を知らないまま社会に潜在する'HCVキャリアが減少したと考えられる．

なお，これまでの血清疫学的調査より，我が国での水平感染および母子感染によるHCVキャリアの新たな発生は，特別な場合を除きほとんど認められないこと(表1)から，HCVキャリア数は更に減少していくことが推察される．しかし，血液透析患者集団など感染のリスクが一般集団の数倍～10^2倍高いと考えられる場合には定期的な感染動向調査および感染予防対策は引き続き十分に行っていく必要があることは言うまでもない．

4. HCVキャリアの自然病態

疫学的な視点から，疾患の自然経過と予後を明らかにすることは有用である．経過が急激で致命的であるのか，あるいは経過が緩やかで生存期間が長いものであるのかを知ることは，予防対策や治療介入を行った際の効果判定に役立つからである．

図3-aに，広島県赤十字血液センターにおいて，献血時の検査を契機に偶然に発見されたHCVキャリア1,019例(平均年齢45.3歳)が医療機関へ受診した時点の肝病態の内訳を示す．後方視的追跡調査で得られた集計結果[13]である．病院初診時に，'異常を認められない'と診断された人は46.2％(471例)にすぎず，自覚症状がないまま肝病態が慢性肝炎以降に進展していた例は，肝がん症例も含み半数を超えており(53.7％)，特に男性に多いことが明らかとなっ