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# The Influence of Alcoholic Liver Disease on Serum PIVKA-II Levels in Patients without Hepatocellular Carcinoma

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**Background/Aims:** Prothrombin induced by vitamin K deficiency or antagonist II (PIVKA-II) is a widely used diagnostic marker for hepatocellular carcinoma (HCC). We evaluated the correlation between alcoholic liver disease (ALD) and serum PIVKA-II levels in chronic liver disease (CLD) patients. **Methods:** We retrospectively reviewed the medical records of 2,528 CLD patients without HCC. Among these patients, 76 exhibited serum high PIVKA-II levels of >125 mAU/mL (group 1). We categorized 76 control patients matched by age, sex, and the presence of liver cirrhosis from the remaining patients who were negative for serum PIVKA-II (group 2). **Results:** Group 1 revealed increased antibiotic usage (23.7% vs 2.6%, p<0.001) and incidence of ALD (60.5% vs 14.5%, p<0.001) as well as elevated aspartate aminotransferase (52.5 IU/L vs 30.5 IU/L, p=0.025) and γ glutamyl transpeptidase (67.5 IU/L vs 36.5 IU/L, p=0.005) levels compared with group 2. Further, group 1 was significantly associated with a worse Child-Pugh class than group 2. In the multivariate analysis, ALD (odds ratio [OR], 7.151; p<0.001) and antibiotic usage (OR, 5.846; p<0.001) were significantly associated with positive PIVKA-II levels. **Conclusions:** Our study suggests that ALD and antibiotics usage may be confounding factors when interpreting high serum PIVKA-II levels in patients without HCC. Therefore, serum PIVKA-II levels in patients with ALD or in patients administered antibiotics should be interpreted with caution. **(Gut Liver, 2015;9:224-230)**

**Key Words:** Prothrombin induced by vitamin K deficiency or antagonist II; Liver diseases, alcoholic; Hepatocellular carcinoma

## INTRODUCTION

Serum concentrations of prothrombin induced by vitamin K absence or antagonist II (PIVKA-II), also known as des-γ-carboxyprothrombin, are often elevated in patients with hepatocellular carcinoma (HCC). PIVKA-II is widely used as a valuable biomarker for the diagnosis of HCC, showing high sensitivity and specificity especially when used in combination with α-fetoprotein (AFP).[1] PIVKA-II has also been reported as a prognostic indicator for HCC patients and has been approved as an effective tumor marker for HCC in Korea, Japan, and Indonesia.[2]

PIVKA-II was first described in 1968 by Niléhn and Ganrot[3] as an abnormal prothrombin found in the plasma of patients treated with a vitamin K antagonist.[3] Prothrombin is primarily synthesized in the liver similarly to other vitamin K-dependent zymogens and has 10 γ-carboxylated glutamic acid (Gla) residues in its N-terminal domain.[2] PIVKA-II exists in various forms with varying numbers of Gla residues.[4] Under abnormal conditions like vitamin K deficiency, PIVKA-II has fewer than 10 Gla residues in the Gla domain due to insufficient reactions.[5]

In 1984, a report showed that PIVKA-II levels in the serum of HCC patients were elevated as assessed by a competitive radioimmunoassay and that the levels did not return to baseline by treatment with vitamin K.[6] Although serum PIVKA-II has been shown to have a diagnostic accuracy of 59% to 84% in differentiating between HCC and liver cirrhosis (LC) patients using cutoff values of 40, 60, or 100 mAU/mL,[7-14] no universal consensus for cutoff values currently exists. However, 125 mAU/mL has been suggested as an optimal cutoff value of PIVKA-II to distinguish between HCC in chronic liver disease (CLD) patients with or without LC.[15]

Although PIVKA-II is a sensitive and specific marker for the

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detection of HCC, 3% to 5% of patients with LC show elevated PIVKA-II levels even without HCC.[16] Several confounding factors such as vitamin K deficiency,[5] administration of warfarin,[17] primary gastric adenocarcinoma,[18,19] graft rejection after liver transplantation,[20] acute hepatic failure,[21] malnutrition, use of antibiotics that alter gut flora, underlying renal failure,[22] coexisting inflammatory bowel disease,[23] and alcoholic liver disease (ALD)[16,24] have been reported to increase the level of serum PIVKA-II in patients without HCC.

Only three studies have reported that serum PIVKA-II levels in patients with ALD are higher than that in patients with viral hepatitis-related CLD.[16,24,25] However, the influence of ALD on CLD patients without HCC who have serum PIVKA-II levels higher than the cutoff value for the diagnosis of HCC has not been evaluated. Therefore, we conducted a retrospective case-control study to evaluate the influence of ALD on high serum PIVKA-II levels in CLD patients without HCC.

## MATERIALS AND METHODS

### 1. Patients

We conducted a retrospective case-control study by reviewing the medical records of 3,858 CLD patients whose serum levels of PIVKA-II were recorded at the Korea University Guro Hospital between January 2005 and March 2012. We excluded 1,330 patients who were diagnosed with concurrent HCC, who were being treated with vitamin K or a vitamin K antagonist. The peak level of serum PIVKA-II was selected in patient with multiple measured PIVKA-II levels and all demographic, laboratory, and clinical data were used at that time.

Although it have been reported that alcohol could increase PIVKA-II level,[16,24,25] there was no proposed cutoff level to identify ALD. Therefore, we tried to find the optimal cutoff to discriminate ALD from other CLD in our 2,528 patients using receiver-operating characteristics (ROC) curve analysis. However, area under ROC (AUROC) was 0.554, the sensitivity and specificity by estimated best cutoff level (53 mAU/mL) was 34.2% and 83.1%. Because AUROC was insignificant level like coin toss, we analyzed PIVKA-II levels according to presence or not of ALD. There was significant difference of median PIVKA-II level between ALD and non-ALD (511 mAU/mL vs 95 mAU/mL, Mann-Whitney U test, $p<0.001$). Because the median PIVKA-II level of whole patients was 93.5 mAU/mL and the cutoff value of 125 mAU/mL has been reported to be optimal for distinguishing between HCC in patients with CLD with or without LC,[15] we decided to set the positive serum PIVKA-II levels as more than 125 mAU/mL. Among the 2,528 patients, 76 patients who were positive for serum PIVKA-II levels were categorized into group 1. We then assigned 76 control patients matched by age, sex, and presence of LC from the remaining 2,452 subjects who had negative serum PIVKA-II levels into group 2. The summarized flow of enrolled patients in this study was presented in Fig. 1.

We defined ALD patients as those who had clinical, laboratory (such as liver function tests abnormalities) or imaging evidence of fatty liver, hepatitis, or LC with a history of heavy alcohol consumption (more than 70 g/day ethanol for men and more than 30 g/day for women for more than 10 years). Other chronic viral hepatitis-related liver disease was defined as those positive hepatitis B surface antigen or antihepatitis C virus antibody for more than 6 months.

### 2. Measurement of serum PIVKA-II levels

Until September 2011, patient serum PIVKA-II concentration was manually measured using the Haicatch® PIVKA-II enzyme-linked immunosorbent assay kits (Sanko Junyaku Co., Ltd., To-

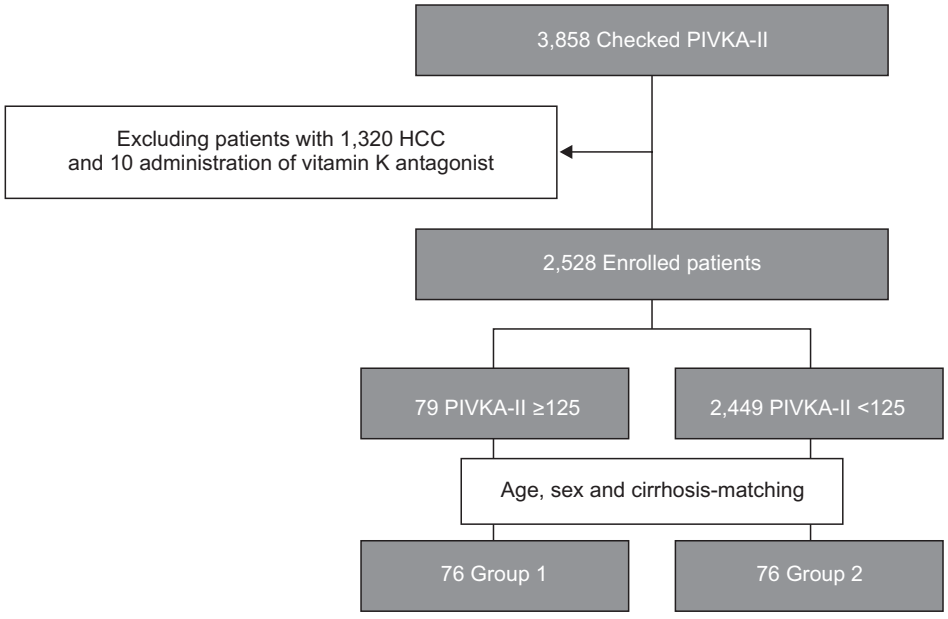


**Fig. 1.** Flow of patients through the study.
PIVKA-II, prothrombin induced by vitamin K absence or antagonist II; HCC, hepatocellular carcinoma.

kyo, Japan) according to the manufacturer's instructions. After September 2011, the Lumipulse® PIVKA-II EISAI (Fujirebio Inc., Tokyo, Japan) was used, which is a fully automated chemiluminescent enzyme immunoassay system.

### 3. Statistical analysis

Categorical and continuous variables were analyzed by the chi-square test and Student t-test, respectively. Linear by linear association was used in the score test for trend. To identify the independent factors associated with elevated serum PIVKA-II levels, univariate and multivariate analysis were performed by logistic regression. Variables reaching statistical significance ($p<0.05$) in univariate analyses were entered into multivariate analyses. A two-tailed p-value of less than 0.05 was defined as statistically significant. All analyses were performed using the SPSS version 18.0 (SPSS Inc., Chicago, IL, USA).

## RESULTS

### 1. Comparison of baseline characteristics

The baseline characteristics of patents in groups 1 and 2 are summarized in Table 1. Men accounted for 76.3% of all patients, and the median age of all patients was 55 years. The number of patients with LC was 52 (68.4%) in each group. The median serum PIVKA-II level significantly differed between groups, with values of 248.5 mAU/mL (range, 125 to 2,000 mAU/mL) in group 1 and 38.0 mAU/mL (range, 10 to 89 mAU/mL) in group 2 ($p<0.001$). However, the median serum level of AFP was not different between the two groups. Group 1 patients had a higher incidence of antibiotic usage (23.7% vs 2.6%,

**Table 1.** Comparison of Baseline Characteristics

| Characteristic | Group 1 (n=76) | Group 2 (n=76) | p-value |
|---|---|---|---|
| Male sex | 58 (76.3) | 58 (76.3) | 1.000 |
| Age, yr | 55 (27–75) | 55 (27–75) | 0.967 |
| Cirrhosis | 52 (68.4) | 52 (68.4) | 1.000 |
| History of heavy alcohol consumption | 49 (64.5) | 21 (27.6) | <0.001 |
| Alcoholic liver disease | 46 (64.5) | 11 (14.5) | <0.001 |
| Viral hepatitis related liver disease | 39 (51.3) | 65 (85.5) | <0.001 |
| Gastric cancer | 0 | 0 | |
| Chronic kidney disease | 6 (7.9) | 2 (2.6) | 0.276* |
| Antibiotics | 18 (23.7) | 2 (2.6) | <0.001 |
| Laboratory parameter | | | |
| PIVKA-II, mAU/mL | 248.50 (125–2,000) | 38.00 (10–89) | <0.001 |
| AFP, ng/mL | 3.45 (0.7–591.0) | 2.8 (0.5–85.2) | 0.214 |
| Platelet, $\times 10^9$/L | 135 (25–276) | 140 (12–318) | 0.148 |
| Prothrombin time, INR | 1.295 (0.91–7.74) | 1.080 (0.79–1.78) | 0.003 |
| Total bilirubin, mg/dL | 1.75 (0.56–33.42) | 1.01 (0.23–4.52) | 0.002 |
| Albumin, g/dL | 3.4 (2.2–4.6) | 4.2 (2.6–4.7) | <0.001 |
| AST, IU/L | 52.5 (19–656) | 30.5 (13–346) | 0.023 |
| ALT, IU/L | 32.5 (6–1,068) | 25 (8–753) | 0.456 |
| GGT, IU/L | 67.5 (10–1,550) | 36.5 (7–387) | 0.005 |
| BUN, mg/dL | 14.5 (4.0–83.0) | 14.95 (6.70–5.0) | 0.263 |
| Creatinine, mg/dL | 0.74 (0.29–10.29) | 0.78 (0.38–10.70) | 0.653 |
| Liver function parameter | | | |
| Ascites, yes | 32 (42.1) | 9 (11.8) | <0.001 |
| Hepatic encephalopathy, yes | 13 (17.1) | 4 (5.3) | 0.021 |
| Child-Pugh class | | | <0.001 |
| A | 36 (47.4) | 66 (86.8) | |
| B | 25 (32.9) | 7 (9.2) | |
| C | 15 (19.7) | 3 (3.9) | |

Data are presented as number (%) or median (min–max).
PIVKA-II, prothrombin induced by vitamin K absence or antagonist II; AFP, α-fetoprotein; INR, international normalized ratio; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ glutamyl transpeptidase; BUN, blood urea nitrogen.
*Fisher exact test.

**Table 2.** The Relationship between Serum PIVKA-II Levels and the Severity of Liver Dysfunction

| Variable | Total (n=152) | Group 1 (n=76) | Group 2 (n=76) | p-value* |
|---|---|---|---|---|
| Ascites | | | | <0.001 |
| None | 112 | 44 (39.3) | 68 (60.7) | |
| Easily controlled | 32 | 27 (84.4) | 5 (15.6) | |
| Poorly controlled | 8 | 5 (62.5) | 3 (37.5) | |
| Encephalopathy | | | | 0.005 |
| None | 136 | 63 (46.3) | 73 (53.7) | |
| Easily controlled | 11 | 8 (72.7) | 3 (27.3) | |
| Poorly controlled | 5 | 5 (100.0) | 0 | |
| Total bilirubin, mg/dL | | | | <0.001 |
| <2 | 113 | 47 (41.6) | 66 (58.4) | |
| 2–3 | 22 | 15 (68.2) | 7 (31.8) | |
| >3 | 17 | 14 (82.4) | 3 (17.6) | |
| Albumin, g/dL | | | | <0.001 |
| >3.5 | 105 | 39 (37.1) | 66 (62.9) | |
| 2.8–3.5 | 23 | 16 (69.6) | 7 (30.4) | |
| <2.8 | 24 | 21 (87.5) | 3 (12.5) | |
| Prothrombin time, INR | | | | 0.003 |
| <1.7 | 140 | 65 (46.4) | 75 (53.6) | |
| 1.7–2.3 | 7 | 6 (85.7) | 1 (14.3) | |
| >2.3 | 5 | 5 (100.0) | 0 | |
| Child-Pugh class | | | | <0.001 |
| A (5–6) | 102 | 36 (35.3) | 66 (64.7) | |
| B (7–9) | 32 | 25 (78.1) | 7 (21.9) | |
| C (10–15) | 18 | 15 (83.3) | 3 (16.7) | |

Data are presented as number (%).
PIVKA-II, prothrombin induced by vitamin K absence or antagonist II; INR, international normalized ratio.
*Linear by linear association.

**Table 3.** The Characteristics of Antibiotics Usage among Patients

| Antibiotics | No. |
|---|---|
| Cephalosporin | 9 |
| Cefotaxime | 7 |
| Ceftriaxone | 1 |
| Cefditoren | 1 |
| Quinolone | 8 |
| Ciprofloxacin | 7 |
| Norfloxacin | 1 |
| Antituberculosis drug | 3 |
| Duration of administration, day | |
| 1–3 | 12 |
| 4–7 | 4 |
| 7–30 | 1 |
| >31 | 3 |
| Cause of administration | |
| Variceal bleeding | 10 |
| SBP treatment or prophylaxis | 3 |
| Tuberculosis | 3 |
| Etc. (HEP, jaundice, acute bronchitis, skin biopsy) | 4 |

SBP, spontaneous bacterial peritonitis; HEP, hepatic encephalopathy.

p<0.001), ALD (60.5% vs 14.5%, p<0.001), and higher aspartate aminotransferase (AST) (52.5 IU/L vs 30.5 IU/L, p=0.023) and γ glutamyl transpeptidase (GGT) (67.5 IU/L vs 36.5 IU/L, p=0.005) levels than group 2 patients. Group 1 patients had a significantly higher incidence of ascites, hepatic encephalopathy, lower incidence of viral hepatitis related liver disease, higher level of total bilirubin and prothrombin time (international normalized ratio [INR]), lower level of serum albumin, and worse Child-Pugh class than group 2 patients (all p<0.005). Detailed descriptions of baseline characteristics of liver function parameters and antibiotics usage are summarized in Tables 2 and 3.

### 2. The effects of alcohol consumption

The proportion of patients with a previous history of heavy alcohol intake was significantly higher in group 1 than in group 2 (49/76 [64.5%] vs 21/76 [27.6%], p<0.001). In addition, the proportions of patients who heavily consumed alcohol consistently before PIVKA-II measurement were significantly different between the two groups (34/76 [44.7%] in group 1 vs 11/76 [14.5%] in group 2, p<0.001). Further, there were more number of patients with ALD in group 1 than in group 2 (46/76 [60.5%] vs 11/76 [14.5%], p<0.001).

### 3. Factors influencing a positive serum PIVKA-II level

Univariate analysis demonstrated that the presence of ALD (odds ratio [OR], 9.061; 95% confidence interval [CI], 4.123 to 19.911; p<0.001) has a significant positive correlation with a positive serum PIVKA-II level. In addition, the Child-Pugh score (OR, 1.617; 95% CI, 1.305 to 2.002; p<0.001) and its components such as prothrombin time (PT) (INR) (OR, 23.316; 95% CI, 4.520 to 120.261; p<0.001), serum albumin (OR, 0.263; 95% CI, 0.146 to 0.473; p<0.001), total bilirubin (OR, 1.896; 95% CI, 1.263 to 2.846; p=0.002), and the presence of hepatic encephalopathy (OR, 3.714; 95% CI, 1.152 to 11.974; p=0.028) or ascites (OR, 5.414; 95% CI, 2.357 to 12.436; p<0.001) all significantly affected the serum PIVKA-II level (Table 4). However, multivariate analysis showed that only the presence of ALD (OR, 7.151; 95% CI, 3.182 to 16.072; p<0.001) and the use of antibiotics (OR, 5.846; 95% CI, 1.189 to 28.741; p<0.001) were independently correlated with a positive serum PIVKA-II level. In the subgroup analysis in patients without LC, only a previous history of heavy alcohol consumption (OR, 6.600; 95% CI, 1.246 to 34.949; p=0.026) was significantly correlated according to the univariate and multivariate analyses (Table 5).

**Table 4.** Logistic Regression Analysis of Factors Influencing Serum PIVKA-II Levels

| Factor | Univariate | | Multivariate | |
|---|---|---|---|---|
| | OR (95% CI) | p-value | OR (95% CI) | p-value |
| Prothrombin time, INR | 23.316 (4.520–120.261) | <0.001 | 2.474 (0.347–17.636) | 0.366 |
| Albumin | 0.263 (0.146–0.473) | <0.001 | 0.92 (0.337–2.516) | 0.872 |
| Total bilirubin | 1.896 (1.263–2.846) | 0.002 | 1.249 (0.939–1.660) | 0.126 |
| Ascites | 5.414 (2.357–12.436) | <0.001 | 0.422 (0.101–1.766) | 0.237 |
| Encephalopathy | 3.714 (1.152–11.974) | 0.028 | 1.320 (0.279–6.248) | 0.726 |
| Viral hepatitis related liver disease | 0.178 (0.082–0.390) | <0.001 | 0.494 (0.186–1.311) | 0.157 |
| Antibiotics use | 11.483 (2.560–51.501) | 0.001 | 5.84 (1.189–28.741) | 0.030 |
| Alcoholic liver disease | 9.061 (4.123–19.911) | <0.001 | 7.151 (3.182–16.072) | <0.001 |
| AFP | 1.005 (0.995–1.016) | 0.335 | - | - |
| AST | 1.009 (1.000–1.017) | 0.041 | 0.997 (0.988–1.006) | 0.502 |
| ALT | 1.001 (0.998–1.004) | 0.470 | - | - |
| GGT | 1.004 (1.001–1.008) | 0.010 | 1.002 (0.998–1.005) | 0.323 |
| Creatinine | 1.062 (0.817–1.380) | 0.654 | - | - |
| CKD (≥grade 3) | 3.171 (0.619–16.241) | 0.166 | - | - |

PIVKA-II, prothrombin induced by vitamin K absence or antagonist II; OR, odds ratio; CI, confidence interval; INR, international normalized ratio; AFP, α-fetoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ glutamyl transpeptidase; CKD, chronic kidney disease.

**Table 5.** Subgroup Analysis of the Factors Influencing the Serum PIVKA-II Levels of Patients without Cirrhosis (N=48)

| Factor | Univariate | | Multivariate | |
|---|---|---|---|---|
| | OR (95% CI) | p-value | OR (95% CI) | p-value |
| Prothrombin time, INR | 18.570 (0.066–5,262.793) | 0.311 | - | - |
| Albumin | 0.764 (0.119–4.908) | 0.777 | - | - |
| Total bilirubin | 1.494 (0.571–3.911) | 0.414 | - | - |
| History of heavy alcoholic intake | 6.600 (1.246–34.949) | 0.026 | 6.600 (1.246–34.949) | 0.026 |
| Viral hepatitis related liver disease | 0.714 (0.142–3.600) | 0.683 | - | - |
| AFP | 1.042 (0.858–1.265) | 0.678 | - | - |
| AST | 1.005 (0.997–1.013) | 0.215 | - | - |
| ALT | 1.001 (0.998–1.005) | 0.486 | - | - |
| GGT | 1.005 (0.998–1.011) | 0.148 | - | - |
| Creatinine | 0.793 (0.413–1.523) | 0.486 | - | - |

PIVKA-II, prothrombin induced by vitamin K absence or antagonist II; OR, odds ratio; CI, confidence interval; INR, international normalized ratio; AFP, α-fetoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ glutamyl transpeptidase.

## DISCUSSION

In this study, we showed that ALD was independently associated with a high level of serum PIVKA-II. This is consistent with the results of previous studies.[16,25] In 1999, Ohhira *et al*.[16] first reported that patients with ALD had higher serum PIVKA-II levels than those with viral hepatitis-related liver disease.

Although several studies have been performed to determine the optimal cutoff level of PIVKA-II for differentiating patients with HCC from those with nonmalignant CLD, the optimal cutoff, which has been reported to range from 40 to 250 mAU/mL, remains unclear.[13,26,27] Similarly, even though it have been reported that ALD could increase PIVKA-II level,[16,24,25] there was no proposed cutoff level to identify ALD. We tried to find the optimal cutoff to discriminate ALD from other CLD using ROC curve analysis in 2,528 patients, but it was revealed that the value of AUROC was not useful to discriminate ALD. In the absence of any definite cutoff level of PIVKA-II to discriminate ALD, because a PIVKA-II value of 125 mAU/mL has high sensitivity and specificity in correctly distinguishing HCC from underlying CLD with or without LC[13] and the median PIVKA-II level of 2,528 patients was 93.5 mAU/mL, we arbitrary set

the positive PIVKA-II level as 125 mAU/mL. However, because PIVKA-II level of 125 mAU/mL have a useful meaning to detect HCC, if there was significant difference of ALD between patients with higher and lower than this level, which could give an indirect evidence that more than 125 mAU/mL of PIVKA-II level should be interpreted with caution in diagnosing HCC.

In this study, we extensively reviewed the medical records of 2,528 CLD patients without HCC. Of these, the incidence of positive PIVKA-II levels (>125 mAU/mL) in CLD patients without HCC was rare at 3% (76/2,528), and these patients were selected to make up the positive PIVKA-II patient group (group 1). We set the control group (group 2) by matching patients on age, sex, and the presence of LC to those of group 1. In multivariate comparison, we found ALD and antibiotics usage independently associated with a positive PIVKA-II level.

It is well known that progressed liver disease or administration of antibiotics like β-lactams can lead to elevated serum PIVKA-II levels.[15] Previous data suggests that the effect of antibiotics is mainly due to changes in microsomal γ-carboxylation activity or endogenous vitamin K levels predisposing patients to hypoprothrombinemia.[28,29] In this study, liver dysfunction-associated parameters were clearly associated to group 1 in the univariate analysis, but these associations disappeared in the multivariate analysis. These findings suggest that the effect of liver dysfunction on the PIVKA-II level is not significant compared to ALD.

The underlying mechanism behind the effect of alcohol on serum PIVKA-II levels remains unclear. It has been suggested that vitamin K deficiency may occur in chronic alcohol abusers.[30] However, no relationship between serum vitamin K concentration and serum PIVKA-II levels was shown in previous studies.[16,25]

In a previous report, Ohhira *et al*.[24] measured serum PIVKA-II levels using two different monoclonal antibodies, 19B7 and MU-3, meaning that two serum PIVKA-II variants could be checked by different immunoassay systems. The authors of that study reported that the ratio of 19B7 to MU-3 was significantly higher in ALD patients than in HCC patients. Therefore, a different variant of PIVKA-II in HCC patients may play a role in ALD. Due to the retrospective nature of the present study, we could not measure serum concentrations of vitamin K or variants of PIVKA-II. Further research is required to obtain information on PIVKA-II production during the process of alcohol metabolism.

Differences in laboratory findings between groups are shown in Table 1. Group 1 had prolonged PT (INR), a higher level of serum total bilirubin, and a lower level of serum albumin than group 2. Thus, there may be more severe liver dysfunction in group 1 patients than in group 2 patients. In addition, group 1 included more number of patients with a history of current heavy alcohol consumption, which may have negatively influenced PT (INR) and the levels of total bilirubin and albumin. Table 1 shows higher serum AST and GGT levels in group 1 than in group 2, while the serum alanine aminotransferase level was not significantly different between the two groups. As serum levels of AST and GGT are predominantly elevated in patients with ALD, we can interpret our findings as a hepatic enzyme pattern related to the consumption of alcohol in these patients.

There are several limitations in our study. Firstly, it was retrospective, case-control study. Secondly, there was a wide distribution of Child-Pugh class in the cirrhotic patients, which could affect the serum PIVKA-II level. However, the relationship between each parameters of Child-Pugh class and serum PIVKA-II levels did not show significance in the multivariate analysis.

In conclusion, ALD was significantly associated with the high serum PIVKA-II levels in patients without HCC. Our study suggests that ALD and antibiotics usage may be a confounding factor while interpreting high serum PIVKA-II levels. Therefore, serum PIVKA-II levels in patients with ALD or who treated with antibiotics should be interpreted with caution. This study advocated the need of further study to find the optimal cutoff level of PIVKA-II in diagnosing HCC who have ALD which is one of main cause of HCC, because ALD could influence PIVKA-II level.

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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