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Studies on Factors Affecting Plasma PIVKA-II Levels in Hepatocellular Carcinoma

Kenji SOGA, Futoshi ARAI, Takashi TSURUYA, Keiko AIKAWA, Muneatsu TOSHIMA, Koichi SHIBASAKI and **Yutaka AOYAGI***

Department of Internal Medicine, School of Dentistry at Niigata, The Nippon Dental University, Hamauracho 1-8, Niigata 951, Japan, *The Third Division, Department of Internal Medicine, Niigata University School of Medicine, Niigata 951, Japan

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Summary. **In order to diagnose hepatocellular carcinoma (HCC), plasma levels of protein induced by vitamin K absence or antagonist-II (PIVKA-II) were determined by an enzyme-linked immunosorbent assay (ELISA). Positive reaction was noted in 34 (68%) of 50 patients with HCC, with PIVKA-II serving as an useful tumor marker in the estimation of HCC staging. On the other hand, the administration of vitamin K and the presence of jaundice were found to be important factors that affect plasma PIVKA-II levels. The administration of antibiotics with N-methylthiotetrazole at a dosage of 2 g/day for 3 days (total 6 g) did not seem to increase plasma concentrations of PIVKA-II levels. Thus, the measurement of the plasma PIVKA-II levels was useful for the diagnosis of HCC except for the disturbance of vitamin K utilization.**

INTRODUCTION

The determination of the serum level of α-fetoprotein (AFP) is widely employed as a serological approach for the diagnosis of hepatocellular carcinoma (HCC). However, AFP alone is not always a reliable marker for HCC, because serum levels of AFP are often negative in patients with HCC, and AFP can often turn positive with regeneration of hepatic cells: for example, in patients with chronic hepatitis or liver cirrhosis.[1,2] To ensure a more reliable diagnosis of HCC, therefore, a more specific marker other than AFP was designed. In recent years, the protein induced by vitamin K absence or antagonist-II (PIVKA-II) that is produced under conditions of vitamin K deficiency has attracted attention as a new marker of HCC.[3,4] In this study, we established plasma PIVKA-II levels as a marker of HCC by an enzyme-linked immunosorbent assay (ELISA) and investigated the factors that affect plasma PIVKA-II levels.

MATERIALS AND METHODS

This study was carried out on 175 patients of whom 50 (38 men and 12 women) had HCC; 42, liver cirrhosis; 25, chronic hepatitis; 23, a malignant tumor with liver metastasis; and 35, a malignant tumor without liver metastasis. Diagnosis of the patients was determined histologically by liver biopsy or the clinical use of ultrasonography, scintigraphy, computed tomography, and selective celiac angiography. Plasma PIVKA-II levels were determined with ELISA kits (EI test mono-P-kit, Eizai Lab., Tokyo, Japan) using anti-PIVKA-II monoclonal antibody, and the test was considered positive when 0.1 arbitrary unit/ml (AU/ml) or more of PIVKA-II was detected.[5,6] The size of HCC was classified according to diagnostic criteria for primary hepatic carcinomas as set by the Japan Liver Cancer Study Group.[7] Briefly, tumors occupying less than 20% of the whole liver, as determined by imaging diagnosis, were classified as E1, 20% to 39% as E2, 40% to 59% as E3, and more than 60% as E4.

RESULTS

1. Plasma levels of PIVKA-II in patients with liver diseases and malignant tumors

The PIVKA-II test was positive in 34 (68%) of 50 patients with HCC, 2 (5%) of 42 patients with liver cirrhosis, none of 25 patients with chronic hepatitis, 3 (13%) of 23 patients having a malignant tumor with liver metastasis, and 2 (6%) of 35 patients having a malignant tumor without liver metastasis. The plasma PIVKA-II levels in the 2 patients with liver cirrhosis were positive at 0.19 and 0.11 AU/ml, respectively, and 2 of 3 positive patients having a

malignant tumor with liver metastasis had severe jaundice (Fig. 1). The sensitivity, specificity, and accuracy of the PIVKA-II test in diagnosis of HCC were 68%, 93%, and 87%, respectively.

2. Relationship between plasma PIVKA-II levels and tumor size

The size of HCC was classified according to diagnostic criteria established by the Japan Liver Cancer Study Group for primary hepatic carcinomas, in order to determine the relationship between plasma PIVKA-II level and tumor size. Five of 14 patients with an E1 tumor and 11 of 14 patients with an E4 tumor were positive for PIVKA-II. However, there was no appreciable correlation between the progression of HCC and plasma PIVKA-II levels (Fig. 2).

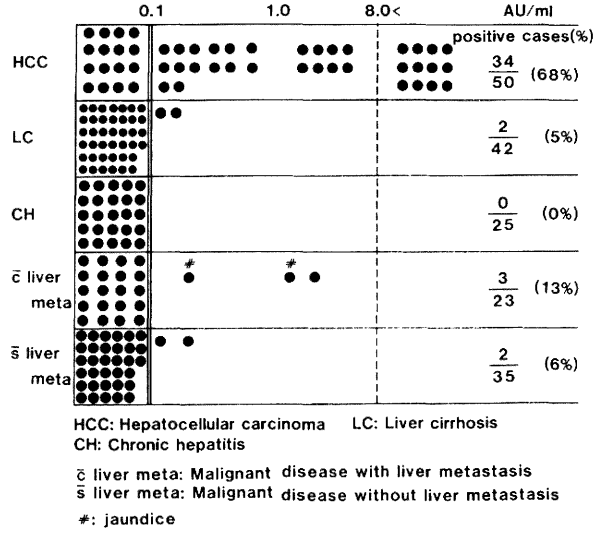

Fig. 1. Plasma levels of PIVKA-II in patients with liver diseases and malignant tumors.

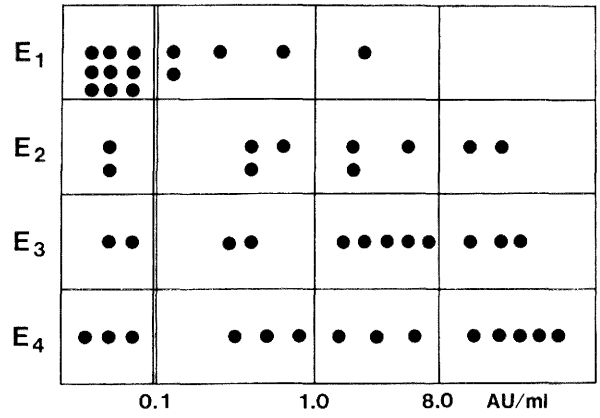

Fig. 2. Relationship between plasma level of PIVKA-II and tumor size. Tumors occupying less than 20% of the whole liver, as determined by imaging diagnosis, are classified as E1, 20% to 39% as E2, 40% to 59% as E3, and more than 60% as E4.

3. Factors affecting plasma PIVKA-II levels

(1) *Changes in plasma PIVKA-II levels after administration of vitamin K*

After three days of the administration of vitamin K2 at a dosage of 30 mg/day by intravenous drip infusion plasma PIVKA-II levels were determined in 10 patients with HCC. In all these patients, plasma PIVKA-II levels decreased to 13%-44% of the initial value 24 h after the administration of vitamin K2, and rose thereafter in connection with the cessation of administration (Fig. 3a). After the consecutive administration of vitamin K2 for more than 30 days, on the other hand, those patients with HCC showed a decrease in plasma PIVKA-II levels or turned negative for PIVKA-II, despite an increase in serum AFP levels and tumor growth (Fig. 3b).

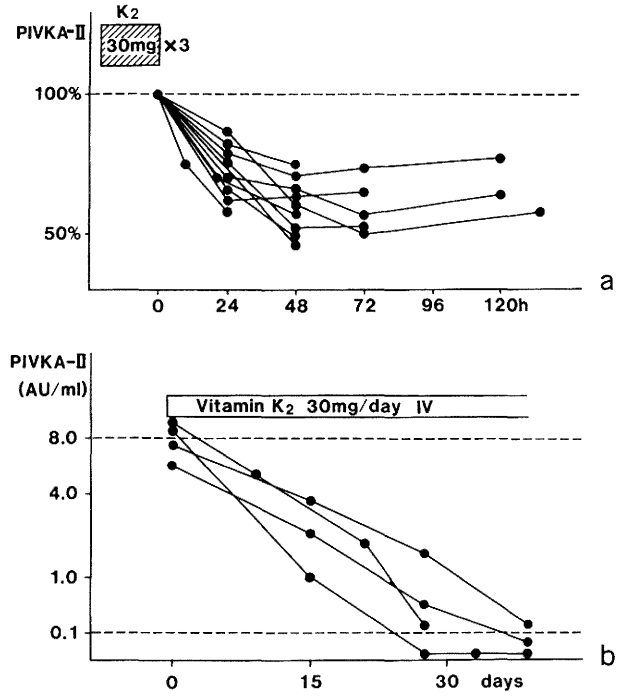

Fig. 3. Changes in plasma PIVKA-II levels after three days of administration by intravenous drip infusion of vitamin K2 at a dosage of 30 mg per day (**a**) and repeated administration of vitamin K2 at a dosage of 30 mg for consecutive days (**b**).

(2) *Changes in plasma PIVKA-II levels after administration of antibiotics of cephems with the N-methylthiotetrazol group*

After three days of administration by intravenous drip infusion of antibiotics of cephems with the N-methylthiotetrazol group (ceftizoxime sodium; CZX and cefoperazone sodium; CPZ) at a dosage of 2g/day, plasma PIVKA-II levels were determined in five patients with HCC. In these patients, plasma PIVKA-II levels rose up from 5% to 15% of the pre-treatment value after 48 h of the administration of CZX and CPZ ($p<0.05$), but tended to return to their initial values after 72 h (Fig. 4).

4. Jaundice and plasma PIVKA-II levels

The relationship between the presence of jaundice and plasma PIVKA-II levels was determined in patients with HCC. Three of 9 patients having a malignant tumor with liver metastasis were positive for PIVKA-II, and 2 of the 3 paitents with positive PIVKA-II were found to have jaundice (Fig. 1). On the other hand, a patient with liver carcinoid who was negative for PIVKA-II on first examination turned positive with the exacerbation of jaundice. Her plasma PIVKA-II level was commensurate with the severity of jaundice (Fig. 5).

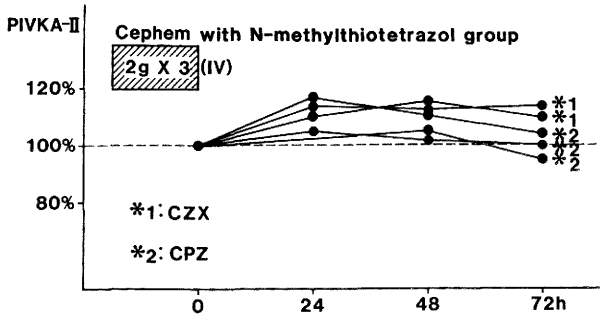

Fig. 4. Changes in plasma PIVKA-II levels after three days of administration by intravenous drip infusion of antibiotics of cephems with the N-methylthiotetrazol group (CZX and CPZ).

DISCUSSION

Liebman et al.[4] reported that PIVKA-II was specifically detected in HCC patients at a high rate. According to them, 69 (91%) of 76 patients with HCC were positive for PIVKA-II when tested by competitive radioimmunoassay. In reports by Fujiyama et al.[3] and Oguro et al.,[8] who used ELISA, plasma levels of PIVKA-II were able to serve as a useful marker for the diagnosis of HCC because the rate of false positivity was extremely low. In the present study, sensitivity, specificity and accuracy were noted to be 68, 93 and 87%, respectively, and the time-course of plasma PIVKA-II levels provided clues not only for diagnosis of HCC but also to determine the progression of HCC. These results additionally supported previous works. However, little is known about the precise mechanism of producing PIVKA-II in HCC. Ono et al.[9] have reported interesting evidence about the regulation mechanism PIVKA-II production. First, in patients with elevated plasma PIVKA-II levels, both immunoreactive prothrombin and PIVKA-II increased significantly in hepatoma tissue as compared with non-cancerous tissue. Second, no significant difference was observed in the endogeneous vitamin K concentrations between

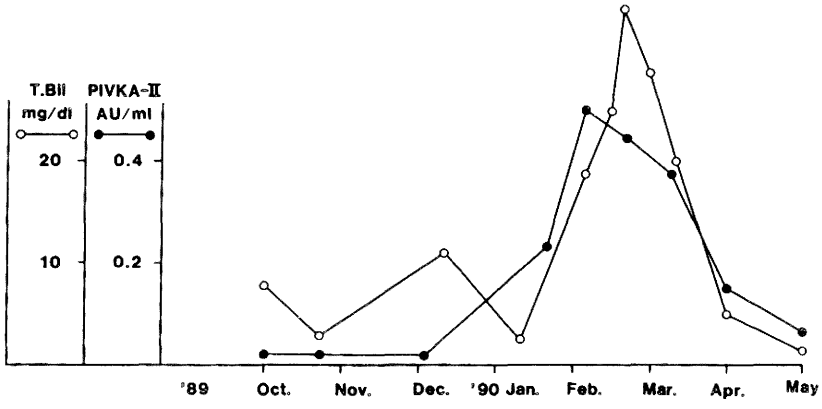

Fig. 5. Clinical course of a patient with liver carcinoid (46-year-old female). PIVKA-II was negative on first examination turned positive with the exacerbation of jaundice.

malignant and benign portions, whether in cases with or without increments of plasma PIVKA-II. They concluded that the overproduction of prothrombin and PIVKA-II in HCC tissue played an important role in the synthesis of PIVKA-II.

In regard to the effect of the administration of vitamin K, PIVKA-II levels decreased all 10 patients with HCC after drip infusion of vitamin K in the present study. As evidenced by Ono et al.[9] vitamin K dificiency was not observed in HCC tissue. However, the administration of vitamin K depressed the PIVKA-II level. The cause of this phenomenon is not clear, and further study is required to solve these problems.

It has been documented that antibiotics with the N-methylthiotetrazol group inhibit vitamin K epoxide reductase in vitamin K cycle.[10,11] In the present study, CPZ and CZX, antibiotics with the N-methylthiotetrazol group, were administered to patients with HCC at dosages of 2 g/day for three days. In these patients, plasma PIVKA-II rose from 5% to 15% of the pre-treatment levels after 48 h ($p<0.05$); however, no significant difference was found between initial plasma PIVKA-II levels and those after 72 h. Thus, the administration of antibiotics with the N-methylthiotetrazol group for 3 days at a dosage of 2 g/day did not seem to increase plasma PIVKA-II levels.

An increase of plasma PIVKA-II levels was observed in 2 out of 3 patients with metastasis to the liver of a malignant tumor and one with liver carcinoid. These patients were found to have jaundice, and there was a correlation between total bilirubin concentrations and PIVKA-II levels. Additionally, PIVKA-II turned negative after the improvement of jaundice. These findings indicated that the mechanism of elevation of PIVKA-II levels in patients with jaundice was considered as a disturbance in the absorption of vitamin K due to the depletion of bile in the intestine.

Accordingly, the measurement of plasma PIVKA-II levels was useful for the diagnosis of HCC, the exception being that the disturbance of vitamin K utilization, such as jaundice or high dosage administration of antibiotics with the N-methylthiotetrazol group, was not observed in patients with HCC.

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