DU-PAN 2 Antigen in Sera of Patients with Liver Diseases

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ABSTRACT. To evaluate the usefulness of serum DU-PAN 2 (an antigen defined by a monoclonal antibody raised against human pancreatic carcinoma cells), serum specimens from 370 cases of hepatobiliary and pancreatic diseases along with 31 normal controls were studied using an enzyme immunoassay. Elevated levels of serum DU-PAN 2 were detected in the serum of 28.3% of the cases with chronic hepatitis (15/53), 36.5% of those with liver cirrhosis (27/74), 48.4% of the hepatocellular carcinoma cases (61/126) and 50% of primary biliary cirrhosis (4/8). Significant differences were noted between patients with chronic inactive hepatitis (17 cases mean 201.4 U/ml) and chronic active hepatitis (36 cases; mean 394.5 U/ml) and, more distinctly, between patients with compensated liver cirrhosis (41 cases; mean 225.1 U/ml) and, those with decompensated liver cirrhosis (33 cases; mean 564.7 U/ml). The highest median levels were seen in patients with primary biliary cirrhosis (922.7 U/ml), and then in those with hepatocellular carcinoma (551.4 U/ml). Using an immunoperoxidase technique on formalin-fixed, deparaffinized liver sections, we showed that DU-PAN 2 reacted with bile-duct epithelium but never stained hepatoma cells. These results suggest that the determination of serum DU-PAN 2 can be useful in evaluating chronic liver diseases.

Key words: DU-PAN 2 — chronic liver disease — hepatocellular carcinoma — immunoperoxidase staining

DU-PAN 2 is an antigen defined by a monoclonal antibody against a human pancreatic adenocarcinoma cell line.[1] This antigen is present on epithelial cells of the normal adult pancreas and gall bladder epithelium. It has also been detected in adenocarcinoma cells of pancreatic and nonpancreatic origin. A recent study has suggested that elevated serum DU-PAN 2 levels can occur in some patients with hepatocellular carcinoma (HCC) and non-malignant hepato-biliary diseases.[2-5] However, only a small number of hepatoblliary disease categories were studied. The present study describes the usefulness of serum DU-PAN 2 in the evaluation of chronic liver diseases. The cellular and tissue distribution of this antigen was also examined using immunoperoxidase tests.

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MATERIALS AND METHODS

Clinical samples

The subjects of this investigation were 370 patients with different hepato-biliary and pancreatic diseases. The sera of 31 healthy individuals who showed no abnormalities in general health screening and liver function tests were used as controls. Sera from patients with acute hepatitis (22), chronic hepatitis (53), liver cirrhosis (74), hepatocellular carcinoma (126), metastatic liver cancer (12) and primary biliary cirrhosis (8), were included. Gallstones (27) and gallbladder and common bile duct cancers (15), chronic pancreatitis (17) and pancreatic cancer (16) were also studied. The diagnosis was made by clinical examinations and laboratory tests in acute hepatitis. Chronic hepatitis was confirmed by standard biochemical and histological criteria and was divided into two group; chronic inactive hepatitis (17) and chronic active hepatitis (36). Liver cirrhosis was diagnosed by clinical, biochemical and laparoscopic examinations and divided into two groups; compensated (41) and decompensated (33) liver cirrhosis. HCC was diagnosed by US-guided tumor biopsy, autopsy, typical angiographic changes and by high serum α-fetoprotein. Primary biliary cirrhosis (PBC) was diagnosed histologically and by positive antimitochondrial antibody. Gallstones were diagnosed from radiological evidence on percutaneous transhepatic cholangiography (PTC), and by computed tomography and angiography. Chronic pancreatitis was diagnosed by elevated serum amylase levels and ERP (endoscopic retrograde pancreatography). The diagnosis of pancreatic cancer was made by CT, angiography, ERP and pathological evidence from surgery or autopsy. Sera were stored at $-40°C$ until assayed.

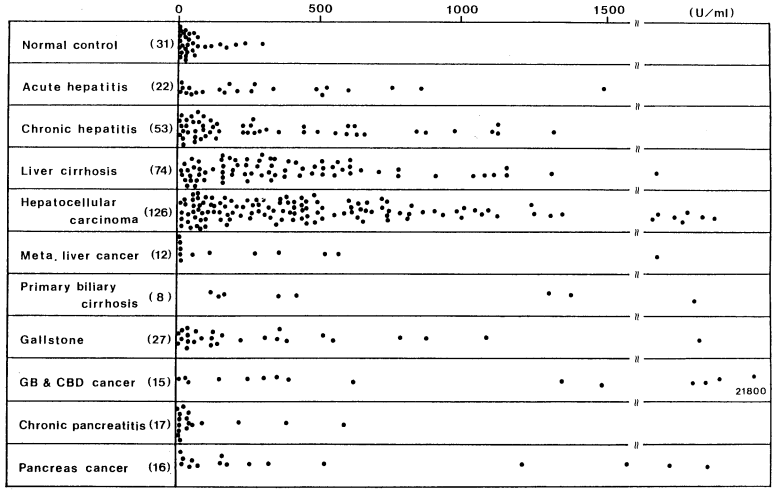

Fig. 1. Distribution of seurm DU-PAN 2 levels in hepato-biliary and pancreatic diseases.

Serum DU-PAN 2 assay

Serm DU-PAN 2 levels were determined by the solid phase enzyme immunoassay (Kyowa Medex, Tokyo). The cut-off level was 400 U/ml, according to Metzgar et al.[1)]

Immunoperoxidase testing

Formalin-fixed, deparaffinized sections of liver obtained by biopsy or autopsy were stained by the indirect peroxidase-labelled antibody method. The deparaffinized sections were treated with 100% methanol containing 0.3% hydrogen peroxide to inhibit endogenous peroxidase and then they were preincubated in 1% non-immunized rabbit seurm for 5 minutes. Then they were incubated for 30 minutes with anti-DU-PAN 2 (provided by Kyowa Medex, Tokyo) diluted 1 : 100. Horseradish peroxidase-labelled IgG fragments mouse polyvalent immunoglobulins (Tago, Tokyo) were used as the second antibody. For a negative control, non-immune mouse sera were utilized in place of primary antibody. Section were immersed in 0.25% 3,3-diaminobenzidine tetrahydrochloride (DAB) solution in 50 mM Tris-HCl buffer (pH 7.6) containing 10 mM hydrogen peroxide and 10 mM sodium azide for about 5 minutes. Finally, the sections were counterstained with 1% methyl green solution buffered by veronal acetate at pH 4.0 for 20 minutes.

Statistical methods

Results were expressed as the mean±SE. Statistical significance was evaluated using the generalized Wilcoxon test for unpaired values.

RESULTS

Serum DU-PAN 2 levels

TABLE 1. Serum DU-PAN 2 levels from 31 normal controls and 370 cases of hepato-biliary and pancreatic disease. The mean values (mean± SE) and positive rates (400 and 1000 U/ml) are shown.

		DU-PAN 2 (U/ml)		
	N	Mean±SE	400<(%)	1000<(%)
Normal control	31	58.0±68.3	0	0
Acute hepatitis	22	322.5±368.4**	7 (31.8)	1 (4.5)
Chronic hepatitis	53	315.2±352.8**	15 (28.3)	4 (7.5)
Liver cirrhosis	74	393.2±370.1**	27 (36.5)	7 (9.4)
Hepatocellular carcinoma	126	551.4±653.0**	61 (48.4)	17 (13.5)
Meta. liver cancer	12	321±535.8	3 (25.0)	1 (8.3)
Primary biliary cirrhosis	8	922.7±1140.4**	4 (50.0)	2 (25.0)
Gallstone	27	395.6±802.3**	6 (22.2)	2 (7.4)
GB & CBD cancer	15	228.1±5482.9**	7 (46.6)	6 (40.0)
Chronic pancreatitis	17	89.8±160.4	1 (5.9)	0 (0)
Pancreas cancer	16	838.5±1559.1**	5 (31.2)	4 (25.0)

** $p<0.01$

Fig. 1 shows the distribution of serum DU-PAN 2 in hepato-biliary and pancreatic diseases, and Table 1 shows the mean value and prevalence. The mean value in 31 normal controls was 50.0±68.3 U/ml with none over 400 U/ml. In liver disease, the highest mean value of DU-PAN 2 (922.7) was in PBC; 50% of the patients had serum DU-PAN 2 levels greater than 400 U/ml. The mean value of DU-PAN 2 in HCC was 551.4 and 48.4% of the patients had values over 400 U/ml. In acute and chronic hepatitis, the mean values were 322.5 and 315.2, respectively. In liver cirrhosis, 27 of 74 patients had DU-PAN 2 levels of over 400 U/ml, with a median of 393.2. In all the liver diseases except metastatic liver cancer, the serum DU-PAN 2 value was significantly higher than in the normal controls. In biliary tract and pancreatic diseases, the prevalence of elevated serum DU-PAN 2 levels was 22.2% in gallstones (mean: 359.6 U/ml), 46.6% in gallbladder and common bile duct cancers (mean: 2280.1 U/ml), 5.9% in chronic pancreatitis (mean: 89.8 U/ml) and 31.2% in pancreatic cancer (mean: 838.5 U/ml).

Serum DU-PAN 2 in chronic liver diseases (Fig. 2)

Chronic hepatitis (53 cases) was divided into two groups; chronic inactive hepatitis, CIH, (17 cases) and chronic active hepatitis, CAH, (36 cases) by laboratory and/or histological findings. As shown in Fig. 2, the serum DU-PAN 2 levels were 201.4±253.4 and 394.5±388.0 U/ml in CIH and CAH, respectively. A statistically significant difference was noted between the two groups ($p<0.05$). Liver cirrhosis, LC, (74 cases) was also divided into two groups; compensated LC (41 cases) and decompensated LC (31 cases) by the presence or absence of ascites, jaundice and hepatic coma. In compensated LC, the value was 255.1±268.9 U/ml, while, in uncompensated LC, it was 564.7±409.1 U/ml. There was a significant difference ($p<0.01$) between the two groups. As for chronic hepatitis, only 2 of 17 CIH patients (11.7%) had DU-PAN 2 values over 400 U/ml, while 14 of 36 CAH patients (38.8%) had serum values over 400 U/ml. In liver cirrhosis, DU-PAN 2 levels of over 400 U/ml were noted in 8 of 41 compensated LC case (19.5%), while in decompensated LC, 17 of 33 patients (51.5%) exceeded 400 U/ml.

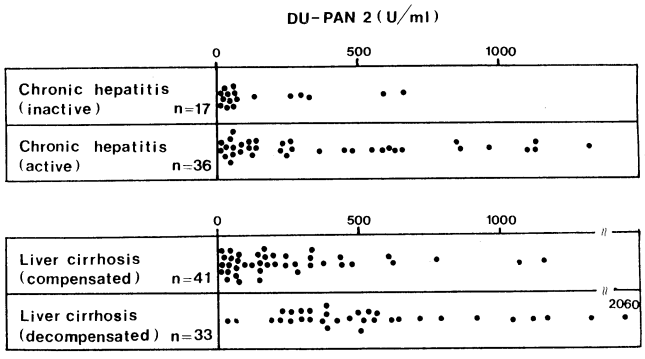

Fig. 2. Comparison of serum DU-PAN 2 values in chronic inactive hepatitis (17 cases) and chronic active hepatitis (36 cases). The mean values were 201.4 and 394.5 U/ml in CIH and CAH, respectively. In liver cirrhosis (LC), compensated LC had a mean value of 255.1 U/ml and decompenstated LC, a mean value 564.7 U/ml. Significant differences were noted between CIH and CAH ($p<0.05$), and also between compensated LC and decompensated LC ($p<0.01$).

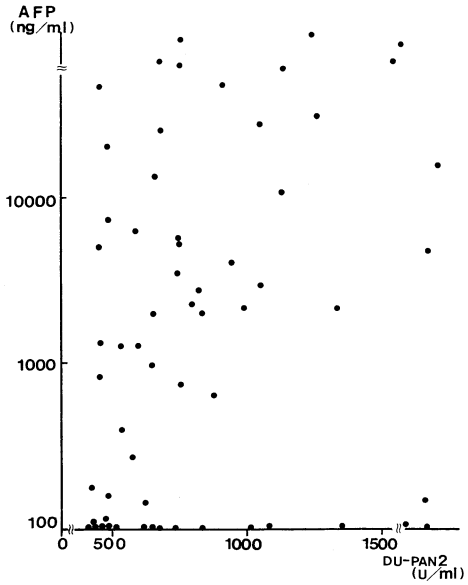

Fig. 3. Correlation between serum DU-PAN 2 (over 400 U/ml) and α-fetoprotein in 61 cases of hepatocellular carcinoma. No significant correlation was found.

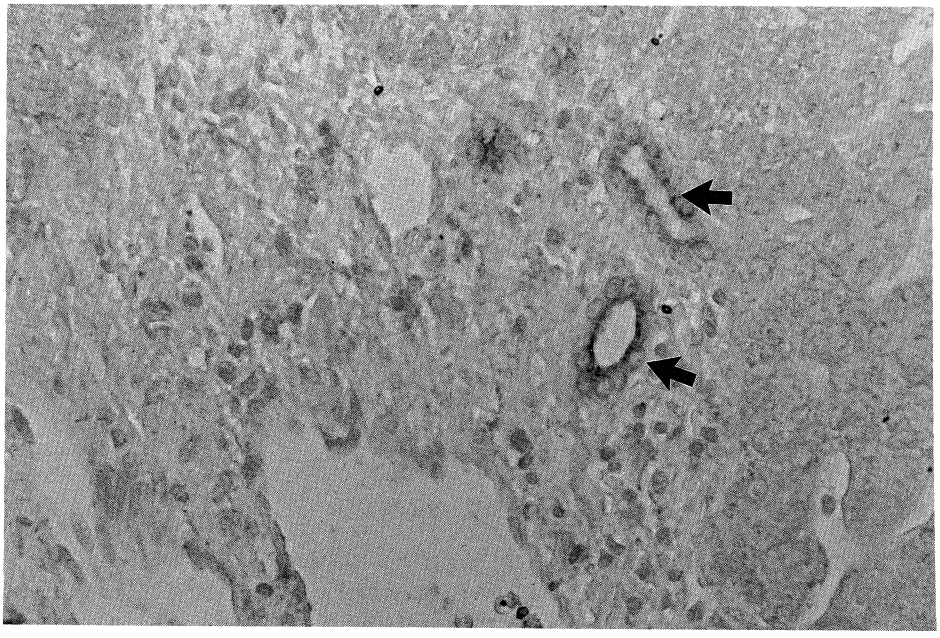

Fig. 4. Paraffin section of liver from a patient with primary biliary cirrhosis stained by the immunoperoxidase technique with DU-PAN 2. The bile ducts (arrows) were stained positively ($\times$200).

Correlation between DU-PAN 2 and α-fetoprotein in HCC (Fig. 3)

In 126 cases of HCC, serum DU-PAN 2 values ranged from 24 to 3960 U/ml, with the mean value being 551.4$\pm$653.0 U/ml. Activities over 400 U/ml

were noted in 61 of 126 cases (48.4%), and over 1000 U/ml in 17 of 126 patients (13.5%). Sixty-one HCC cases with DU-PAN 2 positive (over 400 U/ml) were examined for correlation ($r=0.0434$). In 15 out of 61 patients (24.6%), α-fetoprotein remained below 100 ng/ml.

Enzyme cytochemistry for DU-PAN 2

To ascertain the site of DU-PAN 2 activity in tissues, immunostaining for DU-PAN 2 was performed on 3 liver cirrhosis, 2 PBC, 3 HCC, 1 acute cholecystitis, and 2 pancreatic cancer by the indirect peroxidase-labelled antibody method. Epithelium of the gallbladder and pancreatic cancer tissue were stained heavily; which is consistent with results in a previous paper.[5] Fig. 4 shows ductal staining in PBC. HCC did not stain for DU-PAN 2 antigen (Fig. 5a), whereas adjacent bile ductules were stained (Fig. 5b).

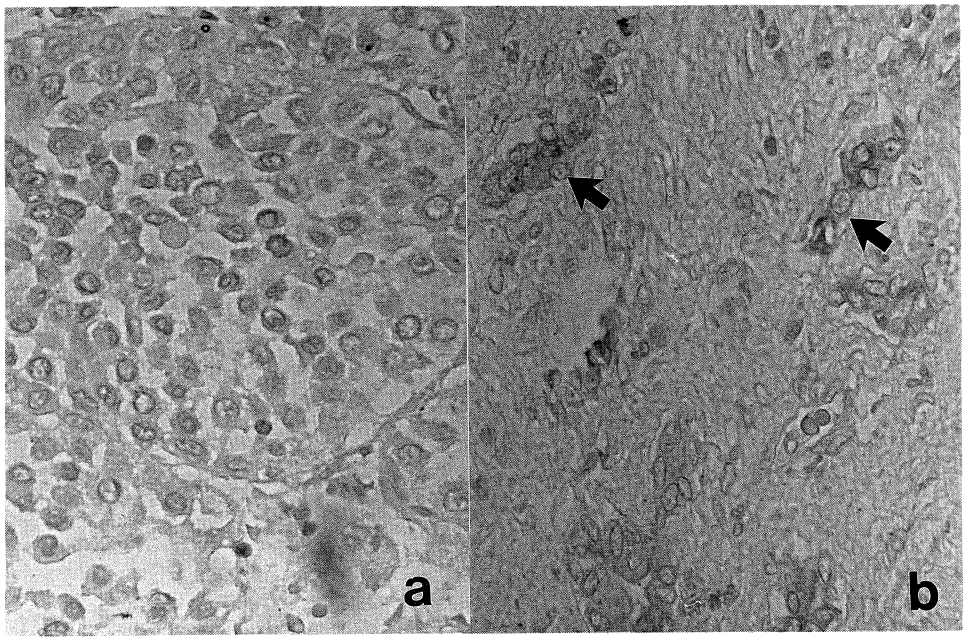

Fig. 5. Paraffin section of liver from a patient with HCC, stained by the immunoperoxidase technique with DU-PAN 2. The hepatoma cells were unstained (a), whereas the adjacent bile ducts (arrows) were stained positively (b) (×200).

DISCUSSION

DU-PAN 2 is an antign which is present in ductal epithelial cells of the pancreas, gallbladder and bile duct and bronchial epithelium.[7] This report and other studies have shown that serum DU-PAN 2 levels are elevated in pancreatic cancer, biliary tract malignancies and HCC.[1-6] The results of this study show that elevated levels of DU-PAN 2 are not restricted to malignant disease. A similar percentage of patients with benign chronic liver disease also have elevated serum DU-PAN 2 levels. The incidence of elevated DU-PAN 2 levels was reported to be highest in pancreatic cancer (66%,[4] 68%,[1] 72%,[2]), but in our study only 5 of 16 pancreatic cancers (31.2%) showed a level of more

than 400 U/ml. The positive rate was highest in HCC (50.0%), follwed by biliary tract malignancy (46.6%). In HCC the incidence of elevated DU-PAN 2 levels was reported to range from 32.3%[8] to 59.6%.[6] In our series 61 of 126 HCC patients (48.4%) exhibited values in excess of 400 U/ml, which is consistent with the report of Haviland (50%).[4] In HCC, 15 out of 61 cases (24.6%) had an elevated DU-PAN 2 level with low α-fetoprotein. In such patients, a combination assay with DU-PAN 2 may contribute to successful screening of HCC. The poor correlation of DU-PAN 2 and α-fetoprotein may be explained by difference in the hepatic distribution of these markers. We and other authors[3] have found that DU-PAN 2 staining is restricted to the bile ducts and rarely to Kupffer cells, but never involves HCC cells. For above reason, Haviland et al.[3] proposed that the abnormal serum DU-PAN 2 levels in HCC patients were secondary to the underlying liver disease. In pancreatic cancer, the high serum levels of DU-PAN 2 tended to correlate with a high degree of strong staining in the cancer cells,[4] while in HCC, the intensity and number of bile ducts stained for DUN-PAN 2 antigen did not correlate with serum DU-PAN 2 levels.[3]

Amog serum DU-PAN 2 levels in non-malignant liver disease, the highest median levels were seen in patients with primary biliary cirrhosis (922.7 U/ml) and the lowest in chronic hepatitis patients (315.2 U/ml). In liver cirrhosis, serum DU-PAN 2 levels were reported to vary over a wide range from 6%[2] to 50%.[3] 27 of 74 LC (36.5%) in this study had elevated levels of more than 400 U/ml. As 11 out of 127 chronic liver diseases (8.6%) showed serum DU-PAN 2 levels of more than 1000 U/ml, we divided 53 chronic hepatitis cases into 17 CIH and 36 CAH, and 74 LC into 41 compensated LC and 33 decompensated LC. In CAH, the median DU-PAN 2 (201.4 U/ml) was lower than that in CAH (394.5 U/ml) ($p<0.05$), which is consistent with the reports of other authors.[9] In liver cirrhosis, the serum DU-PAN 2 level in compensated LC (255.1 U/ml) was significantly lower than that in decompensated LC (564.7 U/ml) ($p<0.01$). An abnormality in the secretion or metabolism of mucin carrying the DU-PAN 2 epitope may play a role in the elevation of serum DU-PAN 2 in chronic liver disease.[4] Another report[5] also proposed that DU-PAN 2 might be returned to the circulation by the obstruction or destruction of the ductules, thus causing high serum levels of DU-PAN 2. In summary, DU-PAN 2 is elevated in a wide spectrum of hepato-biliary diseases and it also could be useful in detecting and monitoring chronic liver diseases.

REFERENCES

1) Metzgar. R.S., Rodriguez, N., Finn, O.J., Lan, M.S., Daasch, V.N., Fernsten, P.D., Meyers, W.C., Sindelar, W.E., Sandler, R.S. and Seigler, H.F.: Detection of a pancreatic cancer-associated antigen (DU-PAN-2 antigen) in serum and ascites of patients with adenocarcinoma. Proc. Natl. Acad. Sci. USA **81**: 5242-5246, 1984
2) Sawabu, N., Toya, D., Takemori, Y. Hattori, N. and Fukui, M.: Measurement of a pancreatic cancer-associated antigen (DU-PAN-2) detected by a monoclonal antibody in sera of patients with digestive cancers. Int. J. Cancer **37**: 693-696, 1986
3) Chang, J., Watanabe, S. and Takeuchi, T.: Clinical significance of serum DU-PAN 2 measurement for the diagnosis of pancreatic cancer. J. Jpn. Panc. Soc. **1**: 69-77, 1986 (in Japanese)
4) Haviland, A.E., Borowitz, M.J., Killenberg, P.G., Lan, M.S. and Metzgar, R.S.: Detection of an oncofetal antigen (DU-PAN-2) in sera of patients with non-malignant

hepatobiliary disease and hepatomas. Int. J. Cancer **41**: 789–793, 1988
5) Suzuki, Y., Ichihara, T., Nakao, A., Sakamoto, J., Takagi, H. and Nagura, H.: High Serum levels of DU-PAN 2 antigen and CA 19-9 in pancreatic cancer: Correlation with immunocytochemical localization of antigens in cancer cells. Hepatogastroenterol. **35**: 128–135, 1988
6) Yamamoto, S., Ideguchi, S., Saito, I., Wada, A., Takatori, K., Yamamoto, R., Ohmoto, K., Ohumi, T., Hino, K. and Hirano, Y.: Clinical significance of serum DU-PAN 2: Measurement in hepato-biliary and pancreatic disease. Kan-Tan-Sui **10**: 759–765, 1989 (in Japanese)
7) Borowitz, M.J., Tuck, F.L., Sindelar, W.F., Fernsten, P.D. and Metzgar, R.S. Monoclonal antibodies against human pancreatic adenocarcinoma-distribution of DU-PAN-2 antigen on glandular epithelia and adenocarcinoma. J. Natl. Cancer Inst. **72**: 999–1003, 1984
8) Ohgami, Y., Kimura, T., Ito, T., Sumii, T. and Ibayashi, H.: Clinical study of tumor maker DU-PAN-2. Rinsho to Kenkyu **64**; 2139–2143, 1987 (in Japanese)
9) Kawasaki, H., Suyama, K. and Horie, Y.: Two cases of primary biliary cirrhosis and hepatocellular carcinoma with elevated serum DU-PAN 2. Rinsho Shokakinaika **5**: 557–562, 1990 (in Japanese)