Diagnosis of Carcinoma of the Pancreas by Assay of Immunosuppressive Acidic Protein

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MATSUNO, S., KOBARI, M., MATSUDA, Y. and SATO, T. *Diagnosis of Carcinoma of the Pancreas by Assay of Immunosuppressive Acidic Protein.* Tohoku J. exp. Med., 1982, **136** (1), 1-10 — To establish a procedure for screening of pancreatic cancer, we assayed the serum level of immunosuppressive acidic protein (IAP) in patients with malignant and benign pancreatic diseases and compared it with the serum level of carcinoembryonic antigen (CEA). In 96% of 46 pancreatic cancer patients, the serum IAP level averaged 928 $\mu\text{g/ml}$; while 500 $\mu\text{g/ml}$ or below was obtained as the normal value in 211 healthy adults. The serum IAP level may be elevated in the presence of non-malignant, inflammatory pancreatic diseases. For the differential diagnosis of pancreatic cancer, therefore, other laboratory data need to be taken into account. The serum IAP level was correlated with the progression of pancreatic cancer and may provide a means to determine whether tumor resection was radical or admitting of recurrence. The serum IAP level in samples obtained from different sites of the portal system did not reflect tumor location in patients with pancreatic cancer. ——— immunosuppressive acidic protein; pancreatic cancer; carcinoembryonic antigen; percutaneous transhepatic portal catheterization

The prognosis of cancer patients depends on early diagnosis and treatment. In pancreatic cancer, however, early diagnosis is difficult and the long-term results of surgical treatment are much poorer than in tumors in other portions of the gastrointestinal tract. This calls for a procedure facilitating the screening for early pancreatic cancer which is simple and highly accurate. Therefore, we determined the serum level of immunosuppressive acidic protein (IAP), which is elevated in cancer patients (Matsuda et al. 1977b), in various pancreatic diseases and compared this level with that of serum carcinoembryonic antigen (CEA). We also studied the value of IAP assay in diagnosing carcinoma of the pancreas.

MATERIALS AND METHODS

The subjects of the present study comprised 125 patients treated in our Department between April 1977 and December 1979. There were 46 patients with carcinoma of the pancreas, 29 with chronic and 6 with acute pancreatitis, 3 each with pancreatic cysts or insulinoma, 26 with carcinoma of the bile duct, and 6 each with carcinoma of the duodenal papilla or gallbladder. The control consisted of 211 healthy adults ranging in age from 18 to 45 years.

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To quantitate serum IAP, we used single radial immunodiffusion, introducing $3.0\ \mu\text{l}$ of serum into wells punched into an agarose plate containing 1–5% anti-IAP serum. The plates were kept wet on a horizontal plane, reacted at 37°C for 48 hr and the diameter of the precipitin ring was measured (Tamura et al. 1978).

In operated patients with cancer of the pancreas or biliary tract, serum IAP assay was performed pre- and post-operatively. The serum of patients undergoing resection was assayed for IAP at intervals following the operation.

To study the relationship between tumor location and the serum levels of IAP and CEA at different marker sites, the blood from 9 patients with pancreatic cancer and one with carcinoma of the duodenal papilla was submitted to simultaneous quantitation of serum IAP and CEA. Peripheral blood samples, and blood collected by percutaneous transhepatic portal catheterization (Göthlin et al. 1974) from the trunk of the portal vein, the superior and inferior mesenteric veins, and the splenic vein were assayed.

RESULTS

In 194 of the 211 (91.9%) control subjects, the serum IAP level was below $500\ \mu\text{g/ml}$ (mean, $351\ \text{mg/ml}$); in 14 (6.7%) it was between 500 and $700\ \mu\text{g/ml}$ and in 3 (1.4%) it was $700\ \mu\text{g/ml}$ or above (Fig. 1). Therefore, the normal range of serum IAP was considered to be $500\ \mu\text{g/ml}$ or below.

Fig. 1. IAP values in normal human serum.

Serum IAP level in malignant and benign pancreatic or biliary tract diseases

As shown in Fig. 2, 44 of 46 (96%) patients with pancreatic cancer, 24 of 26 (92%) patients with bile duct cancer, and all patients with cancer of the duodenal papilla or gallbladder manifested serum IAP levels above the normal range. The incidence of elevated serum IAP levels was also high in patients with inflammatory, benign disorders of the pancreas; all patients with acute pancreatitis or pancreatic cysts and 19 of 29 (66%) of those with chronic pancreatitis had serum IAP levels above the normal range. None of the 3 insulinoma patients showed an elevation in serum IAP (Fig. 2).

$1073.9 \pm 348.4 \mu\text{g/ml}$ if there were many metastatic lesions in both hepatic lobes (H_3).

In patients with no disseminated peritoneal metastasis (P_0), the serum IAP

Fig. 3. Relation between serum IAP values and grade of pancreatic capsular invasion (S), size of tumor (T). S_0 : No infiltration to the pancreatic capsule. S_1 : Tumor barely extended to the surface of the pancreatic capsule. S_2 : Tumor extended beyond the pancreatic capsule. S_3 : Infiltration to the surrounding organ. T_1 : Diameter of the tumor was less than 2 cm. T_2 : Tumor size was between 2 and 5 cm. T_3 : Tumor size was between 5 and 8 cm. T_4 : Tumor size exceeded 8 cm.

Fig. 4. IAP values in serum of pancreatic cancer with liver metastasis (H) and peritoneal dissemination (P). H_0 : No hepatic metastasis. H_1 : Unilobar hepatic metastasis. H_2 : A few scattered metastases in both lobes. H_3 : Multiple metastases in both hepatic lobes. P_0 : No disseminated peritoneal metastasis. P_1 : Dissemination to the peritoneum contiguous to the tumor mass. P_2 : A few metastases in the remote peritoneum. P_3 : Many metastases in the remote peritoneum.

level was $894.6 \pm 299.9 \mu\text{g/ml}$; it was $1058.3 \pm 293.9 \mu\text{g/ml}$ if there was dissemination to the peritoneum contiguous to the tumor mass (P_1), $1237.5 \pm 320.1 \mu\text{g/ml}$ in patients with a few metastases in the remote peritoneum (P_2), and $1305.0 \pm 378.0 \mu\text{g/ml}$ if there were many metastases in the remote peritoneum (P_3) (Fig. 4).

These observations revealed that while there was a relationship between the serum IAP level and tumor size and metastatic involvement of the liver and peritoneum, there was no definite relationship between the IAP level and the degree of infiltration to the pancreatic capsule.

Serum IAP levels after tumor excision

Fig. 5 shows the serum IAP levels, measured at the indicated postoperative intervals, of 2 patients with carcinoma of the pancreatic head, 3 with carcinoma of the duodenal papilla and 2 with carcinoma of the intrapancreatic bile duct.

The serum IAP level of one of the pancreatic cancer patients who had undergone total pancreatectomy decreased from an initial value of $825 \mu\text{g/ml}$ to $430 \mu\text{g/ml}$ one month postoperatively; one year after surgery, a value of $490 \mu\text{g/ml}$ was obtained. The other pancreatic cancer patient manifested recurrence; he died 3 years and 7 months after tumor resection. In this patient, postoperative follow-up revealed a steady increase in the serum IAP level; it was $820 \mu\text{g/ml}$ at 2 years after resection, $830 \mu\text{g/ml}$ 6 months later, and $1105 \mu\text{g/ml}$ at 3 years, at which time there was clinical evidence of recurrence. Immediately prior to the death of the patient, the serum IAP level was $1400 \mu\text{g/ml}$ (Fig. 5).

Fig. 5. Change in serum IAP values in 7 patients after pancreaticoduodenectomy.

In 3 of 4 patients with cancer of the duodenal papilla or intrapancreatic bile duct, we noted a postoperative decrease in serum IAP to near normal levels at one month after tumor resection. However, follow-up study of serum IAP, started 2 years postoperatively, revealed elevated levels in 2 patients (Fig. 5).

Fig. 6. Serum IAP values and CEA values in peripheral blood in patients with pancreatic cancer.

Fig. 7. Portions of the portal system from which blood samples were obtained by percutaneous transhepatic portal catheterization.

1, the trunk of the portal vein; 2, the splenic vein; 3, the superior mesenteric vein; 4, the inferior mesenteric vein.

Comparison of serum IAP and CEA levels in pancreatic cancer patients

Peripheral blood. Serum IAP and CEA levels were determined simultaneously in 46 pancreatic cancer patients. As shown in Fig. 6, 26 of 46 (57%) patients manifested CEA levels of 2.5 ng/ml or above and 44 of 46 (96%) patients had IAP levels of 500 μ g/ml or above.

These results showed that in patients with pancreatic cancer, the incidence of elevated serum IAP levels was higher than of elevated serum CEA levels.

Portal blood. Portal blood samples were obtained from 4 different sites of the portal system (Fig. 7). In individual patients, the serum IAP level exceeded 500 μ g/ml and there was no difference in these levels among different sites of the portal system (Fig. 8). A peripheral blood CEA level of 2.5 ng/ml was obtained in 2 patients; in one of these, serum CEA was below 2.5 ng/ml when the blood was sampled from the superior mesenteric vein. In 3 other patients, blood samples obtained from the trunk of the portal vein revealed a serum CEA level exceeding 2.5 ng/ml (Fig. 9).

Fig. 8. Serum IAP values in the 4 different sites of the portal system and of peripheral blood in 6 patients with cancer of the pancreatic head.

Fig. 9. Serum CEA values in the different sites of the portal system and of peripheral blood in the 6 patients with cancer of the pancreatic head.

TABLE 1. *IAP and CEA values in the portal system and peripheral blood in patients with cancer of the pancreatic body and tail*

Case	Blood from the portal system				Peripheral blood	
	Portal trunk	Superior mesenteric	Inferior mesenteric	Splenic		
1	IAP ($\mu\text{g/ml}$)	840	840	860	670	950
	CEA (ng/ml)	1.7	2.8	2.9	2.2	2.3
2	IAP ($\mu\text{g/ml}$)	650	630		630	910
	CEA (ng/ml)	2.8	3.0		2.8	4.8
3	IAP ($\mu\text{g/ml}$)	710	1045	800	1045	930
	CEA (ng/ml)	0.7	1.2	1.9	0.6	0.6

TABLE 2. *Serum IAP and CEA values in the portal system and peripheral blood in patients with cancer of the duodenal papilla before and after pancreaticoduodenectomy*

		Blood from the portal system				Peripheral blood
		Portal trunk	Superior mesenteric	Inferior mesenteric	Splenic	
Preoperation	IAP ($\mu\text{g/ml}$)	900	800	920	780	825
	CEA (ng/ml)	0.6	0.7	0.7	0.5	0.8
Postoperation	IAP ($\mu\text{g/ml}$)		380		260	530
	CEA (ng/ml)		0.8		0.9	1.0

In all 3 patients with carcinoma of the pancreatic tail, the serum IAP level was above $500 \mu\text{g/ml}$; in 2 of these patients, the portal venous CEA level exceeded 2.5 ng/ml . Comparison of the serum IAP and CEA levels revealed no difference in blood obtained from different sites of the portal system, nor was there any tendency for serum IAP or CEA elevation at the site of the portal system where the tumor was located (Table 1).

Patients with cancer of the duodenal papilla were subjected to percutaneous transhepatic portography 1 week before and 4 weeks after resection of the pancreatic head and duodenum. Assay of serum IAP and CEA in peripheral blood and in samples from different sites of the portal system showed pre- and postoperative CEA values below 1.0 ng/ml . On the other hand, while serum IAP before surgery was as high as $780 \mu\text{g/ml}$ or above at all examined sites of the portal system, it decreased to normal levels postoperatively — to $380 \mu\text{g/ml}$ in blood from the superior mesenteric vein, to $260 \mu\text{g/ml}$ in splenic venous blood and to $530 \mu\text{g/ml}$ in peripheral blood (Table 2).

DISCUSSION

Matsuda et al. (1977a) have shown that the serum and ascitic fluid of tumor-bearing mouse and cancer patients contain an acidic protein having a molecular weight of 59,000 which exerts immunosuppressive activity and migrates at a pH of 3.0–3.4. IAP has been documented to suppress the blastogenesis of PHA- or

Con A-stimulated mouse lymphocytes *in vitro*, to inhibit delayed-type hypersensitivity reactions in mice, to promote tumor growth in tumor-bearing hosts, and to inhibit interferon activity (Matsuda et al. 1978). However, the present study revealed IAP to be present, at a mean concentration of 351 $\mu\text{g/ml}$, in normal human serum, therefore, it cannot be regarded as being specific for cancer.

On the other hand, because the serum IAP level is higher in cancer patients and because of its correlation with the gastric cancer stage, Matsuda and Sato (1979) suggested that serum IAP assay may be useful in evaluating the prognosis of patients who had undergone cancer surgery.

In the present study, we examined the serum IAP levels of patients with pancreatic, and biliary tract diseases. Excepting one pancreatic, 2 bile duct and 2 duodenal papilla cancer patients, the cancers in our study were advanced and unresectable. This may account for our observation that more than 90% of the cancer patients manifested serum IAP levels above the normal range.

We obtained a preoperative serum IAP level of greater than 500 $\mu\text{g/ml}$ in 5 patients whose cancer we consider to have been at a relatively early stage at the time of surgery. This finding suggests that serum IAP assay may be highly valuable in diagnosing cancer of the pancreas or biliary tract. Furthermore, although there was no correlation with the degree of invasion to the pancreatic capsule, the serum IAP level reflected the progress of pancreatic cancer, as it was correlated with the tumor size and the extent of liver metastasis and disseminated peritoneal metastasis.

After the resection of pancreatic or biliary tract cancer, a decline in the serum IAP level was observed; in many cases, the level decreased to normal values at about 1 months after surgery. In one operated pancreatic cancer patient there was recurrence, leading to his death. In this patient, the postoperative serum IAP level increased steadily and the level of CEA, a cancer-specific protein (Sharma et al. 1976; Sugarbaker 1976) also increased. It is of interest to note that the postoperative patterns of serum IAP and CEA were similar in many patients.

While the exact mechanism whereby serum IAP is increased remains obscure, the observation that this level was also increased in patients with acute or chronic pancreatitis suggests that, unlike in other tumor markers such as alpha-fetoprotein (Abelev et al. 1963) and pancreatic oncofetal antigen (Gelder et al. 1978), this mechanism is complicated. Considering that IAP possesses immunosuppressive activity, that there is a correlation between the serum IAP level and the progress of pancreatic cancer, and that serum IAP decreases upon removal of the cancer, it appears that the elevation in serum IAP reflects the extent to which the immunocompetence of the cancer-bearing host is compromised. Therefore, we suggest that serum IAP assay may represent a valid means for estimating the progress of pancreatic cancer and for determining whether tumor resection was radical or admitting of recurrence.

In some patients with cancer of the pancreatic head, the serum CEA level was higher when the blood sample had been taken from the trunk of the portal vein

than from other sites of the portal system. On the other hand, there was no such difference in patients with cancer of the pancreatic tail. In either type of pancreatic cancer, we noted no difference regarding the serum IAP level, irrespective of the portal site from which the blood was sampled.

Although prior to the inception of this study we had hypothesized that, depending on the location of the tumor, there may be difference between IAP and CEA levels in the portal blood of pancreatic cancer patients, our present results do not bear out this hypothesis. Neither IAP nor CEA was elevated in veins close to the tumor site. Our observation that in pancreatic cancer patients, the incidence of increased peripheral blood IAP levels was higher than of increased CEA levels leads us to suggest that serum IAP assay may be useful in the screening of pancreatic cancer.

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