

Elevated serum ribonuclease in patients with pancreatic cancer

(biochemical marker/pancreatic organ specific/detection of pancreatic cancer)

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ABSTRACT Serum RNase (ribonuclease) of normal persons and of patients with pancreatitis, carcinoma of pancreas, or other neoplasms was determined with poly(C) as substrate. Strikingly abnormal elevations occur in the serum RNase of patients with pancreatic cancer. There is no elevation in the serum RNase level of patients with pancreatitis. Average serum RNase values of 52 normal persons, 10 patients with pancreatitis, 30 patients with pancreatic cancer, 28 patients with breast cancer, 11 patients with lung cancer, 20 patients with colon cancer, six patients with stomach cancer, and four patients with liver cancer, respectively, were 104, 120, 383, 131, 173, 197, 194, and 152 units/ml of serum. Ninety percent of the patients with pancreatic cancer were above the level of 250 units/ml of serum and 90% of all patients with varied cancers were below this level. In the presence of severe renal insufficiency, marked elevation of serum RNase was also observed. Serum RNase, because of its unique specificity, pancreatic origin, and its abnormal elevation in sera of patients with pancreatic cancer, serves as a reliable biochemical marker of carcinoma of the pancreas in the presence of normal renal function.

Carcinoma of the pancreas is difficult to diagnose because the existing methods are either insensitive or limited in applicability. For this reason, diagnosis is almost always too late. History, physical examination, and roentgenographic, endoscopic, echographic, and isotopic scanning techniques are far from accurate. Serum amylase is an unreliable parameter (1). Although biopsy is possible, such highly invasive testing is extremely limited in its applicability. Pancreatic cancer is fatal within 12 months for 90% of affected patients and constituted 5.3% of estimated deaths in 1975. Earlier and more reliable diagnosis would allow new approaches to treatment.

RNase is one of several enzymes elaborated by the pancreas. The properties and structure of bovine pancreatic RNase (ribonuclease I; ribonuclease 3'-pyrimidino-oligonucleotidohydrolase; EC 3.1.4.22) have been extensively studied (2). In contrast, information on human pancreatic RNase is meager. Recent studies in this laboratory revealed striking similarities between RNase of human pancreas and that present in human serum (3). Both enzymes have their pH optimum at 6.5 with poly(C) as substrate. Their action on poly(C) is inhibited by poly(A) and poly(G). They are absolutely dependent on phosphate or citrate for their activity. They are thermostable at pH 4.2 and thermolabile at pH 8.5. They are highly specific to secondary phosphate esters of cytidine 3'-phosphates.

These biochemical similarities imply that human pancreas is the source of human serum RNase. This conclusion is at variance with earlier studies (4, 5) which suggested that leukocytes are the source of serum RNase. In our studies, the RNase present in leukocytes is highly specific to secondary phosphate esters of uridine 3'-phosphates and has very little activity toward secondary phosphate esters of cytidine 3'-phosphates (6). Hence, white cells could not be the source of serum RNase.

The results presented in this paper show abnormally elevated RNase in the sera of patients with pancreatic cancer. This

further supports the proposition that serum RNase is of pancreatic origin. The abnormal activity of serum RNase serves as a biochemical indicator of pancreatic cancer.

MATERIALS AND METHODS

Reagents. Poly(C) was purchased from Schwarz/Mann, Orangeburg, N.Y. All other reagents used in this investigation were of reagent grade.

Patients. The diagnosis of cancer of patients included in this study was proven by biopsy or by autopsy.

Serum. Venous blood, drawn either from volunteer laboratory workers or patients, was allowed to clot at room temperature for 1 hr and centrifuged at $750 \times g$ for 15 min at room temperature. The serum was removed with a capillary pipette and assayed immediately or stored at -20° .

RNase Assay. Because human serum RNase is highly specific to secondary phosphate esters of cytidine 3'-phosphates, its assay with poly(C) as a substrate is more sensitive than with RNA, which is hydrolyzed only partially. Cleavage of poly(C) by serum RNase was followed by the formation of mono- and oligonucleotides, which were separated from partially digested poly(C) fragments by acid precipitation. The concentration of acid-soluble products was measured at 278 nm (3). Detailed assay procedure is given in the legend to Table 1.

One RNase unit is defined as that amount which renders 1 μ mol of poly(C) acid-soluble in 1 min at 37° and at pH 6.5 (U_{37°).

RESULTS

The normal level of serum RNase was 103 ± 24 units/ml of serum. Serum RNase activity was stable, and about 12% of its original activity was lost when stored at room temperature for 72 hr. The time of venipuncture or eating had no effect on serum RNase activity.

Results in Table 1 show strikingly abnormal elevations in the serum RNase of patients with pancreatic cancer. Serum RNase values of 52 normal persons ranged from 63 to 160 units/ml of serum. Of 30 pancreatic cancer patients studied, two had values of 99 and 150 units of RNase per ml of serum and the remaining 28 had values ranging from 247 to 714 units/ml of serum. The patient who had 99 units/ml of serum had a rare adenocystic carcinoma of the pancreas without evidence of disease after resection. Of 10 patients with pancreatitis, one had 206 units of RNase per ml of serum and the rest had values ranging from 67 to 150 units/ml of serum. Of 28 patients with breast cancer, three patients had values of 244, 250, and 275 units/ml of serum and the rest had values ranging from 75 to 193 units/ml of serum. Of 20 patients with colon cancer, three had values of 252, 295, and 300 units of RNase per ml of serum and the rest had values ranging from 80 to 220 units/ml of serum. Of 11

Table 1. Serum RNase of normal persons and patients with pancreatitis and varied neoplasms

Donor	No. of patients	Average RNase units per ml of serum	Standard deviation	Standard error	Probability that normals equal
Normal	52	104	24.3	3.4	
Pancreatitis	10	120	39.4	12.5	0.17
Pancreatic cancer	30	383	145.2	26.5	10^{-10}
Miscellaneous cancer	69	164	58.9	7.1	10^{-6}
Breast	28	131	49.1	9.3	
Colon	20	197	53.2	11.9	
Lung	11	173	57.5	17.3	$(10^{-7})^*$
Stomach	6	194	61.4	25.1	
Liver	4	152	44.6	22.3	
Kidney cancer †	4	628	89.4	44.7	

Reaction mixtures consisting of 0.05 ml of poly(C) (100 µg), 0.15 ml of phosphate-borate buffer (0.1 M with respect to phosphate), pH 6.5, and 0.05 ml of serum that had been diluted 200-fold were incubated at 37° for 15 min and then transferred to an ice bath. To each tube was added, with mixing, 0.25 ml of cold 1.2 M HClO₄ containing 0.02 M lanthanum nitrate. After 20 min at 0°, the precipitates were removed by centrifugation at 12,100 × g for 30 min in the cold. The supernatants were diluted with H₂O and their absorbances were measured at 278 nm. Enzyme and substrate blanks were always run side by side.

* Probability that miscellaneous cancers = pancreatic cancer.

† Serum creatinine values were elevated in each of these patients, suggesting false positive values (see text).

patients with cancer of the lung, two had values of 244 and 272 units of RNase per ml of serum and the rest had values ranging from 80 to 201 units/ml of serum. Serum RNase values of six patients with stomach cancer ranged from 92 to 260 units/ml of serum. Serum RNase of four patients with liver cancer ranged from 117 to 216 units/ml of serum.

The individual values of normal persons, patients with pancreatitis, patients with pancreatic cancer, and others with varied cancers were plotted as cumulative percentages of total persons in each group (Fig. 1). At a threshold RNase value of 250 units/ml of serum, all normal subjects and all patients with pancreatitis are excluded; 90% of the patients with pancreatic

cancer are above the threshold level of 250 units, and 90% of all patients with varied cancers are below this level.

Significant increases in serum RNase levels of patients who did not have pancreatic cancer were also observed. Serum RNase activity of four patients with carcinoma of kidney ranged from 545 to 732 units/ml of serum (Table 1). Their serum creatinine values ranged from 1.4 to 2.3 mg/100 ml of serum. Sera of six patients with renal failure, obtained just before dialysis, were also investigated. Their serum RNase ranged from 1730 to 3500 units/ml of serum and their creatinine values ranged from 9 to 17 mg/100 ml of serum. Simultaneous serum RNase and creatinine values were also available for 26 patients

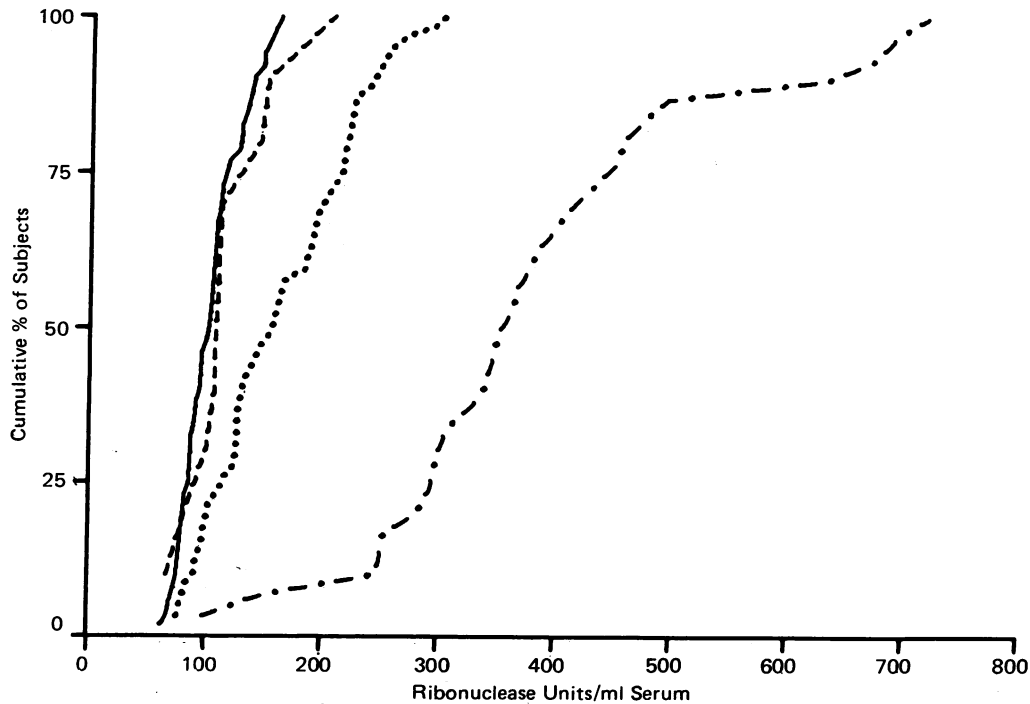


FIG. 1. Serum RNase activities of normal persons and patients plotted as cumulative percent of all persons studied in each group. Normal subjects, n = 52 (—); pancreatitis, n = 10 (- - -); varied cancers, n = 69 (· · ·); pancreatic cancer, n = 30 (- · · ·).

with pancreatic cancer; while their serum RNase ranged from 99 to 714 units/ml of serum, their serum creatinine values remained normal, 0.6 to 1.4 mg/100 ml of serum (mean \pm SD, 0.98 ± 0.26 mg). It is evident from these results that, in addition to pancreatic cancer, substantial elevations in serum RNase can also occur in renal insufficiency. The increased serum RNase levels observed in patients with kidney cancer could be due to renal insufficiency and, hence, may not be related to cancer. Elevations in serum RNase were also observed in two patients with carcinoma of gall bladder, in two patients with ampullary carcinoma, and in one patient with ovarian carcinoma, all of whom had upper abdominal masses and were jaundiced. These elevations could result from interference with the normal excretion of pancreatic RNase due to obstruction of ducts.

DISCUSSION

Human serum RNase, because of its unique specificity, pancreatic origin, and its abnormal elevation in sera of 90% of patients with pancreatic cancer, could serve as a biochemical marker in carcinoma of the pancreas. In the presence of serum RNase values above 250 units/ml, intensive clinical scrutiny is imperative to establish the diagnosis of pancreatic cancer. Correlation of elevated pancreatic RNase with early disease as a diagnostic screening tool and as a monitor of therapeutic results is under investigation. Since serum RNase, which is ordinarily excreted by the renal route (Reddi, unpublished data), increases in the presence of renal insufficiency, an interpretation of its elevation due to pancreatic cancer depends on the presence of normal renal function.

The increased RNase in the serum of pancreatic cancer patients might represent an increased enzyme synthesis by the proliferating tumor cells or might be due to the synthesis of a new species of RNase. It has been shown that neoplastically transformed tissues are capable of synthesizing RNase that differs qualitatively and quantitatively from that existing in their normal counterpart tissues (7). Recently, an oncofetal antigen complex present only in fetal pancreas and in adult pancreatic carcinoma has been reported (8). However, its nature and properties are not known.

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