

Sensitivity and Specificity of Canine Serum Total Amylase and Isoamylase Activity Determinations

Robert M. Jacobs, Cheryl L. Swenson, Deborah J. Davenport and Robert J. Murtaugh*

ABSTRACT

Serum isoamylases were determined prospectively in dogs with pancreatic and extrapancreatic diseases. Mean serum isoamylase determinations were significantly different ($p < 0.05$) between normal dogs and dogs with pancreatitis and exocrine pancreatic insufficiency. The sensitivity of serum isoamylase determination exceeded that of total amylase activity for the diagnosis of pancreatitis. Serum isoamylase determinations were less influenced by extrapancreatic diseases compared to total amylase activity when used in the diagnosis of pancreatic disease. Neither serum isoamylase determination nor total amylase activity had adequate sensitivity to support their use in the diagnosis of exocrine pancreatic insufficiency. There were significant ($p < 0.05$) linear correlations between isoamylase determinations, total amylase activity, and trypsin-like immunoreactivity concentration.

RÉSUMÉ

Cette expérience visait à déterminer, de façon prospective, les isoamylases sériques, chez des chiens atteints de maladies pancréatiques ou extrapancreatiques. La détermination de la moyenne des isoamylases sériques, chez des chiens normaux et chez d'autres qui souffraient de pancréatite ou d'insuffisance du pancréas exocrine, afficha une différence significative ($p < 0,05$). La sensibilité de la détermination des isoamylases sériques dépassa celle de l'activité de l'amylase totale, pour le

diagnostic de la pancréatite. La détermination des isoamylases sériques subit moins d'influence par les maladies extrapancreatiques que celle de l'activité de l'amylase totale, lorsqu'on les utilise pour le diagnostic des maladies du pancréas. La détermination des isoamylases sériques et celle de l'activité de l'amylase totale n'affichèrent pas une sensibilité adéquate pour qu'on puisse les utiliser dans le diagnostic de l'insuffisance du pancréas exocrine. On enregistra des corrélations linéaires significatives ($p < 0,05$) entre la détermination des isoamylases sériques, celle de l'activité de l'amylase totale et la concentration de l'immunoréactivité semblable à celle de la trypsine.

INTRODUCTION

Most canine sera contain at least three isoamylases, termed isoamylase-2, -3 and -4, migrating from the most anodal to the most cathodal respectively. Isoamylase-3 and -4 account for approximately 13.5% and 71.4% of the total serum amylase activity, respectively (1,2). Isoamylase-1, the fastest migrating isoamylase, is present inconsistently and can account for up to 2.0% of the total amylase activity in canine serum (1). Experimental evidence supports the pancreatic origin of canine isoamylase-3 while serum isoamylase-4 is produced apparently in both the pancreas and small intestine. In an experimental model of canine pancreatitis, the ratio of serum isoamylase 3/4 appeared to be a useful calculation for describing the changes in serum isoamylases (2).

Serum isoamylase analysis is of some importance in the accurate diagnosis of pancreatitis and exocrine pancreatic insufficiency (EPI) in people (3-5). In a preliminary study of pancreatic and extrapancreatic disease in dogs it appeared that serum isoamylase analysis might be helpful in the diagnosis of pancreatic diseases (1). In this prospective study we compared the sensitivity and specificity of serum total amylase activity and isoamylase analysis in the diagnosis of canine pancreatic disease. Additionally, we examined the correlation of serum isoamylase activity and trypsin-like immunoreactivity (TLI) concentration since the serum TLI concentration has proven usefulness in the diagnosis of EPI (6).

MATERIALS AND METHODS

Dogs greater than one year of age were assigned prospectively to each of the following categories: normal ($n = 75$), enteric disease ($n = 14$), hepatic disease ($n = 35$), renal disease ($n = 16$), EPI ($n = 10$), and pancreatitis ($n = 11$). Dogs were considered normal if they were admitted for elective surgery or well-health examinations, if they were normal upon physical examination, and had normal biochemical and hematological profiles. Dogs with enteric disease, hepatobiliary disease, and renal disease had their diagnoses and the absence of pancreatic disease confirmed by surgical biopsy or necropsy. Enteric diseases included foreign body ($n = 3$), bacterial overgrowth induced experimentally (7) by an established procedure (8) ($n = 5$),

*Department of Pathology, University of Guelph, Guelph, Ontario N1G 2W1 (Jacobs), Department of Pathobiology, The Ohio State University, 1925 Coffey Road, Columbus, Ohio 43210 (Swenson), Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute, Blacksburg, Virginia 24061 (Davenport) and School of Veterinary Medicine, Tufts University, 200 Westboro Road, North Grafton, Massachusetts 01536 (Murtaugh).

This work was supported in part by a grant from the Canadian Veterinary Research Trust Fund.

Submitted November 2, 1987.

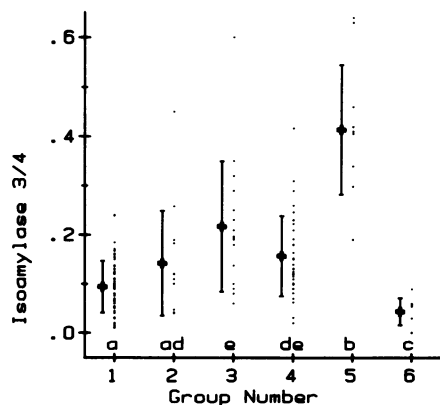


Fig. 1. Distribution of serum isoamylase 3/4 ratios among six groups of dogs. Bars represent mean $\pm$ standard deviation. Solid circles represent individual dogs. Some data points overlap. Group numbers represent dogs without disease or with disease or organ dysfunction as follows: 1 = normal ($n = 75$), 2 = enteritis ($n = 14$), 3 = renal disease ($n = 16$), 4 = hepatic disease ($n = 35$), 5 = pancreatitis ($n = 11$), and 6 = exocrine pancreatic insufficiency ($n = 10$). Groups with different letters are significantly different ($p < 0.05$).

granulomatous enteritis ($n = 2$), bacterial enteritis ($n = 1$), and parvoviral enteritis ($n = 3$). Hepatobiliary diseases included bile duct rupture ($n = 2$), chronic active hepatitis ($n = 8$), acute necrotizing hepatitis ($n = 5$), chronic fibrosing hepatitis ($n = 2$), vascular anomalies associated with shunts ($n = 6$), cirrhosis ($n = 4$), and steroid hepatopathy ($n = 8$). Renal diseases were glomerulonephritis ($n = 3$), chronic interstitial nephritis ($n = 5$), renal calculi ($n = 2$), amyloidosis ($n = 1$), acute renal failure ($n = 2$), and cancer of the urogenital tract ($n = 3$). Of the ten dogs with EPI, six had juvenile pancreatic atrophy and had been maintained on replacement therapy. These six dogs had life-long signs of EPI. The four remaining dogs had acquired EPI diagnosed by unequivocal abnormal results in tests of digestive function and required replacement enzyme therapy to alleviate the signs of enzyme insufficiency. The diagnosis of pancreatitis in 11 dogs was confirmed at necropsy; gross lesions were not present in any organs except the pancreas. The pancreatic lesions included severe edematous pancreatitis ($n = 3$), and necrotizing and/or hemorrhagic pancreatitis ($n = 8$).

Blood samples were collected at initial presentation and allowed to clot

at room temperature. The serum was harvested and stored frozen at -20°C . Total serum amylase activity was determined by nephelometry (Perkin Elmer Inc, Coleman Instrument Division, Oak Brook, Illinois). Serum isoamylase activities were determined by an established procedure (9) as used previously in dogs (2). Zymograms were scanned in a densitometer at 600 nm and the ratios of isoamylase-3/4 calculated. Interassay variability for the isoamylase 3/4 ratios was determined by measurements on a pooled normal canine serum repeated 30 times. The data were not normalized to give equivalent serum concentrations of urea nitrogen or creatinine between subjects since prerenal and renal azotemia do not have a consistent effect on serum amylase activity (10).

The isoamylase 3/4 ratio was compared with the TLI concentration in canine sera obtained from ten dogs. The serum TLI concentration was measured by radioimmunoassay (6).

The isoamylase 3/4 ratio data were transformed to Gaussian form using the arc sine (χ) transformation, then analyzed for significant differences by the one way analysis of variance followed by Duncan's test. Differences between groups with $p < 0.05$ were considered significant. Sensitivity and specificity were calculated by established procedures (11). Significance of the regression analyses were assessed by analysis of variance.

RESULTS

The distribution of isoamylase 3/4 ratios among the six groups of dogs are shown in Fig. 1. Based on the determination of isoamylase 3/4 ratios the two groups of dogs with pancreatic disease were distinguishable from one

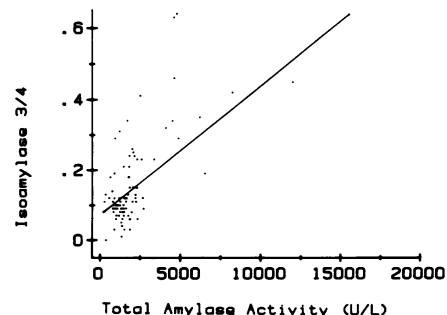


Fig. 2. Correlation of serum isoamylase 3/4 ratio and total amylase activity ($n = 91$). The solid circles represent individual dogs. Some data points overlap. The solid line indicates the regression line ($p < 0.05$, $r = 0.65$).

another, from normal dogs, and from dogs with extrapancreatic disease ($p < 0.05$). The isoamylase 3/4 ratio reference range (± 2 S.D.) for the 75 normal dogs was 0.012 to 0.220 for the normalized data. The coefficient of variation for isoamylase 3/4 ratios in a pooled normal canine serum repeated 30 times was 12%.

There was a significant linear correlation between total serum amylase activity and isoamylase 3/4 ratio ($p < 0.05$) (Fig. 2). The coefficient of determination was 0.42. Of the ten dogs in which the serum TLI concentration was measured nine had signs suggesting EPI and serum TLI concentrations below $2 \mu\text{g/L}$. One dog had surgery for removal of a metastatic insulinoma and had marked hyperamylasemia, normolipemia and a serum TLI concentration of $16.6 \mu\text{g/L}$. There was a significant linear correlation of isoamylase 3/4 ratio and serum TLI concentration for those data points with serum TLI concentrations less than $2 \mu\text{g/L}$ ($p < 0.05$) (Fig. 3). For these data the coefficient of determination was 0.46.

TABLE I. The Sensitivity and Specificity of Determinations of Total Serum Amylase Activity and the Isoamylase 3/4 Ratio for the Detection of Pancreatic Disease, Pancreatitis and Exocrine Pancreatic Insufficiency in Dogs

Determination	Disease	Sensitivity	Specificity
Total Serum Amylase Activity	Pancreatic Disease	0.53	0.58
	Pancreatitis	0.78	0.77
	Exocrine Pancreatic Insufficiency	0.17	0.76
Isoamylase 3/4 Ratio	Pancreatic Disease	0.57	0.77
	Pancreatitis	0.91	0.77
	Exocrine Pancreatic Insufficiency	0.20	0.67

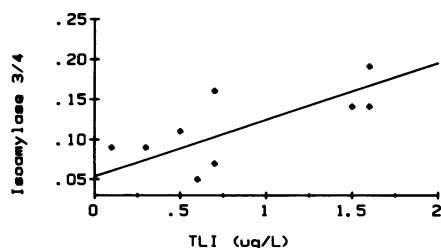


Fig. 3. Correlation of serum isoamylase 3/4 ratio and trypsin-like immunoreactivity (TLI) concentration ($n = 9$). The solid line indicates the regression line ($p < 0.05$, $r = 0.68$). One additional data point had a serum isoamylase 3/4 ratio of 0.21 and a TLI concentration of $16.6 \mu\text{g/L}$.

The sensitivity and specificity of the measurements of total serum amylase activity and isoamylase 3/4 ratio for the detection of pancreatic disease, pancreatitis, and EPI are shown in Table I.

DISCUSSION

The measurement of serum isoamylase 3/4 ratio appeared to be more useful overall than total serum amylase activity in the detection of pancreatic disease. This difference was most evident in the greater sensitivity of the isoamylase 3/4 ratio for detection of pancreatitis. The measurement of isoamylase 3/4 ratio appeared to be less influenced by extra pancreatic disease compared with total serum amylase activity. Support for this lies in the higher specificity associated with the isoamylase 3/4 ratio for pancreatic disease.

Although isoamylase 3/4 ratios in dogs with EPI were decreased significantly ($p < 0.05$) compared with the ratio in normal dogs, there was overlap. Isoamylase 3/4 ratios and total serum amylase activity below the reference ranges also were seen in sera of some dogs with extrapancreatic disease. Some of the lack of sensitivity of these tests is due probably to their

relatively wide reference ranges. This problem is well recognized with respect to total serum amylase activity and appears to be due to biological variation. In the case of isoamylase 3/4, the ratio in normal dogs is low and the electrophoretic technique may not yield adequate resolution at the low end. Since the sensitivity of both tests for EPI was quite low in their present form, they are of limited usefulness in detection of the insufficient state.

There was a significant ($p < 0.05$) positive correlation between the total serum amylase activity and isoamylase 3/4 ratio. This result was not unexpected, since in experimental studies of canine pancreatitis total serum amylase and isoamylase activities closely paralleled one another (2). However, in the present study the coefficient of determination for the regression was only 0.42. In other words, only 42% of the variation of isoamylase 3/4 ratio was attributable to the same factors resulting in changes in the total serum amylase activity. This was most likely due to the inclusion, in the present study, of dogs with a multitude of extrapancreatic diseases known to potentially influence total serum amylase activity. From these data it is clear that the isoamylase 3/4 ratio is influenced by extrapancreatic disease, but not to the same degree or frequency as total serum amylase activity.

The significant linear correlation of serum TLI concentration and isoamylase 3/4 ratio indicates the two tests are changed similarly as a result of a relative lack of pancreatic parenchyma. In contrast, a dog with a markedly increased serum TLI concentration and an isoamylase 3/4 ratio at the upper end of the reference range suggests that serum TLI and isoamylases may not be released into the blood in a parallel manner during an active destructive process. Dogs with reduced pancreatic reserve will be

examined in future studies to elucidate the relationship of functional pancreatic mass to analytes measured in the peripheral blood.

ACKNOWLEDGMENTS

The authors thanks Dr. D.A. Williams, University of Florida (Gainesville) for his willingness to cooperate in this project.

REFERENCES

1. JACOBS RM, HALL RI, ROGERS WA. Isoamylases in clinically normal and diseased dogs. *J Vet Clin Pathol* 1982; 11: 26-32.
2. MURTAUGH RJ, JACOBS RM. Serum amylase and isoamylase and their origins in healthy dogs and dogs with experimentally induced acute pancreatitis. *Am J Vet Res* 1985; 46: 742-747.
3. LEVITT MD. Clinical use of amylase clearance and isoamylase measurements. *Mayo Clin Proc* 1979; 54: 428-431.
4. JENSEN DM, ROYSE VL, BONELLO JN, SCHAFFNER J. Use of amylase isoenzymes in laboratory evaluation of hyperamylasemia. *Dig Dis Sci* 1987; 32: 561-567.
5. NASRALLAH SM, MARTIN DM. Serum isoamylase as a test for pancreatic insufficiency. *Gut* 1983; 24: 161-164.
6. WILLIAMS DA, BATT RM. Diagnosis of canine exocrine pancreatic insufficiency by the assay of serum trypsin-like immunoreactivity. *J Small Anim Pract* 1984; 24: 583-588.
7. DAVENPORT DJ. Clinicopathologic parameters of a surgical model of small intestinal bacterial overgrowth (SIBOG) and evaluation of the usefulness of a PABA — Cholic conjugate in the detection of SIBOG in the dog. M.Sc. Thesis. The Ohio State University, Columbus, 1986.
8. BISHOP RF. Bacterial flora of the small intestine of dogs and rats with intestinal blind loops. *J Pathol Bacteriol* 1962; 87: 189-196.
9. LECLERC P, FOREST J. Electrophoretic determination of isoamylases in serum with commercially available reagents. *Clin Chem* 1982; 28: 37-40.
10. WAGNER AE, MACY DW. Nephelometric determination of serum amylase and lipase in naturally occurring azotemia in the dog. *Am J Vet Res* 1982; 43: 697-699.
11. MARTIN SW. The evaluation of tests. *Can J Comp Med* 1977; 41: 19-25.