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Comparative evaluation of biochemical and imaging modalities in diagnosis of acute pancreatitis in dogs[#]

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Abstract

Diagnosis of exocrine pancreatic diseases in dogs is challenging. The anatomical location of pancreas, wide variations in clinical presentation, non specific clinico-pathologic parameters and lack of a single accurate diagnostic test together contributes to the difficulty in diagnosis of acute pancreatitis. In the present research, the effectiveness of haematology, estimation of serum biochemical parameters, species specific SNAP cPL and imaging techniques in the diagnosis of pancreatitis was compared. Serum lipase and serum amylase were significantly elevated in the cases screened for acute pancreatitis. SNAP cPL test was found positive in 27.58 per cent of the suspected cases of pancreatitis. No significant changes could be observed in the pancreas on abdominal radiography and ultrasonography. Estimation of serum lipase and amylase and SNAP cPL test aid in more accurate diagnosis of acute pancreatitis when compared with other diagnostic modalities.

Keywords: Acute pancreatitis, serum amylase, serum lipase, SNAP cPL test

Acute pancreatitis is the inflammation of exocrine pancreas characterised by oedema and necrosis and not associated with permanent histologic pancreatic variations (Newman *et al.*, 2006). It is the most common exocrine pancreatic disease in companion animals (Xenoulis, 2015). Universally, a gold standard test is still lacking in the diagnosis of acute pancreatitis and is at present performed using a battery of diagnostic modalities which includes history, clinical signs, physical examination findings, laboratory diagnostic tests including estimation of serum amylase, serum lipase, species specific pancreatic lipase immunoreactivity and various imaging study findings (Cridge *et al.*, 2021). Semi-quantitative colourimetric reactions are currently available as in-clinic tests for the diagnosis of canine acute pancreatic lipase with high sensitivity (91 to 94 percent) and specificity (71 to 78 percent) (McCord *et al.*, 2012). The primary ultrasonographic features of pancreatitis include hypoechoic pancreas with hyperechogenic surrounding mesentery, enlarged or irregular pancreas, dilated pancreatic or biliary ducts and abdominal effusion (Hecht and Henry, 2007). Nonspecific clinical signs

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and absence of a confirmatory diagnostic test makes the diagnosis of pancreatitis always challenging. Hence the present study was aimed to compare the diagnostic accuracy of various serum biochemical parameters and abdominal imaging techniques in the diagnosis of acute pancreatitis in dogs.

Materials and methods

The study was conducted in dogs presented to University Veterinary Hospitals at Mannuthy and Kokkalai of Kerala Veterinary and Animal Sciences University from August, 2023 to May, 2024. Cases brought with the history of anorexia, vomiting, lethargy, diarrhoea, melena, haematochezia and abdominal pain were selected for detailed study. Peripheral blood smear examination, haematology, serum biochemistry, SNAP cPL, faecal sample examination and imaging techniques were performed in all the selected cases. Cases positive only on SNAP cPL test was categorised as primary pancreatitis and cases with positive SNAP cPL test along with changes in other diagnostic parameters studied was categorized as secondary acute pancreatitis.

Serum biochemistry

Serum lipase (U/L) (modified methylresorufin method), amylase (U/L) (modified CNPG2 method), creatinine (mg/dL), glucose (mg/dL), alanine aminotransferase (ALT) (IU/L) and alkaline phosphatase (ALP) (IU/L) was estimated using semiautomatic biochemical analyser, Alpha Chem Touch of Alpha Technologies. Canine trypsin like immunoreactivity (cTLI) was estimated by canine trypsin like immunoreactive protein ELISA kit (Origin Diagnostics and Research, Kerala).

SNAP cPL test

Serum pancreas specific lipase was estimated in hyperlipasaemic cases using SNAP cPL test (IDEXX Laboratories, Inc). Three drops of the serum sample were added to a new sample tube, four drops of conjugate were added and the tube was capped and mixed thoroughly by inverting 3-5 times. The entire content was transferred to the SNAP cPL device's sample well. After waiting 30-60 seconds for the sample to reach the activation circle of the device the activator was pressed firmly and results were read within 10 minutes of activation. The intensity of colour developed at sample spot was compared with the reference spot in the device. The result was interpreted as normal canine pancreatic lipase (cPL) level, if the colour intensity of the sample spot was lighter than the reference spot. If the colour intensity of the sample spot was equal to or darker than that of the reference spot, the cPL level was considered as abnormal.

Abdominal radiography

Abdominal radiographic examination was

performed in all the selected cases. A right lateral view radiograph of the abdomen was obtained with the dogs in right lateral recumbency with the fore limbs and hind limbs parallel to each other and stretched cranially and caudally respectively.

Abdominal ultrasonography

Ultrasonography of the pancreas was performed using Esoate MyLabX8eXP and MyLabX7VET ultrasound scanners with 4-15 MHz linear and 1-5 MHz micro convex transducers as per the standard imaging protocol described by Mattoon and Nyland (2015). Abdominal ultrasonographic examination was performed with the animal in left lateral, right lateral and dorsal recumbency. Right lateral recumbency was used for examination of left lobe of pancreas and left lateral recumbency for the examination of the right lobe of the pancreas. Dorsal recumbency was adopted in deep chested breeds in which the pancreas was difficult to be located. The right lobe of the pancreas was identified by locating the duodenum as the target on a transverse image adjacent to right abdominal wall and right kidney. The triangular right lobe of the pancreas was visualised adjacent to the medial and ventral aspects of the duodenum. Branches of the pancreaticoduodenal vein and artery could be seen as one or two circular anechoic structures in the right lobe of the pancreas on sagittal view and was confirmed by Doppler examination. The pancreatic body was located dorsocaudal to the pylorus in close proximity to the portal vein. The left lobe was located in the acoustic window formed by stomach cranially, spleen dorsally and transverse colon caudally. The statistical analysis of data obtained was carried out using SPSS version 24.0.

Results and discussion

A total of 29 dogs were presented to the small animal medicine unit during the study period with the clinical signs suggestive of acute pancreatitis. Serum lipase and amylase levels were found to be elevated in 13 cases. Four azotaemic animals were excluded from the study as both serum lipase and amylase could be elevated in cases of renal insufficiency. Nine cases were found positive for SNAP cPL test. One case was excluded from the study as the owner was not willing for remaining diagnostic evaluation and eight cases (27.58 per cent) were studied in detail. Though, pancreatitis is the most common disease of exocrine pancreas in dogs (Xenoulis, 2015), the low occurrence of acute pancreatitis in the study might be due to the lack of a single specific test and vague clinical presentation (Berman et al., 2020 and Cridge et al., 2021). Acute pancreatitis is considered to be a monopathogenic disease in dogs (Lazarov, 2020).

All the cases diagnosed as acute pancreatitis in the present study was found in association with the multi-organ dysfunction associated with the various etiologies diagnosed and was concluded as secondary acute pancreatitis. This was in correspondence with the findings of Abhilaasha et al. (2020) who observed that the prevalence of secondary acute pancreatitis (64.3 per cent) was more when compared to primary acute pancreatitis (35.7 per cent). Acute pancreatitis observed in the present study was secondary to Babesia gibsoni infection (n=5), Ehrlichia canis infection (n=1), leptospirosis (n=1) and cholecystitis (n=1). Elevated serum amylase and lipase levels were observed in babesiosis and ehrlichiosis (Cridge et al., 2021). Similar observations were made by Karasova et al. (2022) who reported Systemic inflammatory response syndrome (SIRS) leading to pancreatitis in dogs with babesiosis. Multisystemic pathogenic nature of the leptospiral organism would result in elevated lipase level in pancreatitis (Griebsch et al., 2022).

Clinical signs observed in all the eight cases were lethargy, anorexia, vomiting, pale mucous membrane. Abdominal pain was noticed in two animals and they exhibited praying posture, humped back and restricted activity. Statistically significant difference in appetite and lethargy exhibited by diseased dogs was observed in comparison with healthy control on Mann-Whitney U test.

On statistical analysis (Table 1), significant increase in serum lipase level (672.80±112.82 U/L) was observed at p≤ 0.01 and serum amylase (2291.39±427.05 U/L) at p \leq 0.05 level in the suspected cases in comparison with the healthy control (Table 1). Serum amylase and lipase concentrations could be significantly elevated in acute pancreatitis as reported by Mansfield (2013). Nonpancreatic diseases like renal diseases, diabetes mellitus, lymphosarcoma and haemangiosarcoma also resulted in serum amylase levels to be higher than the normal reference level (Hoffmann and Solter, 2008). Diseases like renal failure, enteritis and gastroenteritis, hepatic disorders, bile duct carcinoma and lymphosarcoma of the gastrointestinal tract were reported to be associated with elevated lipase level (Hoffmann and Solter, 2008 and Mansfield, 2013). Estimation of serum amylase and lipase could be used as a diagnostic tool of pancreatitis provided with the exclusion of other conditions that elevates the enzyme concentrations. Statistical evaluation of serum canine trypsin like immunoreactivity (cTLI) level had no significant difference from healthy control. The level of cTLI was usually found to be elevated during early phase of pancreatic inflammation and rapidly declines to normal (Mansfield, 2013).

Eight animals (27.58 per cent) were found positive for pancreatitis on SNAP cPL test (IDEXX Laboratories, Inc). The intensity of colour developed at the sample spot was darker than the reference spot in SNAP cPL test (IDEXX Laboratories, Inc) (Fig. 1). This was in concurrence with Xenoulis and Steiner (2016) who observed that SNAP cPL (IDEXX Laboratories, Inc) was an in-clinic rapid test with better accuracy in the diagnosis of acute pancreatitis of dogs since the test was pancreas and species specific. False positive SNAP cPL test (IDEXX Laboratories, Inc) could be obtained in conditions including gastrointestinal foreign body, septic peritonitis and hepatic diseases (Haworth et al., 2014). Obtaining a negative SNAP cPL (IDEXX Laboratories, Inc) result has good correlation of having diseases other than pancreatitis (McCord et al., 2012).



Fig. 1. SNAP cPL test showing intense colour at sample spot (arrow)

Haematologic evaluation of cases diagnosed with acute pancreatitis revealed significant decrease in haemoglobin (9.3±1.37 g/dL) and haematocrit (25.03±3.96 %) levels in comparison with the healthy control. Anaemia observed was due to the haemolysis caused by the primary pathogens *viz B. gibsoni, Ehrlichia canis* and leptospira. Similar findings were reported by Thrall *et al.* (2012).

Serum biochemical parameters including glucose, creatinine, alanine aminotransferase (ALT) and alkaline phosphatase (ALP) depicted no significant difference from the healthy control on statistical analysis (Table 2). The findings suggested that pancreatitis does not cause changes in these parameters.

 Table 1. Serum amylase, serum lipase and cTLI values of dogs with acute pancreatitis compared with healthy control (Mean±SE)

Parameters	Diseased dogs (n=8)	Healthy animals (n=6)	t- value	p- value
Serum amylase (U/L)	2291.39±427.05	1155.38±128.12	2.23 [*]	0.05
Serum lipase (U/L)	672.80±112.82	51.59±7.61	5.49**	0.00
cTLI (μg/L)	44.24±6.69	38.96±4.36	0.66	0.52

**significant at 0.01 level, * significant at 0.05 level

Parameters	Diseased dogs (n=8)	Healthy animals (n=6)	t- value	p- value
Glucose (mg/dL)	102.19±15.42	82.94±4.34	1.20	0.27
ALT (IU/L)	51.04±17.82	20.14±1.56	1.73	0.13
ALP (IU/L)	517.34±260.03	48.95±17.05	1.54	0.15
Creatinine (mg/dL)	2.26±1.19	1.13±0.04	0.82	0.43

Table 2. Serum biochemistry values of dogs with acute pancreatitis compared with healthy control (Mean±SE)

**significant at 0.01 level, * significant at 0.05 level

No specific pancreatic lesions were observed in the abdominal radiograph of the diseased dogs in the present study. Increased soft tissue opacity and decreased serosal details in the cranial abdomen, displacement of the stomach and duodenum, gaseous dilatation of loops adjacent to the pancreas and ascites are the radiographic features expected in acute pancreatitis. A normal pancreas is difficult to be imaged on radiography due to silhouetting with the adjacent abdominal structures and the deepseated anatomical location of the pancreas (Thrall, 2018). Radiography remains the initial imaging tool of choice for acute pancreatitis to rule out other diseases (Xenoulis, 2015).

Abdominal ultrasonography visualised a normal iso-to-hypoechoic pancreas in all the eight cases of acute pancreatitis in the present research (Fig. 2 and 3). Abdominal ultrasonography is a commonly used imaging tool for evaluation of canine pancreas (Penninck and



Fig.2. Transverse image of normal iso-to-hypoechoic right pancreatic lobe (yellow circle) with pancreaticoduodenal duct (arrow) and adjacent duodenum (a) using linear transducer (4-15MHz) d'Anjou, 2015) which helps to rule out other conditions that may present with similar clinical signs. Puccini Leoni *et al.* (2020) observed that significant ultrasonographic findings of acute pancreatitis might not be observable on the first day of presentation and should be reassessed every third or fourth day since ultrasonographic changes might be visible after a few days. Slight changes in pancreatic echo texture are also difficult to be detected ultrasonographically (Xenoulis, 2015). Absence of ultrasound findings in the present study might also be due to the secondary origin of pancreatitis in all the eight cases. (Hecht and Henry, 2007).



Fig. 3. Sagittal view of normal iso-to-hypoechoic right pancreatic lobe (yellow circle) with pancreaticoduodenal duct (arrow), pancreaticoduodenal vein on colour Doppler examination and adjacent duodenum (a) using linear transducer (4-15MHz).

Comparative study of the diagnostic tests is depicted in table 3.

Table 3. Com	parison of	different	diagnostic	modalities	in acute	pancreatitis

SI. No.	Diagnostic tests	Percentage of cases in which significant changes identified (per cent)
1.	Serum amylase and lipase	44.83
2.	SNAP cPL	31.03
3.	cTLI	6.90
4.	Abdominal radiography	0
5.	Abdominal ultrasonography	0

Conclusion

Effectiveness of various tests in the diagnosis of acute pancreatitis was assessed in the present research. No single test could confirm acute pancreatitis but elevation of serum lipase and amylase and positive SNAP cPL test (IDEXX Laboratories, Inc) was found to be superior to other diagnostic modalities for screening and diagnosis of pancreatitis. Interpretation of the results of different diagnostic modalities is critical for an accurate diagnosis.

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Conflict of interest

The authors declare that they have no conflict of interest.

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