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RESEARCH ARTICLE

Exploring the Diagnostic Potential of miR-216a and miR-375 for Detecting Acute Pancreatitis in Canine Model

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ABSTRACT

Acute pancreatitis (AP) is a severe inflammatory condition of the pancreas that can result in organ dysfunction, severe illness, and potentially death. However, the absence of a standardized screening test poses challenges in detecting acute pancreatitis. This study aimed to assess the efficacy of two microRNAs, miR-216a and miR-375, as potential biomarkers for acute pancreatic injury. The study also compared their effectiveness with traditional biomarkers, including hemato-biochemical markers, pancreatic ultrasound examination, and histopathological examination, in a canine pancreatitis model six hours after caerulein infusion. Fourteen healthy mongrel dogs were randomly assigned to two groups: the control group (n=7), which received intravenous saline, and the acute pancreatitis group (n=7), which received intravenous caerulein. Dogs in the caerulein group exhibited clinical symptoms such as anorexia, vomiting, diarrhea, and lethargy. Caerulein infusion significantly elevated erythrogram parameters and serum amylase, lipase, ALT, AST, and ALP activities. Molecular analysis revealed a significant up-regulation of miR-216a and miR-375 in the caerulein-treated group compared to the control group. Ultrasonographic examination of the caerulein-treated group demonstrated an enlarged hypoechoic pancreatic structure and a thickened corrugated duodenal wall. Histopathological investigation confirmed the presence of acute exocrine pancreatitis in the caerulein-treated group. The findings of this study demonstrate the potential of miR-216a and miR-375 as effective biomarkers for detecting acute pancreatitis in mongrel dogs. These microRNAs could offer valuable insights into the early diagnosis of acute pancreatitis, complementing the existing diagnostic methods.

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INTRODUCTION

Acute pancreatitis (AP) is a pancreatic inflammatory condition that poses a significant global burden in terms of clinical dysfunction syndromes, morbidity, and mortality (Lee *et al.,* 2023). Severe acute pancreatitis, characterized by extensive pancreatic necrosis and multiple organ failure, contributes to a considerable fatality rate of around 30% among AP patients (Khan *et al.,* 2023). Early and accurate diagnosis of AP plays a critical role in guiding appropriate treatment strategies and improving patient outcomes.

In case of dogs, pancreatitis often manifests with nonspecific clinical symptoms such as vomiting, diarrhea, and dehydration (Lee *et al.,* 2023). The release of digestive enzymes due to exocrine pancreatic damage not only affects the pancreas but also impacts other organ systems (Walkowska *et al.,* 2022). Although histopathology serves as the gold standard for pancreatitis diagnosis, the invasiveness and associated risks of

surgical pancreatic biopsy make it less suitable for routine use (Tirkes *et al.,* 2022). Currently, the diagnosis of pancreatitis in dogs relies on abdominal ultrasonography, alongside general clinical pathology and measurement of serum amylase activity, total serum lipase activity, trypsin-like immunoreactivity, and pancreatic lipase immunoreactivity (Walkowska *et al.,* 2022). However, these diagnostic methods often lack the desired accuracy in diagnosing canine pancreatitis. Consequently, there is a pressing need to explore and develop novel diagnostic techniques that can enhance the accuracy and reliability of pancreatitis diagnosis in dogs.

MicroRNAs (miRNAs) are a class of noncoding RNAs that play a crucial role in gene expression regulation by binding to mRNA molecules and interfering with their translation (Shang *et al.,* 2023). These single-stranded nucleotides are involved in various physiological and pathological processes, including cell growth, development, and apoptosis (Azizi Dargahlou *et al.,* 2023). Upon pancreatic injury, the levels of miR-216a, miR-216b, miR-217 (enriched in the exocrine pancreas), and miR-375 (enriched in the endocrine pancreas) are known to increase (Lee *et al.,* 2023). The intricate regulatory systems involving these miRNAs make them essential contributors to the molecular mechanisms underlying acute pancreatitis (Azizi Dargahlou *et al.,* 2023). Previous studies have shown that miR-216a exhibits high selectivity in pancreatic tissue, with significantly higher concentrations compared to other tissues (Shang *et al.,* 2023). This observation has been consistently replicated in various animal models, including rats (Bregalda *et al.,* 2023), dogs (Lee *et al.,* 2023), and humans (Patel *et al.,* 2023). Similarly, miR-375, a microRNA known for its crucial role in the function of islet beta cells, has also been implicated in the diagnosis of acute pancreatitis (Patel *et al.,* 2023). It is highly expressed in islet beta cells but comparatively lower in pancreatic acinar cells (Lee *et al.,* 2023).

Currently, there is a lack of consensus on the most effective noninvasive diagnostic approaches for canine pancreatitis. Therefore, the objective of this study was to evaluate and compare the potential of two specific microRNAs, miR-216a and miR-375, as biomarkers for detecting acute pancreatic injury. The effectiveness of these microRNAs as biomarkers was assessed in comparison to traditional biomarkers, including hemato-biochemical markers, pancreatic ultrasound examination, and histopathological examination, in a canine pancreatitis model.

MATERIALS AND METHODS

Ethical statements: The animal experimentation protocol conducted in this study was approved by the Institutional Animal Ethics Committee of the Faculty of Veterinary Medicine, Kafrelsheikh University, under Approval No. KFS-IACUC/5/8/2021.

Animals: In this study, fourteen adult Mongrel dogs (8 females and 6 males), aged 1–2 years and weighing 10–20 kg, were recruited. The dogs had been clinically examined and acclimatized for at least one week before the commencement of the study. All dogs exhibited normal physical examination findings, including body temperature, body weight, and demeanor, indicating their overall health. The dogs were individually housed in separate cages and provided with dry food following the feeding guidelines set by the National Research Council. They had unrestricted access to water throughout the study duration.

Experimental design: The study comprised fourteen dogs divided into two equal groups: the control group (N=7) and the treated group (N=7). The control group received a 2-hour intravenous infusion of normal saline at a rate of 3 mL/kg/h. In the treated group, acute pancreatitis was induced by continuous intravenous infusion of cerulein (in saline) at a rate of 7.5 µg/kg/h into the cephalic vein and administered at a rate of 3 mL/kg/h for 2 hours (Lim *et al.,* 2014). The dogs were closely monitored for the manifestation of clinical signs associated with acute pancreatitis, such as vomiting and diarrhea. Both groups underwent identical diagnostic tests for acute pancreatitis, and their outcomes were compared to assess significant differences in accuracy.

Blood sampling, hematological and serum biochemical analysis: Two blood samples were collected from the right cephalic vein of each animal, 6 hours after the administration of normal saline and caerulein. The first set of blood samples was collected in EDTA-containing tubes for hematological analysis. This analysis included measurements of red blood cell count (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), white blood cell count (WBCs), and a differential leukocyte count encompassing neutrophils, lymphocytes, monocytes, and eosinophils (Feldman *et al.,* 2000). The second set of blood samples was collected in simple Eppendorf® tubes without anticoagulant. These samples were then centrifuged at 3000 rpm for 15 minutes at 4°C. These serum samples were utilized for assessing both biochemical parameters and microRNA quantification. Serum biochemical parameters, including amylase, lipase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total proteins, albumin, glucose, urea, and creatinine, were measured using the Cobas C & Cobas E® system (France). Insulin level was assessed using the Siemens-immulite-2000/xpi® analyzer (Germany) with semi-automation (BioSystems BTS-302®, Barcelona, Spain).

Micro RNA quantification: The relative quantification of pancreatic miRNA-216a and miRNA-375 in the serum was performed using specific primers for miRNA-216a (UAAUCUCAGCUGGCAACUGUG (Rouse *et al.,* 2017), and miRNA-375 (UUUGUUCGUUCGGCUCGCGUGA (Rouse *et al.,* 2017), along with a universal reverse primer included with the Quanti-Mir RT kits (SBI, System Biosciences®, California, USA).

Ultrasonographic examination: Ultrasonographic examination was conducted using the Mindray Z5® ultrasound machine (Shenzhen, China) equipped with an 8.5 MHz linear transducer (Avante *et al.,* 2018). Prior to the examination, all dogs were sedated with Xylaject®

(Xylazine, Adwia Pharmaceutical Co) at a dose of 1ml/10kg body weight. Pre- and post-intervention images of the pancreatic lobes were obtained for comparison after the infusion of normal saline or caerulein.

Histopathological examination: To conduct the histopathological examination, the dogs that received caerulein infusion were euthanized and necropsied at the end of the experiment. Laparotomy was performed under general anesthesia with ketamine (10 mg/kg, IV). The samples were collected and immediately fixed in 10% buffered formalin for further examination.

Statistical analysis: The data were presented as mean ± SD for both the control and treated groups. A t-test was used to compare the two groups, and differences with a p-value of less than 0.05 were considered significant. Statistical analysis was performed using GraphPad Prism™ version 9.0 (GraphPad Software®, San Diego, California, USA).

RESULTS

Clinical signs: Prior to the experiment, both groups did not exhibit any abnormal clinical signs. The control dogs did not show any adverse clinical signs throughout the experiment. While the caerulein-infused dogs displayed anorexia, vomiting, diarrhea, and lethargy after receiving the caerulein infusion.

Hematological and Serum biochemical findings: Following the caerulein infusion, the erythrogram revealed a significant increase in RBC count, Hb, and PCV compared to the control group (Fig. 1A). The leukogram showed a significant increase in total leukocyte count, neutrophil count, and monocyte count, along with a significant decrease in lymphocyte count. The eosinophil count showed a non-statistical decline in the caerulein group compared to the control group (Fig. 1B).

Serum biochemical analysis demonstrated a significant increase ($P<0.05$) in enzyme activities such as amylase, lipase, AST, ALT, and ALP in the caerulein group compared to the control group (Fig. 2). Additionally, there was a significant increase ($P<0.05$) in total proteins, albumin, urea, creatinine, and insulin levels (Fig. 3), while the serum glucose level showed a significant decline ($P<0.05$) in the caerulein group compared to the control group (Fig. 3).

Micro RNA expression: The qRTPCR results showed a significant ($P<0.05$) increase in the expression levels of miRNA-216a (Fig. 4A) and miRNA-375 (Fig. 4B) in the serum of dogs infused with caerulein, indicating the presence of acute pancreatitis compared to the control group.

Ultrasonographic examination: In the healthy dogs, the right pancreatic lobe was examined as a reference point between the right kidney and duodenum, while the left pancreatic lobe was examined between the stomach and left kidney or between the splenic vein and portal vein. The control group showed no observable changes in the right and left pancreatic lobes, as depicted in Fig. 5A and 5B. In dogs infused with caerulein, the pancreatic lesions were most prominent 2-4 hours after infusion. These lesions included glandular enlargement, corrugation of the duodenum, and a hyperechoic peripancreatic mesentery, as illustrated in Fig. 5C. Throughout the examination, the presence of peripancreatic fluid collection and an enlarged pancreas above normal size were observed. The pancreatic parenchyma exhibited hypoechoic regions with uneven edges. Additionally, a hyperechoic mesentery with duodenal corrugation, as shown in Fig. 5D, indicated the presence of acute pancreatitis.

Histopathological examination: The pancreatic acinar histology of the control dogs appeared normal, as depicted in Fig. 6A. In the caerulein-infused dogs, clear indications of acute hemorrhagic pancreatitis were observed (Fig. 6B), including interstitial hemorrhage, infiltration of inflammatory cells, fat necrosis, and damage to blood vessels. The caerulein-infused dogs displayed interstitial hemorrhage, along with inflammatory cell infiltrations primarily composed of neutrophils, as well as fat necrosis in the pancreas (Fig. 6C). The parenchyma exhibited coagulative necrosis, with inflammatory cells observed in the superficial layers of the gland (Fig. 6D). The inflammatory response appeared more severe in the interlobular spaces and septal tissue compared to within the lobules. The caerulein-infused dogs also showed hemorrhages, thrombosing vasculitis, and fat necrosis (Fig. 6E).

DISCUSSION

Acute pancreatitis is a common inflammatory disease of the pancreas in dogs (Lee *et al.,* 2023). The diagnosis of acute pancreatitis can be challenging due to the lack of a definitive diagnostic test. In this study, we investigated the potential of caerulein-induced pancreatitis in dogs as a model for evaluating diagnostic techniques for acute pancreatitis. Dogs treated with caerulein developed clinical signs consistent with acute pancreatitis, including vomiting, weakness, diarrhea, and abdominal discomfort. These clinical signs are similar to those reported in spontaneous cases of acute pancreatitis in dogs (Lee *et al.,* 2023). Hematological analysis revealed significant differences between the treatment and control groups, with higher RBC counts, Hb, and PCV in the treatment group. The leukogram results indicate a potential stress condition, which could be attributed to elevated levels of endogenous or exogenous corticosteroids. Increased corticosteroids can lead to specific changes in the leukogram, such as neutrophilia, lymphopenia, monocytosis, and eosinopenia, as described by Lowe *et al.* (2008). Additionally, in cases of acute pancreatic injury, a significant increase in total leukocyte count may be observed due to the local activity of caerulein, even in the absence of a systemic reaction, as noted by Su *et al.* (2006).

Serum enzyme activity of amylase, lipase, AST, ALT, and ALP was significantly higher in caerulein-infused dogs compared to control dogs. Amylase and lipase are enzymes produced by the pancreas that are

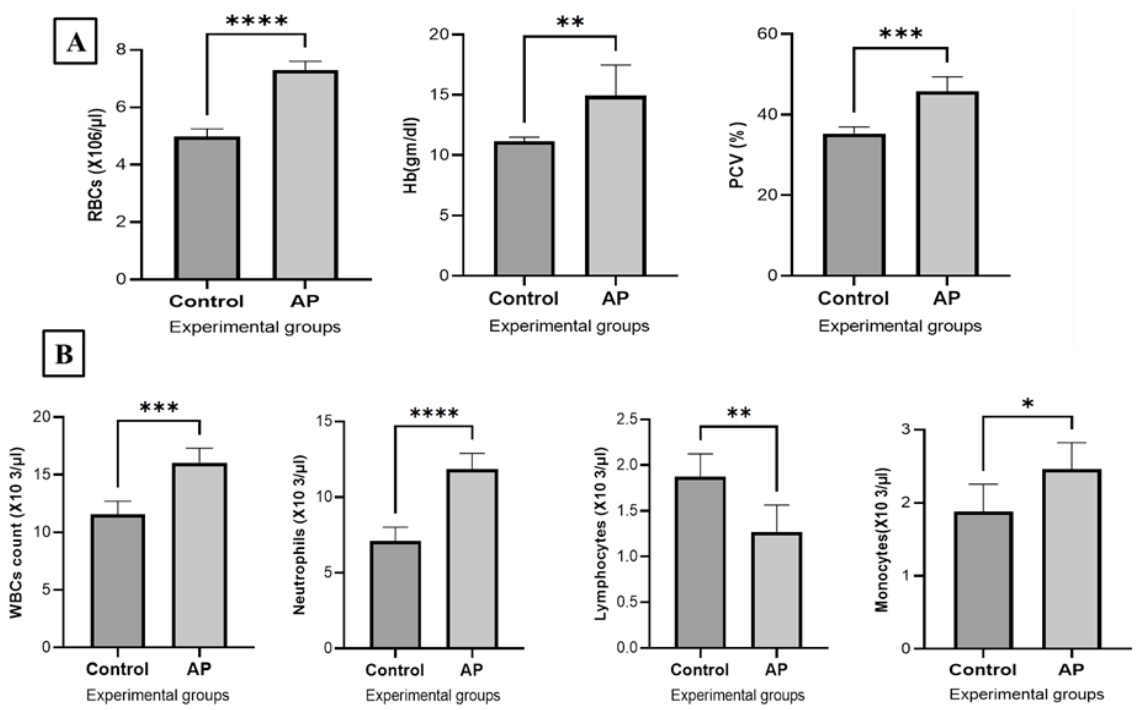

Fig. 1: Hematological parameters in both the control and the caerulein treated dogs after 6 hours of caerulein infusion. Values are expressed as mean ± SD. Bars with *,**,***,**** are significantly different from each other at P < 0.05, 0.01, 0.001, 0.0001 respectively.

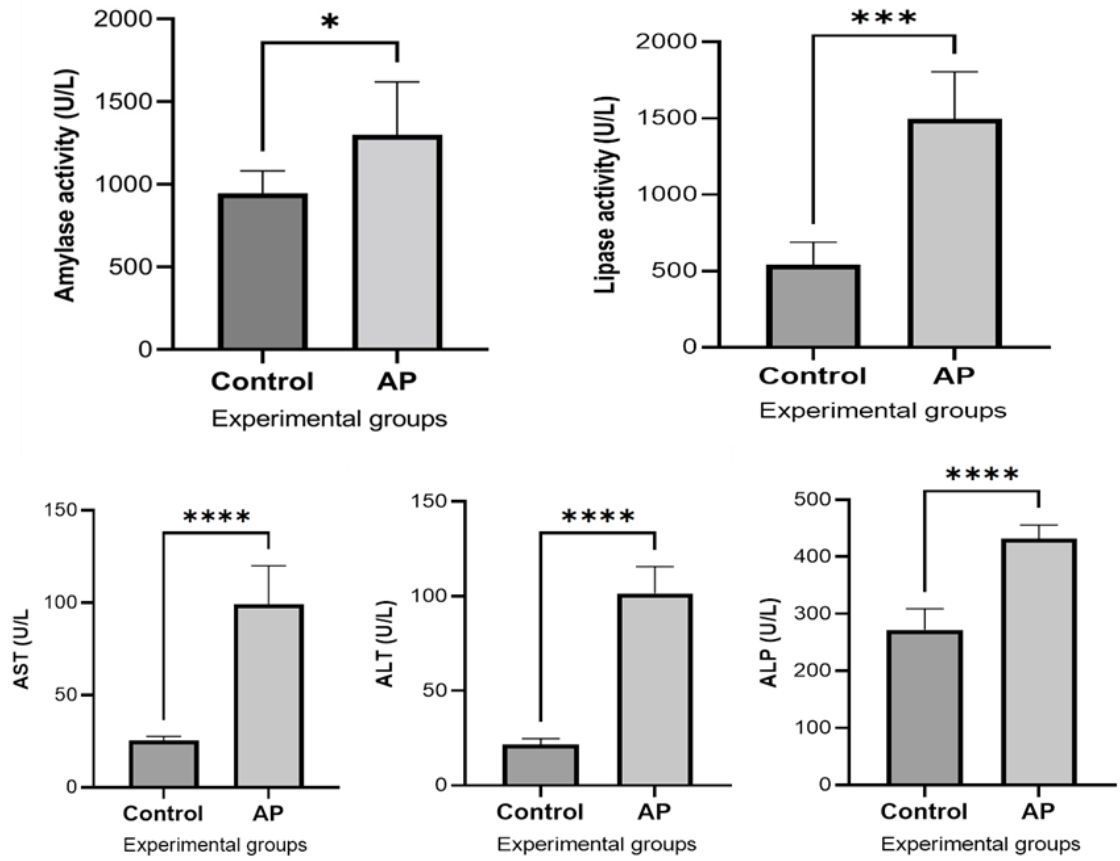

Fig. 2: Serum pancreatic and liver enzyme activities in both the control and the caerulein treated dogs after 6 hours of caerulein infusion. Values are expressed as mean ± SD. Bars with *,***,**** are significantly different from each other at P < 0.05, 0.001, 0.0001 respectively.

commonly used to diagnose acute pancreatitis. However, their specificity is limited, as they can be elevated in other conditions such as salivary gland disease, intra-abdominal inflammation, and diabetic ketoacidosis (Trivedi *et al.*, 2011).

The increase in liver enzymes AST, ALT and ALP in caerulein-infused dogs is likely due to the release of inflammatory mediators from the pancreas. These mediators can cause liver damage, leading to the release of liver enzymes into the bloodstream. Furthermore,

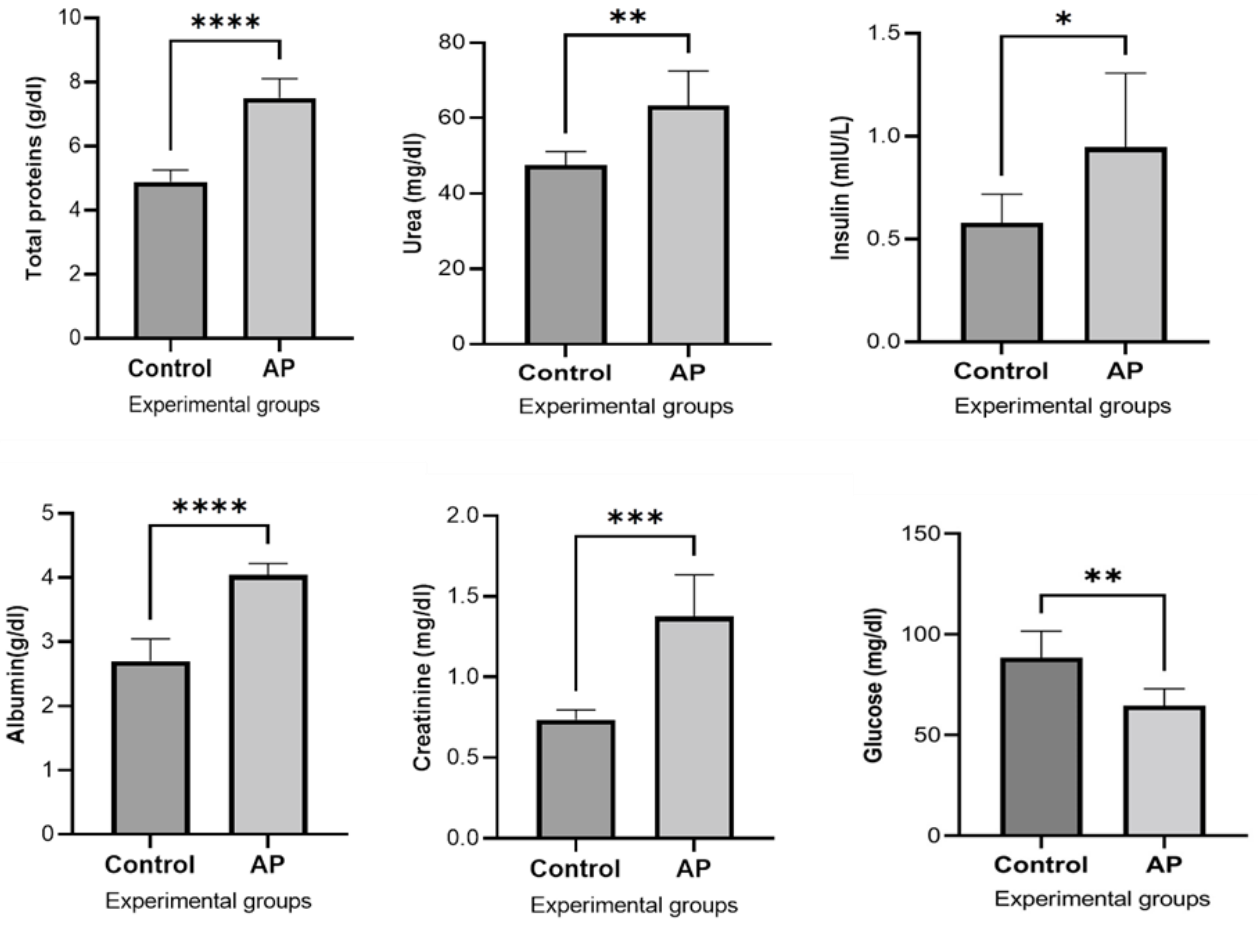

Fig. 3: Serum biochemical parameters in both the control and the caerulein treated dogs after 6 hours of caeruline infusion. Values are expressed as mean ± SD. Bars with *,**,***,**** are significantly different from each other at P<0.05, 0.01, 0.001, 0.0001 respectively.

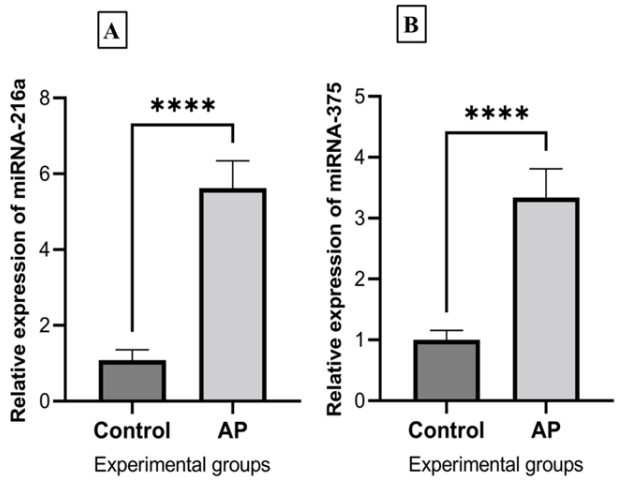

Fig. 4: Changes in relative expression of miRNA216a (A) and miRNA375 (B): in both the control and the caerulein treated dogs. Values are expressed as mean ± SD. Bars with **** are significantly different from each other (P≤ 0.001).

these inflammatory mediators can directly impact hepatic cells through the portal vein and also stimulate Kupffer cells to release TNF, IL-1, IL-6, and other cytokines that contribute to the development of local and systemic inflammatory responses, as discussed by Folch-Puy (2007). During the inflammatory response, there is an increase in vascular permeability, leading to the extravasation of intravascular fluid into the peritoneal cavity. This fluid loss reduces perfusion pressure in the pancreas, causing microcirculatory changes that contribute to pancreatic necrosis (Bassi *et al.,* 1994). On the other hand, the rise in serum total proteins may be attributed to caerulein administration. Caerulein is associated with elevated levels of CRP, SAA and haptoglobin (Yoon *et al.,* 2020), and it has been observed that caerulein administration increases blood total proteins by approximately 50% of total protein synthesis within three hours of injection (Sans *et al.,* 2003). The treated dogs showed a notable increase in BUN and creatinine levels. This increase could be attributed to prerenal azotemia, which is caused by dehydration resulting from vomiting and diarrhea in caerulein-treated dogs (Simpson *et al.,* 1992). It is worth noting that higher levels of blood creatinine and urea were found to be associated with an increased case fatality rate (Marchetti *et al.,* 2017).

In the current study, the treated dogs demonstrated a noteworthy decrease in blood glucose level accompanied by a significant increase in the insulin level. The increase in the insulin level in caerulein-infused dogs is consistent with hyperinsulinemia which is a common complication of acute pancreatitis (Pendharkar *et al.,* 2016). Several factors may contribute to the occurrence of hyperinsulinemia in acute pancreatitis. The inflammatory process associated with acute pancreatitis can disrupt normal pancreatic cell function, including the insulin-producing beta cells (Gray *et al.,* 2010). This disruption can trigger an excessive release of insulin, contributing to

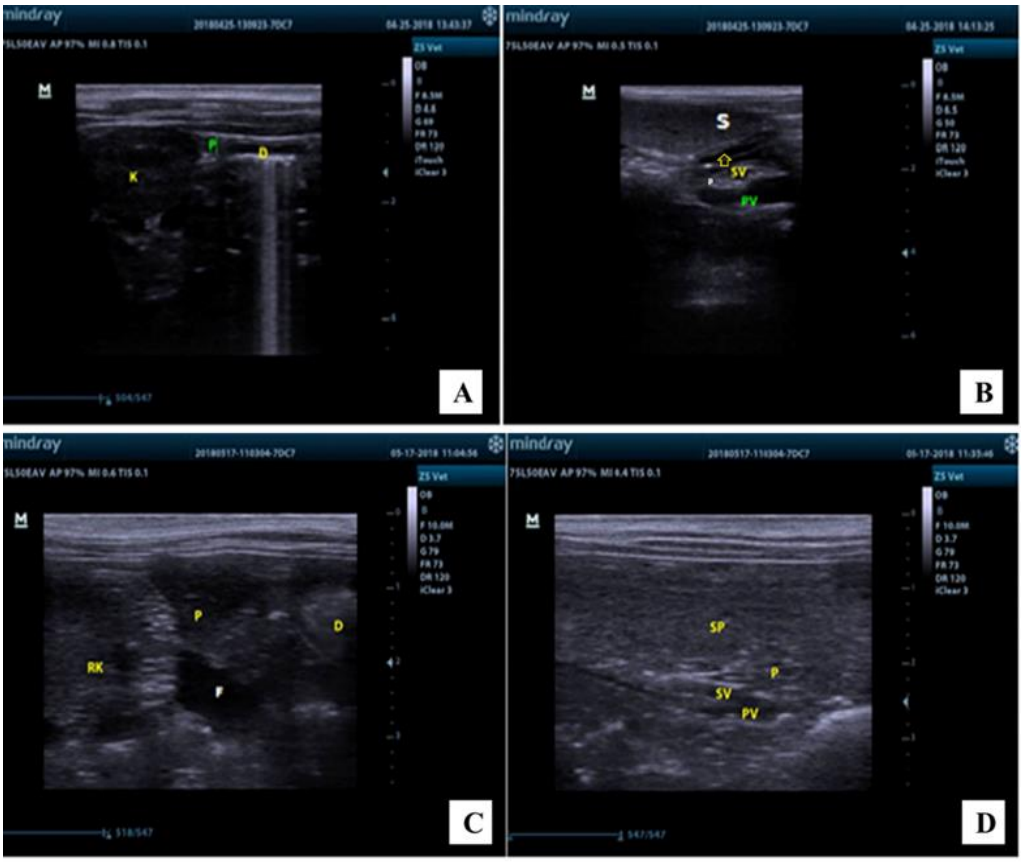

Fig. 5: Ultrasonographic appearance of the pancreas in both the control and the caerulein treated dogs. **(A)** Normal appearance of pancreas between right kidney and duodenum. **(B)** Normal appearance of pancreas at left side between splenic vein and portal vein. **(C)** Ultrasonographic of the pancreas in the caerulein treated dogs showing glandular swelling, corrugation of the duodenum, peri-pancreatic fluid accumulation. (D) Ultrasonographic image of left lobe pancreas between splenic vein and portal vein showing an increase in size of pancreas. Arrow refers to splenic vein, D= duodenum, S= spleen, F= fluid, RK=right kidney; P= pancreas; PV= Portal vein; SP= spleen; SV= splenic vein.

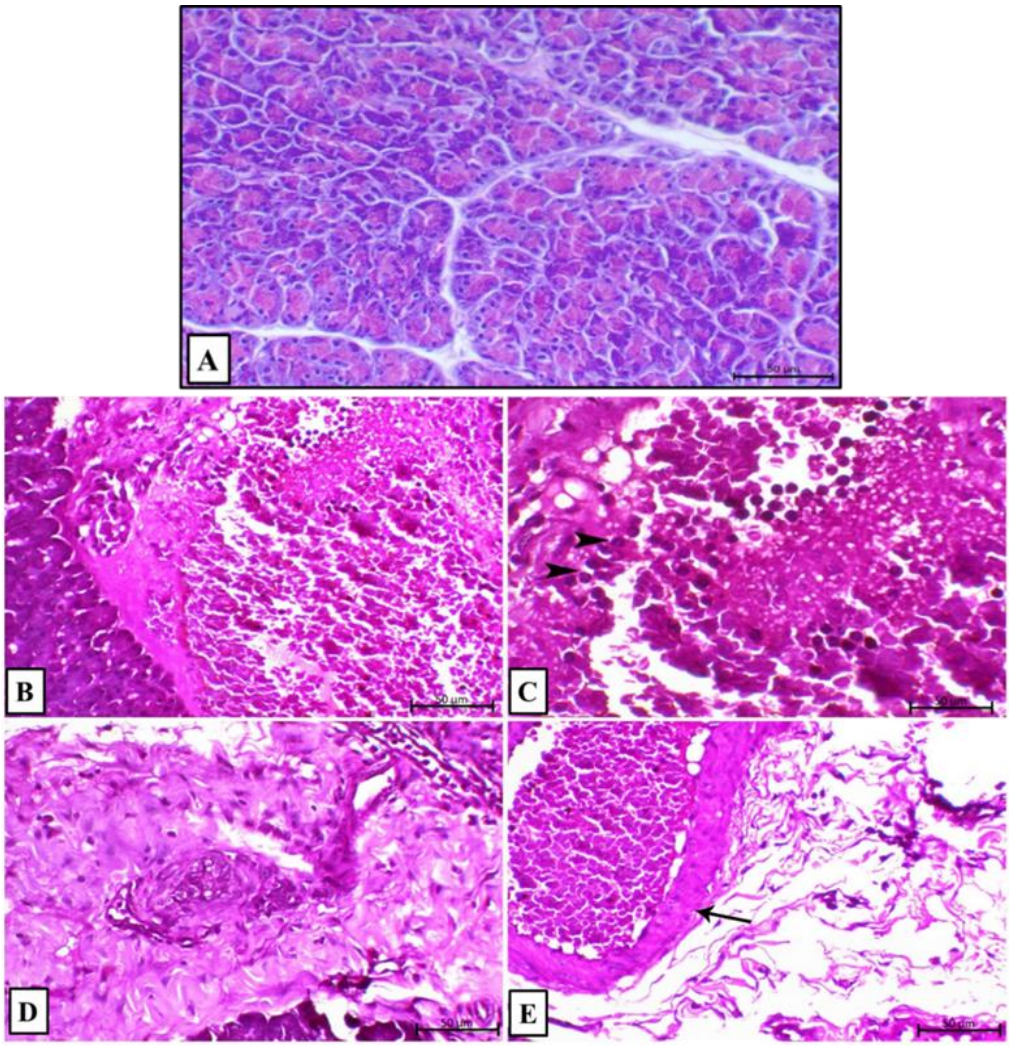

Fig. 6: Histopathologic examination of pancreatic tissue in both the control and the caerulein treated dogs. (A) Normal histology of pancreatic acinar cells in the control group. (B) The pancreas in caerulein-infused dogs showing necrosis of pancreatic parenchyma together with inflammatory cells infiltrations. (C) The pancreas in caerulein-infused dogs showing severe necrosis of pancreatic parenchyma. (D) The pancreas in caerulein-infused dogs showing interstitial hemorrhage. (E) This figure is showing higher magnification of figure D showing interstitial hemorrhage (H&E, x200), bar = 50µ.

hyperinsulinemia. Additionally, the stress response elicited by acute pancreatitis may also play a role (Zhang *et al.,* 2021). However, it is important to note that the precise mechanisms underlying hyperinsulinemia in acute pancreatitis are not fully elucidated and require further investigation.

Diagnostic ultrasonography is a useful tool for diagnosing acute pancreatitis in dogs, but it does have some practical limitations (Walkowska *et al.,* 2022). These limitations include the potential obstruction of pancreas evaluation by gases in the gastrointestinal system, the difficulty in applying appropriate pressure for imaging due to abdominal discomfort from pancreatitis, and the reliance on operator skill (Feliciano *et al.,* 2014). The ultrasonographic appearance of the pancreas in dogs treated with caerulein showed an expanded, irregular, hypoechoic region, consistent with previous findings on acute pancreatitis (Granger *et al.,* 2015). Ultrasound abnormalities in the pancreas, such as edema, necrosis, or bleeding, can occur as a result of pancreatitis (Walkowska *et al.,* 2022). Modest edema and corrugation in the duodenum were also observed through ultrasonography. However, duodenal corrugation is a non-specific sign that can be caused by other conditions like enteritis or peritonitis (Lim *et al.,* 2014).

Histopathologic analysis of pancreatic tissue is considered the most accurate method for diagnosing pancreatitis in dogs (Cridge *et al.,* 2018). In this study, acute hemorrhagic pancreatitis was observed in dogs treated with caerulein, with a more pronounced inflammatory response in the intralobular spaces and septal tissue compared to the lobules. This finding suggests that caerulein primarily affects the exocrine pancreas rather than the endocrine pancreas, consistent with previous observations in animal models of caerulein-induced pancreatitis (Tirkes *et al.,* 2022).

In this study, two microRNAs, miR-216a and miR-375, were investigated as potential biomarkers for the diagnosis of acute pancreatitis. Significant upregulation of miR-216a and miR-375 was observed in caerulein-treated dogs compared to control dogs. Similarly, Lee *et al.* (2023) demonstrated that miR-216a and miR-216b expression is primarily localized in pancreatic acinar cells rather than islet cells, and that serum levels of both miRNAs are significantly elevated in pancreatic damage. Additionally, Kong *et al.* (2010) reported that miR-216a is abundantly expressed in pancreatic tissue, with a concentration 128 times higher than in the second highest expressing tissue, the kidney. L-arginine has been shown to increase serum miR-216a levels in rats, and its specificity is superior to that of amylase and lipase (Kong *et al.,* 2010). Given the known role of miRNAs in regulating immune cell activities, it is not surprising that miRNAs are implicated in the pathogenesis of AP. Additionally, Lee *et al.* (2023) compared the diagnostic performance of exocrine pancreas-enriched miRNAs miR-216a, miR-216b, and miR-217, as well as endocrine pancreas-enriched miRNAs miR-375 and miR-148a, to serum amylase and lipase in the diagnosis of acute pancreatitis. They found that the miRNAs exhibited equivalent or better sensitivity, a wider range of reactivity, and a stronger correlation with acinar cell damage than amylase and lipase. These findings support the use of miR-216a as a potential biomarker for the early detection of pancreatitis in both animal models and human patients.

Conclusions: In conclusion, caerulein induces acute pancreatitis in dogs and has a toxic effect on pancreatic cells. MiR-216a and miR-375 have emerged as promising circulating blood biomarkers for canine acute pancreatitis, offering a faster and less invasive alternative to histopathology. While a comprehensive diagnosis of acute pancreatitis requires a combination of clinical indicators, laboratory findings, imaging, and histological evaluation, miR-216a and miR-375 stand out as superior candidate biomarkers for early pancreatic damage in dogs. The significant elevation of these miRNAs provides substantial evidence to support their potential as biomarkers for the clinical diagnosis of acute pancreatitis.

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Declaration of competing interest: The authors declare no conflict of interest with this research.

Authors' contribution: All authors contributed to this present work: RM, MZ, MA, MN, IO and NG designed the study. RM, NG, AH, NE and MA performed the experiments. MZ, IO and MA analyzed the data. RM, MZ, IO and MA drafted the manuscript. All authors revised the manuscript. All authors read and approved the final manuscript.

REFERENCES

Avante ML, da Silva PD, Feliciano MA, *et al.,* 2018. Ultrasonography of the canine pancreas. Revista MVZ Córdoba 23:6552-6563.

Azizi Dargahlou S, Iriti M, Pouresmaeil M, *et al.,* 2023. MicroRNAs; their therapeutic and biomarker properties. Cellular Mol Biomed Reports 3:73-88.

Bassi D, Kollias N, Fernandez-del Castillo C, *et al.,* 1994. Impairment of pancreatic microcirculation correlates with the severity of acute experimental pancreatitis. J American Coll Surg 179:257-263.

Bregalda A, Carducci C, Viscomi MT, *et al.,* 2023. Myelin basic protein recovery during PKU mice lifespan and the potential role of microRNAs on its regulation Neurobiol Dis 180:106093.

Cridge H, MacLeod A, Pachtinger G, *et al.,* 2018. Evaluation of SNAP cPL, Spec cPL, VetScan cPL rapid test, and precision PSL assays for the diagnosis of clinical pancreatitis in dogs. J Vet Int Med 32:658-664.

Feldman B, Zinkl J and Jain N, 2000. Schalm's Veterinary Hematology Lippincott Williams and Wilkins. Philadelphia, Baltimore.

Feliciano M, Maronezi M, Pavan L, *et al.,* 2014. ARFI elastography as a complementary diagnostic method for mammary neoplasia in female dogs–preliminary results. J Small Anim Pract 55:504-508.

Folch-Puy E, 2007. Importance of the liver in systemic complications associated with acute pancreatitis:the role of Kupffer cells. The Journal of Pathology:A J Pathol. Soc. Great Britain and Ireland 211:383-388.

Granger LA, Hilferty M, Francis T, *et al*., 2015. Variability in the ultrasonographic appearance of the pancreas in healthy dogs compared to dogs with hyperadrenocorticism. Vet Radio Ultrasound 56:540-548.

Gray SL, Donald C, Jetha A, *et al.,* 2010. Hyperinsulinemia precedes insulin resistance in mice lacking pancreatic β-cell leptin signaling. Endocrinology 151:4178-4186.

Khan U, Petrechko O, Sagheer S, *et al.* 2023. Acute pancreatitis and myocardial infarction:a narrative review. Cardiolology 148:1-11.

Kong X-Y, Du Y-Q, Li L, *et al.,* 2010. Plasma miR-216a as a potential marker of pancreatic injury in a rat model of acute pancreatitis. World J Gastroenterol 16:4599.

Lee D, Yun T, Koo Y, *et al.,* 2023. Evaluation of serum miR-216a and miR-375 as biomarkers in dogs with acute pancreatitis. J Vet Int Med. 37:92-100.

Lim S, Nakamura K, Morishita K, *et al.,* 2014. Qualitative and quantitative contrast-enhanced ultrasonographic assessment of cerulein-induced acute pancreatitis in dogs. J Vet Int Med 28:496-503.

Lowe A, Campbell K, Barger A, *et al.,* 2008. Clinical, clinicopathological and histological changes observed in 14 cats treated with glucocorticoids. Vet Record 162:777-783.

Marchetti V, Gori E, Lippi I, *et al.,* 2017. Elevated serum creatinine and hyponatraemia as prognostic factors in canine acute pancreatitis. Aust Vet J 95:444-447.

Patel HR, Diaz Almanzar VM, LaComb JF, *et al.,* 2023. The Role of MicroRNAs in Pancreatitis Development and Progression. Int J Mol Sci 24:1057.

Pendharkar SA, Asrani VM, Xiao AY, *et al.,* 2016. Relationship between pancreatic hormones and glucose metabolism:a cross-sectional study in patients after acute pancreatitis. Am J Physiol Gastrointest Liver Physiol 311:G50-G58.

Rouse R, Rosenzweig B, Shea K, *et al.,* 2017. MicroRNA biomarkers of pancreatic injury in a canine model. Experim Toxicol Pathol 69:33-43.

Sans MD, DiMagno MJ, D'Alecy LG, *et al.,* 2003. Caerulein-induced acute pancreatitis inhibits protein synthesis through effects on eIF2B and eIF4F. Am. J. Physiol. Gastrointest. Liver Physiol 285:G517-G528.

Shang R, Lee S, Senavirathne G, *et al.,* 2023. microRNAs in action: biogenesis, function and regulation. Nat Rev Genet 24:816-833.

Simpson K, Starkey R, Dibartola S, *et al.,* 1992. The effect of subtotal nephrectomy on circulating trypsin-like immunoreactivity, amylase, lipase, and gastrin in the dog. J Vet Int Med 6:128.

Su KH, Cuthbertson C and Christophi C, 2006. Review of experimental animal models of acute pancreatitis. Hpb 8:264-286.

Tirkes T, Saeed OA, Osuji VC, *et al.,* 2022. Histopathologic correlation of pancreatic fibrosis with pancreatic magnetic resonance imaging quantitative metrics and Cambridge classification. Abd Radiol 47:2371-2380.

Trivedi S, Marks S, Kass P, *et al.,* 2011. Sensitivity and specificity of canine pancreas-specific lipase (cPL) and other markers for pancreatitis in 70 dogs with and without histopathologic evidence of pancreatitis. J Vet Int Med 25:1241-1247.

Walkowska J, Zielinska N, Tubbs RS, *et al.,* 2022. Diagnosis and treatment of acute pancreatitis. Diagnostics 12:1974.

Yoon J-S, Kim S, Kang J-H, *et al.,* 2020. Alterations in serum protein electrophoresis profiles during the acute phase response in dogs with acute pancreatitis. Canadian J Vet Res 84:74-78.

Zhang AM, Wellberg EA, Kopp JL, *et al.,* 2021. Hyperinsulinemia in obesity, inflammation, and cancer. Diabetes Metab J 45:285-311