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MARKERS FOR HEPATOCELLULAR DAMAGE AND AFFECTING THE BILIARY TRACT IN THE COURSE OF ACUTE PANCREATITIS IN DOGS

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ABSTRACT

The purpose of this study was to determine the values of AST, ALT and alkaline phosphatase as markers for hepatocellular damage and / or involvement of the biliary tract during acute pancreatitis (AP) in the dog. The studies were performed on 83 dogs with spontaneous acute pancreatitis divided into two groups, depending on the outcome of the disease, and 12 clinically healthy dogs, with experimentally induced acute pancreatitis by 2 different methods.

A statistically significant difference was observed in the values of the three studied parameters, both between the two spontaneous and between the two experimental groups of animals. The results suggest that severe pancreatic inflammatory conditions can lead to secondary liver toxicity and partial biliary obstruction.

Key words: pancreatitis, dog, ALT, AST, alkaline phosphatase.

Introduction

In the cases of biliary pancreatitis, bilirubin, cholestatic enzymes and aminotransferases are significantly increased (Carter et al., 2000; Kondratenko, 2008). Hepatic ischemia, topical inflammatory mediators and toxic pancreatic mediators in the portal circulation lead to hepatocellular damage with elevated liver enzymes (alanine-ALT and aspartate aminotransferase AST) in both dogs and cats (Hill and Van Winkle, 1993; Hess et al., 1998; Van den Bossche et al, 2010). Threefold increase in serum bilirubin or persistent increase in liver tests over time have important diagnostic value (Kondratenko, 2008; Van den Bossche et al, 2010; Zuobiao, 2011). According to a number of authors, a two to fivefold increase in bilirubin is detected in 30 to 50% of dogs and in up to 65% of cats with cholestasis (Hill and Van Winkle, 1993; Hess et al., 1998; Gerhardt et al., 2001; Washabau, 2001). This cholestasis may subsequently progress to inflammation or fibrosis of the pancreas, which impedes (partially or completely) the patency of the common bile duct. (Bunch, 2003; Watson, 2004; Mix and Jones, 2006). Cholestasis may lead to increased AST, ALT and alkaline phosphatase (ALP) (Watson, 2004).

One of the most common uses of ALP is as a marker for intra- and extra-hepatic obstruction. Initially, ALP was thought to increase due to the inability of the liver cells to release the enzyme via bile (Wachstein and Zak, 1946), but it is now accepted that ALP activity is increased due to increased enzyme synthesis (Kaplan and Righetti, 1970; Elspeth, 1985; Center, 2009). In cholestasis, an increase in bilirubin (mainly free) is accompanied by an increase in ALP, while in hepatocellular damage, ALT and total and free bilirubin are increased, but ALP does not increase significantly (Elspeth, 1985; Morag, 2002; Wang et al., 2009). Because the proportions of total and free bilirubin in hepatocellular damage overlap with those observed in primary cholestasis, ALP is of particular value in differential diagnosis. (Elspeth, 1985; Wang et al., 2009). In cases of acute biliary obstruction, ALT is most significantly increased (over 150 U/L) (Watson, 2004).

The purpose of this study is to determine the value of AST, ALT, and alkaline phosphatase as markers for hepatocellular damage and / or involvement of the biliary tract during acute pancreatitis in the dog.

Materials and methods

The study included 83 dogs with spontaneous acute pancreatitis divided into two groups, depending on the outcome of the disease (survivors and nonsurvivors), and 12 clinically healthy dogs in which experimental acute pancreatitis was induced. Group A included 22 animals (with lethal outcome). In 12 patients in this group, the disease ended in death within 48 hours of the initial examination, and another 10 were euthanized at a different stage in the development of the disease due to a pessimistic prognosis. Group B included 61 animals (survivors). Primary clinical trials and blood sampling for haematological and biochemical analysis were performed at the time the animals were admitted to the clinic. The exact onset of the disease could not always be determined with absolute precision. In our estimation and the anamnestic data collected, the majority of spontaneous cases were submitted for primary medical examination between the 72nd and the 96th hour since the onset of suffering. For experimental reproduction of acute pancreatitis, 12 clinically healthy mongrel dogs, of both sexes, aged 4–5 years, weighing 13.5 to 18 kg, were provided by the shelter of Stara Zagora Municipality. They were divided into two experimental groups (C and D) with 6 dogs each. In group C, we caused acute pancreatitis by ligation of *ductus pancreaticus*, and in group D acute pancreatitis was reproduced by introducing oleic acid into the *ductus pancreaticus minor*. The experiments were conducted in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Specific Purposes and the current Bulgarian laws and regulations.

Prior to the start of the experiments, all animals were vaccinated and treated against endo- and ectoparasites. Daily clinical and periodic laboratory (blood and urine) tests were performed for 14 days, which confirmed their good health. Unlimited access to drinking water was provided and they were fed dry granulated food according to their type and weight.

Haematology, ultrasound, and clinical studies were performed in all animals at 0, 24, 48, 72, and 96 hours from the start of the experiments.

Changes in the levels of a number of organ-specific enzymes (amylase, creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and pancreatic amylase), acute-phase proteins (fibrinogen, and C-reactive) and blood glucose were studied in dynamics. Changes in the clinical status and blood count of the experimental animals were monitored. On the 5th day of the start of the experiments, the dogs were euthanized, a pathological examination was performed, and were obtained samples for histological analysis. Thiopental and potassium chloride administered sequentially intravenously were used to perform the euthanasia. The main part of the research was carried out in the Departments of Internal Diseases, Veterinary Surgery and General Pathology at the Faculty of Veterinary Medicine. Laboratory analyses were performed at the Laboratory Diagnostic Center (LDC) at the Faculty of Veterinary Medicine at Trakia University.

The test results obtained at the 0th hour were used as control values in the statistical processing of the results from all groups.

Data were statistically processed by ANOVA (one-way analysis of variance) (Statistica for Windows, Stat Soft Inc., USA 1993). All results are presented as mean and standard error of the

mean (mean ± SEM). The statistical significance of parameters according to time was determined in the LSD test at $p < 0.05$.

Results

The results of the haematological and biochemical tests showed a definite increase (relative to the control values) of all the enzymes tested (Table 1). A statistically significant difference between the two spontaneous groups ($p < 0.001$) and the control group: respectively ($p < 0.001$) – for group A and ($p < 0.01$) – for group B, was found at the levels of alkaline phosphatase. An increase was reported in 100% of the animals from group A, most of them dramatically. Moreover, at a rate of 66 ± 36 U/L (Kaneko et al., 2008), only five animals in the group (<23%) had ALP values less than 1000 U/L. The mean for the group was 3954.5 ± 563 U/L, with a maximum of 9327 U/L and a minimum of 228 U/L. In the group of survivors, an increase in the average ALP value of 348.6 ± 30.3 U/L was also observed, but these values were far from extreme. The maximum reading for this group was 1012 U/L and the minimum was 46 U/L. Only in one animal from group B (1.6%), it was reported value of ALP over 1000 U/L.

Table 1: Haematological values and correlation coefficients between experimental groups A and B in dogs with spontaneous OP. (* reference values by Kaneko et al., 2008).

Indicator	p	Control (n =12)	Group A (n =22)	Group B (n =61)
Total amylase	**<0.001**	668.0 ± 53.34 U/L	4731.2 ± 637.12 U/L[a]	2293 ± 253 U/L[a]
Pancreatic amylase	**<0.001**	266.92 ± 23.87 U/L	3779.5 ± 503.99 U/L[a]	1972 ± 233 U/L[a]
Lipase	**<0.001**	13 - 200 U/L*	1623 ± 203 U/L	1031 ± 93 U/L
Alkaline phosphatase	**<0.001**	86.5 ± 14.62 U/L	3954.5 ± 563 U/L[a]	348.6 ± 30.3 U/L[b]
ASAT	**<0.001**	20.78 ± 1.35 U/L	228.3 ± 17.51 U/L[a]	155.8 ± 9.03 U/L[a]
ALAT	**<0.001**	26.05 ± 3.05 U/L	422.5 ± 46.7 U/L[a]	128.32 ± 6.31 U/L[a]

Statistical reliability of the experimental groups compared to the control: [a]p<0.001; [b]p<0.01

Similarly, the mean values of ALP changed, reaching their highest level in the two experimental groups at the 96th hour from the beginning of the experimental procedures (Fig. 3). In the ligatures group, the maximum mean was 4030.2 U/L and a statistically significant change ($p < 0.05$) was recorded at the 72nd and 96th hours relative to the zero hour. The highest individual value was reached at the 72nd hour – 10560 U/L. In the oleic acid group (group D), the maximum mean was 924.5 U/L and a statistically significant change ($p < 0.001$) was also reported at the 72nd and 96th hours from the start of the experiment relative to the zero hour. The highest individual value in this group was reported at the 96th hour – 1254.4 U/L.

In the study of hepatic transaminases (AST and ALT) statistically significant differences ($p < 0.001$) both between the two spontaneous groups and the control group were found. The mean value of AST into group of nonsurvivors was 228.3 ± 17.51 U/L and in survivors group it was 155.8 ± 9.03 U/L. The maximum value for the indicator (374 U/L) was reported in group A representative. The lowest value (24 U/L) was found in group B. The mean value of ALT in group A was 422.5 ± 46.7 U/L, with variations from 73 to 792 U/L. In group B, values ranged from 26 to 318 U/L and the mean was 128.32 ± 6.31 U/L.

AST and ALT values in groups C and D maintained a tendency for a gradual and continuous increase throughout the experiment (Figures 1 and 2). The highest mean of AST in group C

(189.20 U/L) was reported at 72 hours from the start of the experiment. A statistically significant change ($p < 0.001$) from baseline was observed at 72 hours and 96 hours. The highest individual score for this group (386 U/L) was again found at 72 hours. The highest mean value for AST in group D (131.8 U/L) was reported at 96 hours from the start of the experiment. A statistically significant change ($p < 0.001$) from baseline values in this group was detected as early as 24 hours and remained so until the end of the experiment. The highest individual score for the group (173.6 U/L) was found at 96 hours.

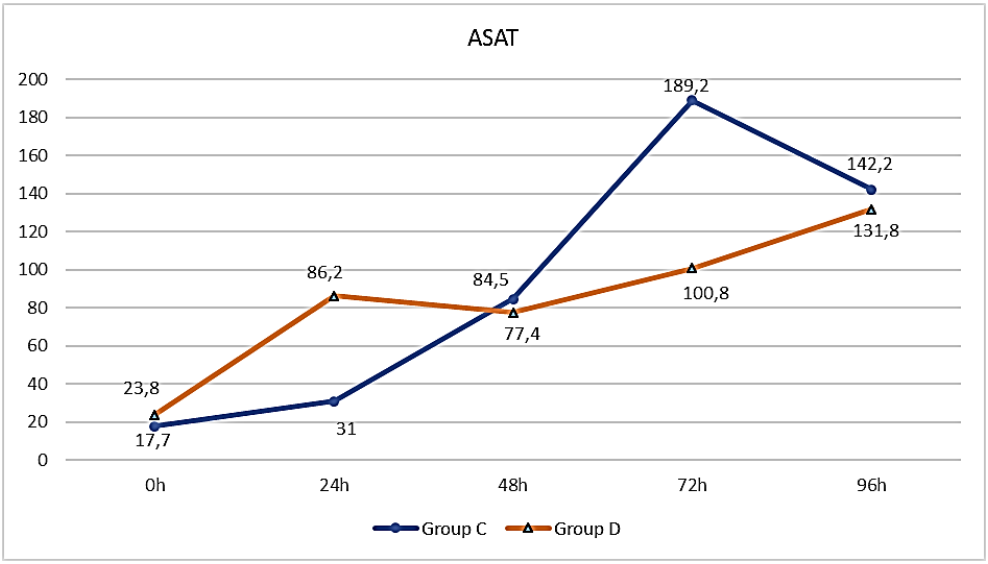

Figure 1: Plasma ASAT levels (U/L) in dogs with experimentally induced AP by placing ligatures on the pancreatic duct (Group C) and introducing oleic acid into the pancreatic duct (Group D).

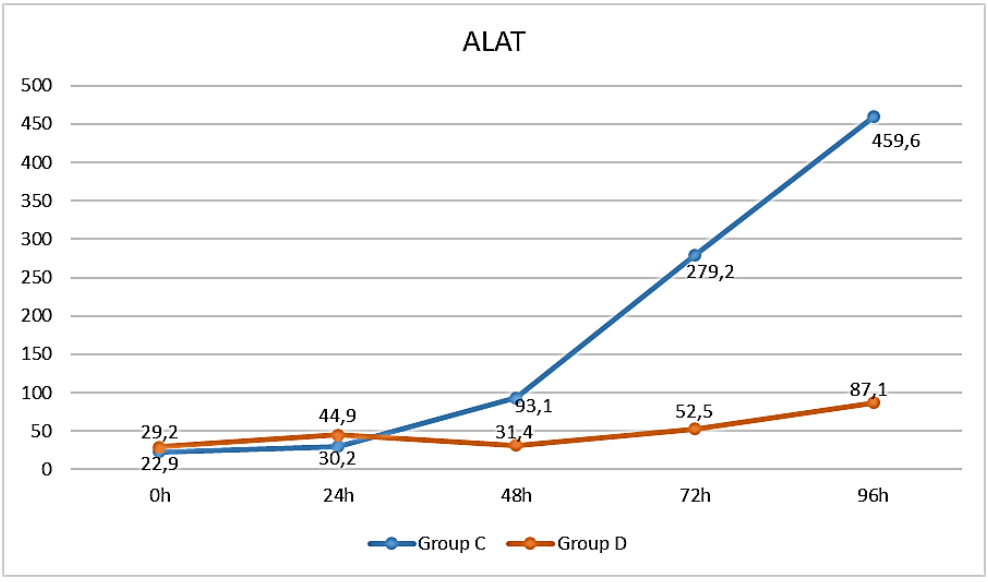

Figure 2: Plasma ALAT (U/L) levels in dogs with experimentally induced AP by placing ligatures on the pancreatic duct (Group C) and introducing oleic acid into the pancreatic duct (Group D).

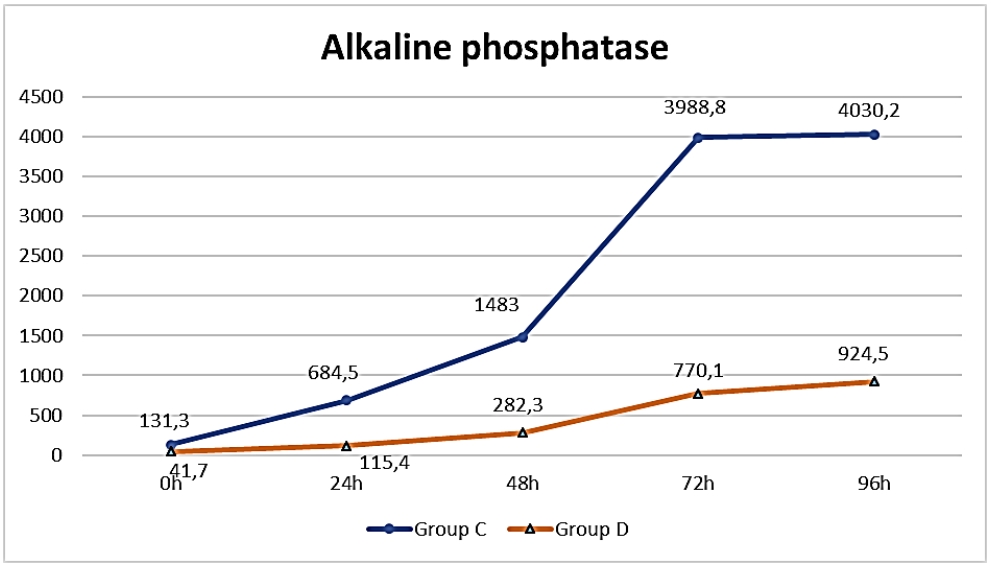

Figure 3: Plasma levels of AlP (U/L) in dogs with experimentally induced AP by placing ligatures on the pancreatic duct (Group C) and introducing oleic acid into the pancreatic duct (Group D).

Increased ALT activity was more pronounced in the group C (Fig. 2). A statistically significant change from baseline was found at 72 h ($p < 0.05$) and 96 h ($p < 0.001$). The highest mean was recorded at the 96th hour – 459.6 U/L, with the highest individual score – 573 U/L again at the same hour. A statistically significant increase ($p < 0.001$) in plasma ALT levels in the group D was detected only at the 96th hour from the start of the experiment. Maximum averages and individual values of 87 U/L and 111.3 U/L were also considered at this stage. The difference ($p < 0.001$) found in the maximum mean values of the two groups was more than 5 times in favor of test group C.

Discussion

One of the most commonly mentioned predictors of AP severity is liver transaminase values. Many of the proposals for prognostic scales, in parallel with pancreatic and renal indices, include a threefold increase in AST, ALT, or ALP (Ruaux and Atwell, 1998; Ruaux, 2000; Kalli et al., 2009).

To date, the mechanism leading to an increase in hepatic transaminases in AP remains unclear, despite the reported increases in ALT in choledocholithiasis (Nathwani et al., 2005). Mossberg и Ross (1963) suggest three different mechanisms for explaining liver transaminase elevation after an obstructive process: 1) regurgitation of transaminases from obstructed bile ducts in the liver sinusoids, 2) increased enzyme production and 3) hepatocyte transaminase secretion in response to elevated intraluminal pressure.

Assuming the above authors' assertion that plasma ALT levels rise sharply with biliary obstruction, we can easily justify the large difference in ALT values in groups A and C on the one hand, and B and D on the other. The ligatures placed on the pancreatic ducts in Group C undoubtedly lead to pancreatic and biliary hypertension, with subsequent biliary regurgitation. For Group A, we can assume that this is biliary hypertension due to the external pressure of the effluent pancreatic structures.

We also found a significant deviation from the AST values, but the differences between the groups are not so drastic. On the other hand, AST is present in many tissues other than hepatobiliary,

a fact that renders it unusable in the diagnosis of biliary AP. However, a ten-fold increase above normal values is associated mainly with hepatic ischemia and biliary obstruction (Matull et al., 2006). In the course of our studies, there was not such a significant increase in this indicator, but a clear increase in all experimental groups supports the claim of other authors that the inflammation of the pancreas exacerbates hepatocellular damage observed in liver obstruction (Murr et al., 2002; Zuobiao et al., 2011).

In a clinical study in humans, Güngör and team (2011) found a significant increase in five biochemical parameters that correlated with biliary AP. One of these indicators is alkaline phosphatase (ALP). Other authors also cite ALP as a significant variable in bile pancreatitis, in addition to indicators such as ALT, amylase, GGT, total and direct bilirubin (Davidson et al., 1988; Chang et al., 1998).

The degree of ALP elevation may also have diagnostic value. A magnification of up to 10 times the normal values indicates the presence of liver lesions (Moore and Feldman, 1974; Syakalima et al., 1998). Values up to 100 times normal are found in extra-hepatic biliary obstruction (Guelfi et al., 1982). Similar to the results obtained for ALT values, ALP also found a drastically large difference in values for experimental groups A and C on the one hand, and B and D on the other.

Our results do not support Morag's (2002) assertion that elevated levels of ALP, ALT, and bilirubin in AP can only be established as a chance finding if there is concomitant hepatitis. The values we found in group A may partially correspond to this statement, but in group C the presence of liver pathology was excluded before the start of the experiment. According to the same author, in extrahepatic obstruction, the activity of ALP increases extremely and reaches values from 10,000 to 50000 U/L, but other liver enzymes remain normal or slightly increased. (Morag, 2002). He also claims that with intrahepatic biliary obstruction, both ALP and parenchymal enzymes are significantly increased.

Rather, we can agree with Hoffmann and Dorner (1977), who, after an experimental study, suggested that expressed inflammatory conditions, including pancreatitis, may lead to secondary liver toxicity and partial biliary obstruction. In such cases, ALP values are much higher than elevated values in bone diseases, although there is evidence that acute pancreatitis may result in an increase in ALP of bone origin. (Rogers, 1976).

Conclusion

- Elevated levels of liver enzymes (ALP, ALT and AST) during AP may be indicators of biliary aetiology or involvement of the biliary tract in the pathological process.
- Severe pancreatic inflammatory conditions can lead to secondary liver toxicity and partial biliary obstruction.
- A significant increase in liver enzyme levels (ALP, ALT and AST) in the course of acute pancreatitis can be considered as a poor prognostic sign.
- Examination of biliary pathology should be performed in all cases of AP in order not to delay treatment and to avoid secondary complications.

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