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VALUE OF CARDIAC MARKERS IN DOGS WITH CHRONIC MITRAL VALVE DISEASE

BAKIREL U and GUNES S

Faculty of Veterinary Medicine, Istanbul University, Istanbul, Turkey

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The aim of this study was to assess the role of serum cardiac troponin I (cTnI), troponin T (cTnT), lactate dehydrogenase (LDH) and MB isoenzyme of CK (CK-MB) concentrations in the detection of cardiac injury in dogs with chronic mitral valve disease (CMVD). Fifty-five dogs with echocardiographic diagnosis of CMVD defined as cardiac thrill (Ct) and non-cardiac thrill (nCt) by auscultation-palpation, and ten control dogs were studied. Serum was extracted from a blood sample from each dog. Analysis of serum cTnI, cTnT, LDH and CK-MB concentrations were performed. Mean ± SD serum cTnI concentration was 1.9 ± 0.38 ng/mL in group-Ct and 1.75 ± 0.27 ng/mL in group-nCt, and cTnI concentrations in both the subgroups were significantly higher than in the control dogs (P<0.01). cTnT and CK-MB concentrations were 0.031 ± 0.02 ng/mL and 83.5 ± 88.4 IU/L in group-Ct and 0.024 ± 0.01 ng/mL and 51.6 ± 47.4 IU/L in group-nCt, respectively. Mean cTnT and CK-MB concentrations in group-Ct were significantly higher than in control dogs (P<0.01, P<0.05). LDH concentration was 918 ± 136.4 IU/L in group-Ct and 351 ± 140.7 IU/L in group-nCt. A significant difference in serum LDH concentrations was observed between the 3 studied groups (P<0.05). In this study, compared with clinically healthy dogs, significant increases in serum cTnI, cTnT, LDH and CK-MB concentrations were detected in dogs with CMVD. The findings of this study indicate that the biochemical markers activity elevated with severity of the disease in dogs with CMVD. Measurement of cardiac troponins, as the newer markers than LDH and CK-MB concentrations, may be useful in the detection of cardiac injury and prognosis in dogs with CMVD.

Key words: troponin I, troponin T, LDH, CK-MB, dog

INTRODUCTION

Lactate dehydrogenase (LDH) and creatine kinase (CK) are sensitive markers of myocardial cell damage, as well as being parameters of high specificity for myocardial injury in humans. So is MB isoenzyme of CK (CK-MB) for animals (Wu and Ford, 1999; Church *et al.,* 2007). However, skeletal muscle injury can also result in increased serum LDH, CK and CK-MB values (Scober *et al*.,

1999; Mellor *et al.,* 2006). Thus, attention has focused on more specific cardiac markers. The cardiac isoforms of troponin I (cTnI) and T (cTnT) are the new generation of biochemical markers that indicate myocardial injury (Adin *et al.,* 2005; Church *et al.,* 2007). In dogs, cats and common laboratory animals, serum troponin concentrations has been investigated as a marker of cardiac injury in different cardiac disorders (Kittleson *et al.,* 1984; O'Brien *et al.,* 2006; Prosek *et al*., 2007). However, chronic mitral valve disease results with mitral regurgitation and a few reports on assessment of myocardial damage with biochemical markers are present in these dogs (Oyama and Sisson 2004; Spratt *et al*., 2005).

The aim of this study was to evaluate the value of cTnI, cTnT, LDH and CK-MB in the detection of cardiac injury in dogs with chronic mitral valve disease.

MATERIALS AND METHODS

Fifty-five dogs diagnosed of chronic mitral valve disease in Istanbul University, Faculty of Veterinary, Department of Internal Medicine between January 2000 and October 2007 and ten healthy control dogs were studied. All dogs were evaluated based on physical examination, x-ray, 12-lead ECG and echocardiography. The 2-dimensional and M-mode echocardiographic studies were made by an expert using SDU-350A and transducer micro-convex of 3.5 - 5.0 MHz.

Inclusion criteria for control dogs entered in the study were small breed dogs considered to be free of any disease on the basis of historical information and full clinical examination. The identification of MR by echocardiography, grade III-IV/IV left apical systolic murmurs by auscultation and cardiac thrill by palpation were the criteria for dogs with CMVD. Exclusion criterions were large breed dogs with the presence of myocardial disease of any aetiology; systemic or congenital disease, or both; multivalvular disease, acquired cardiovascular disorders affecting the mitral valve. The control group consisted of small breed dogs in the age range 6-15 years old. CMVD group was divided into two subgroups as cardiac thrill (Ct) and non-cardiac thrill (nCt) by taking into account murmur intensity and thrill. CMVD group consisted of 5 males, 5 females and 37 males, 18 females, respectively.

Blood samples (4-5 mL) drawn from the jugular or cephalic vein were collected into tubes as recommended by the manufacturer on the same day of the examination. Samples were centrifuged to separate serum and plasma, and analyses were done instantly as real-time. Serum biochemical analyses of LDH (IFCC, Italy), CK-MB (Randox, Italy) cardiac troponin I (Siemens healthcare diagnostic, USA) and cardiac troponin T (Roche-Diagnostic, USA) were studied. Serum LDH concentrations were determined using an auto-analyzer (TMS 1024®, Clinical analyzer, Japan). Serum cTnI was measured by the enzymoimmunofluorometric method (Dade Behring®, Rueil-Malmaison, France). According to the manufacturer's information, the minimal detectable concentration is 0.01 ng/mL. cTnT and CK-MB concentrations were determined by an electro-chemiluminiscence immunoassay system (Elecsys-1010®, Roche-

Diagnostic, Singapore). The lower detection limits of the assay stated by the manufacturer are 0.01 ng/mL and 1.0 IU/L, respectively.

Results were expressed as mean ± standart deviation (mean ± SD). Student's t-test was used to compare serum concentrations of cTnI, cTnT, LDH and CK-MB in healthy dogs with CMVD. The parameters were compared between control group and subgroups of CMDV (nCt and Ct) using two-tailed student's t-test or one-way ANOVA with Bonferroni multiple comparison test. Values of $P<0.05$ were considered to be statistically significant.

RESULTS

The study comprised 65 dogs with healthy (n=10) and chronic mitral regurgitation (n=55). Mean age was 11.6 ± 2.2 years with a total of 42 males (76%) studied. Chronic cough, tachypnea or dyspnoea, tachycardia, holosystolic murmur (III-VI/VI) in the left ventricular apex and cardiac thrill in 22 dogs were present. Enlargement of the left atrial (88%) and left ventricular end-diastolic and end-systolic chamber dimensions (46%), were noticed, and the mitral regurgitation due to CMDV was detected by echocardiography in 55 dogs. Dogs in the CMDV group had significantly larger hearts than those in the control group. Abnormal echocardiographic wall motion as hyperkinetic or ineffective contractions were present in thirty-one patients.

Statistics for cTnI, cTnT, LDH and CK-MB concentrations for the control and chronic mitral valve disease groups are summarized in Table 1. Mean cTnI concentrations of both subgroups were significantly higher than of control dogs ($P<0.01$), but differences between the subgroups were insignificant. Mean cTnT and CK-MB concentrations in dogs with cardiac thrill were significantly higher than in control dogs ($P<0.01$ and $P<0.05$). Although there is a trend toward higher values in dogs with CVDM, there was no significant difference between both control and non-cardiac thrill group; and non-cardiac thrill groups and cardiac thrill group. Mean of the LDH level in control dogs was significantly lower than in dogs with chronic mitral valve disease and LDH of dogs in the cardiac thrill group as a subgroup of CMDV group was significantly higher than of non-cardiac thrill dogs ($P<0.05$).

Table 1. Serum cTnI, cTnT, LDH and CK-MB concentrations in dogs with healthy and chronic mitral valve disease (mean ±SD)

	Control (n=10)	Chronic mitral valve disease		P value
		nCt (n=33)	Ct (n=22)	
cTnI (ng/mL)	0.03 ± 0.01^{a}	1.75 ± 0.27^{b}	1.9 ± 0.38^{bc}	P<0.01
cTnT (ng/mL)	0.013 ± 0.05^{a}	0.024 ± 0.01^{ab}	0.031 ± 0.02^{b}	P<0.01
LDH (IU/L)	187 ± 47.3^{a}	351 ± 140.7^{b}	918 ± 136.4^{c}	P<0.05
CK-MB (IU/L)	17.4 ± 5.8^{a}	51.6 ± 47.4^{ab}	83.5 ± 88.4^{b}	P<0.05

^{a,b,c} : Statistical difference between columns with different alphabets
nCt: non-cardiac thrill; Ct: cardiac thrill; cTnI: cardiac troponin I; cTnT: cardiac troponin T; LDH: lactate dehydrogenase; CK-MB: isoenzyme of creatine kinase

DISCUSSION

Chronic mitral valve disease (CMVD) is the most common acquired heart disease in dogs. The disease can also develop secondary to myocardial disease and other cardiac disorders causing volume overload of the left side of the heart. In these circumstances, valvular insufficiency results from the combined effects of chamber dilatation, enlargement of the mitral annulus, and ventricular or papillary muscle dysfunction (Kittleson and Kienle, 1998; Sisson and Kvart, 1999). The myocardial dysfunction includes any process affecting the function of cardiac myocytes and/or myocardial interstitium that prevents the cardiac ventricles from normal physiologic contraction or relaxation (Sisson and Kvart, 1999; Urabe *et al.,* 1992). In dogs with CMVD, cardiac contractility due to myocardial injury decreases slowly, but progressively and inexorably (Scober *et al.,* 1999). However, severity of intrinsic myocardial impairment in dogs with naturally occurring mitral regurgitation has probably been underestimated (Kittleson *et al.,* 1984). When it results in congestive heart failure, CMVD becomes an important cause of morbidity and mortality in dogs (Linklater *et al.,* 2004).

Troponin is a complex of subunits (C, T, and I) that binds to tropomyosine of the thin filament of striated tissue, and regulates skeletal and cardiac muscle contraction (Wu and Ford, 1999). The cardiac isoforms of cTnI and cTnT are new generation specific biochemical markers for myocardial contraction process that indicate myocardial injury (Adin *et al.,* 2005; Linde *et al.,* 2006). LDH and CK-MB are sensitive markers for myocardial cell damage in humans and animals (Wu and Ford, 1999). It is our hypothesis that one or more of these markers will be elevated at presentation of CMVD compared with established normal values of control dogs.

Based on previous studies investigating cTnI levels in dogs with heart failure, Oyama and Sisson (2004) described that the highest cTnI level was found in animals with acquired heart disease and congenital heart disease with the most severe clinical signs. Similarly, Spratt e*t al*. (2005) suggested that cTnI levels were significantly elevated in dogs with acquired mitral valve disease, dilated cardiomyopathy and pericardial effusion, although blood cTnI levels also varied with the severity of heart failure. These workers reported that eighty-one per cent of control animals had cTnI levels below 0·05 ng/mL, compared with significant elevations in animals with acquired heart disease (Spratt e*t al*., 2005). The above findings showed a positive relationship between circulating troponin I levels and severity of heart disease and suggested that measurements of serum cTnI concentrations might be useful in defining both the presence and severity of heart failure. However, no statistical analysis was attempted to develop a CMVD classification in dogs by cardiac markers. Oyama and Sisson (2004) found a significant difference in cTnI levels between dogs with mitral valve disease and normal controls. A significant difference was also found in serum cTnI levels in dogs with acquired mitral valve disease compared with control dogs, similarly to the present study (Spratt e*t al*., 2005). The research by Pelander *et al.* (2002) found a much broader range of cTnI levels in healthy dogs. Furthermore, these workers found that some dogs with CMVD also had elevated cTnI levels, although

a large number of symptomatic dogs had low blood troponin concentrations. Adams *et al*. (1993) proposed cTnI as a confirmative analysis while elevations of CK-MB may be indicative of myocardial injury in humans. Diniz *et al*. (2007) reported that cTnI and CK-MB serum analyses were sensitive and specific indicators of cardiac damage in dogs with blunt chest trauma. Serum CK-MB and LDH levels marginally increased in this study on dogs with CMVD with or without cardiac thrill. Compared with cTnI, diagnostic sensitivity of CK-MB and LDH were lower. In Dobermans affected with idiopathic dilative myocardiopathy, lower concentrations of troponin T at the myocardial level had been reported by O'Brien *et al*. (1997). Sato *et al.* (2001) had reported that the return to normal levels of cTnT following therapy also had a beneficial impact on prognosis, that they were significantly higher in patients with dilative myocardiopathy in survival times. Linklater *et al.* (2004) presenting cTnI, cTnT, BNP or pro-BNP levels in fifteen dogs with Class IV CHF due to CMVD and showed that 1 of 15 dogs had elevated cTnT, 6 had elevated cTnI. They suggested that elevation of cTnI and cTnT were no consistent and further studies need to be performed to evaluate these markers in dogs with CHF due to MR. In this study; comparing the values of CMVD group with normal laboratory values, elevated serum cTnI concentrations in 6 dogs of Ct group and 4 dogs of nCt group and elevated serum cTnT concentrations in 4 dogs of Ct group and 1 dog of nCt group were detected. Comparison of these results with the control group showed that both cTnI and CtnT were elevated significantly.

The findings of this study indicate that the biochemical markers activity elevated with the severity of the disease in dogs with CMVD. Measurement of cardiac troponins (cTnI, cTnT) as the newer markers than LDH and CK--MB concentrations may be useful in detection of cardiac injury and prognosis in dogs with CMVD.

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Address for correspondence:
Prof. Dr. Utku Bakirel
Department of internal medicine
Faculty of Veterinary, Istanbul University
Avcilar, 34851, Istanbul
Turkey
E-mail: utkubak@istanbul.edu.tr

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VREDNOSTI SRČANIH MARKERA KOD PASA SA HRONIČNO OBOLELIM MITRALNIM ZALISCIMA

BAKIREL U i GUNES S

SADRŽAJ

Cilj ove studije je bio da se utvrdi značaj koncentracija serumskog srčanog troponina I (cTnI), troponina T (cTnT), aktivnost laktat dehidrogenaze (LDH) i MB izoenzima kreatinkinaze (CK--MB) u otkrivanju oštećenja srca kod pasa sa hroničnom miralnom insuficijencijom (CMVD). Pedeset i pet pasa sa ehokardiografskom dijagnozom CMVD je podeljeno na grupu sa srčanim treperenjem (Ct) i grupu sa treperenjem koje nije srčanog porekla (nCt) na osnovu nalaza auskultacijom i palpacijom. U studiju je bilo uključeno i deset zdravih pasa. U uzorcima krvnog seruma određivane su koncentracije cTnI, cTnT, LDH i CK--MB. Srednje vrednosti (±SD) koncentracije serumskog cTnI su bile 1,9±0,38 ng/mL u grupi Ct i 1,75±0,27 ng/mL u grupi nCt. Koncentracije cTnI u obe ove podgrupe su bile značajno veće nego kod kontrolnih jedinki ($P<0.01$). Koncentracije cTnT i CK-MB su bile 0,031±0,02 ng/ml i 83,5±88,4 IU/L u grupi Ct i 0,024±0,01 ng/ml i 51,6±47,4 IU/L u grupi nCt, respektivno. Srednje vrednosti koncentracije cTnT i CK-MB u grupi Ct su bile značajno veće u odnosu na kontrolne jedinke ($P<0.01$, $P<0.05$). Aktivnost LDH je bila 918±136.4 IU/L u grupi Ct i 351±140.7 IU/L u grupi nCt. Razlike u aktivnosti LDH su bile značajne između sve tri grupe pasa ($P<0.05$).

Naši nalazi ukazuju da se koncentracija (ili aktivnost) ispitivanih srčanih markera povećava sa stepenom oštećenja srca pasa sa CMVD. Određivanje koncentracije srčanih troponina kao novijih markera u odnosu na LDH i CK-MB može da bude od koristi u detekciji srčanih oštećenja i postavljanju prognoze.