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ORIGINAL ARTICLE

Cardiac troponin I, fructosamine, and cardiovascular parameters in dogs with diabetes mellitus

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ABSTRACT. Diabetes mellitus (DM) is a common endocrinopathy in dogs, however, the relationship between canine DM and cardiomyopathy is still unclear. The aims of this study were: to evaluate serum troponin I concentrations in diabetic dogs under treatment with insulin; to evaluate the hypothesis that the time of DM diagnosis could influence the troponin I concentration; and to evaluate the relationship between biochemical and cardiovascular parameters in diabetic dogs. This is a cross-sectional study including diabetic canine patients under NPH insulin treatment. Troponin I concentration, systolic blood pressure (SBP), complete blood count, serum fructosamine concentration, biochemical profile, and Doppler echocardiography assesments were carried out in each patient, as well as in age- and size- matched healthy controls. All diabetic dogs had concentrations of troponin I below the limits of detection of the assay. There was no difference between cardiovascular parameters between diabetic and healthy control dogs. There was no correlation between time of DM diagnosis and cardiovascular parameters. There was a significant positive correlation between SBP and fructosamine in diabetic dogs (r=0.54; $P<0.01$). Diabetic dogs receiving insulin treatment, regardless of the time of diagnosis, do not have significantly elevated serum troponin I. The results also suggest that fructosamine levels can be associated with high blood pressure, suggesting a possible correlation between fructosamine levels and vascular complications.

Key words: blood pressure, cardiac biomarkers, echocardiography, endocrinology.

INTRODUCTION

Diabetes mellitus (DM) is a common endocrinopathy in dogs, characterised by hyperglycaemia and glycosuria. The effects of DM in human cardiovascular system include a particular cardiomyopathy, called diabetic cardiomyopathy, that can lead to congestive heart failure (Bell 2003, Schaan and Portal 2004) and increase the risk of death (Stamler *et al* 1993). Fructosamine levels seem to be one factor associated with these complications (Selvin *et al* 2011, Shafi *et al* 2013). However, the relationship between DM and cardiomyopathy in dogs is still unclear.

Cardiac troponin I (cTnI) is a highly sensitive biomarker specific to myocardial cell damage. In healthy canines, minimal to null levels of cardiac troponin are detected in the blood (Oyama 2015). High concentrations of cTnI have been previously reported in dogs with cardiac and extracardiac diseases (Lobetti *et al* 2008, Porciello *et al* 2008, Kocaturk *et al* 2012, Silvestrini *et al* 2012, Hamacher *et al* 2015), but the levels of cTnI in dogs with diabetes have not been investigated.

The aims of this study were: 1) to evaluate serum cTnI concentrations in diabetic dogs under treatment with insulin; 2) to evaluate the hypothesis that the time of DM diagnosis could influence the cTnI concentration and blood pressure; and 3) to evaluate the relationship between biochemical and cardiovascular parameters.

MATERIAL AND METHODS

This is a cross-sectional study including diabetic canine patients referred to the endocrinology service of a university veterinary hospital. The Animal Research Ethics Committee of the Universidade Federal do Rio Grande do Sul approved the study (protocol number 29879), and signed consent was obtained from all owners authorising participation in the study. A total of eighteen client-owned dogs with DM and twelve age- and size-matched healthy controls were included. The diagnosis of DM was based on medical history, clinical signs, physical examination, and clinical pathology. All dogs with DM were being treated twice daily with NPH insulin (dose ranging from 0.25 to 1.2 U/kg, mean 0.62 U/kg, standard deviation 0.24 U/kg). DM control was evaluated by the medical team and rated as poor (n = 1), fair (n = 2), and good (n = 15) on the basis of the following criteria: water intake (mL/24 h), thirst and urination control, body weight stability, serum fructosamine concentration, and biochemical profile. Concurrent diseases such as hyperadrenocorticism, endocardiosis, cardiomyopathies, and nephropathies, as well as the use of drugs that could interfere with the evaluated parameters, such as glucocorticoids, vasodilators, and diuretics were the exclusion criteria for the study. Assessment of the serum cTnI concentration, systolic blood pressure (SBP), complete blood count (CBC), serum fructosamine concentration, and biochemical profile and Doppler echocardiography were carried out in each patient, including dogs in the healthy control group.

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Vacuum blood samples were aseptically collected from the jugular vein with a 21-gauge needle. The samples were collected in ethylenediaminetetraacetic acid tubes and plain red-top tubes for assessment of the CBC and biochemistry profile, respectively. The samples from the plain red-top tubes were centrifuged for 10 min, and the serum was collected after clot retraction for 30 min at room temperature. The biochemical profile (colorimetric assay, CM-200, Wiener) was a measure of the albumin, alanine aminotransferase (ALT), total calcium, total cholesterol, creatinine, alkaline phosphatase (ALP), phosphorus, fructosamine, glucose, urea, and triglycerides levels.

Sera were stored at -20 °C for up to six months for cTnI analysis, which was performed using an electrochemiluminescence immunoassay (I-STAT). Both the inter-assay and intra-assay coefficient of variation were less than 10%. cTnI concentrations below the detection limit of the assay (0.16 ng/mL) were assigned the value of the lower detection limit.

For the Doppler echocardiographic examination (Sonosite Micromaxx®, sector transducer frequencies of 1-5 and 4-8 MHz), one-dimensional, two-dimensional, and Doppler wave modes were used. The following parameters were evaluated by echocardiography: right atrial dimension; left atrial dimension (LA); aortic root diameter (Ao); LA:Ao ratio; interventricular septum thickness in diastole; left-ventricular free-wall thickness in diastole; left-ventricular internal diameter in systole and diastole; right ventricular internal diameter in diastole; fractional shortening; ejection fraction (EF); and blood flow through the mitral, tricuspid, aortic, and pulmonary valves. The left-ventricular filling pattern was used to assess diastolic function by measuring the transmitral early (E) and late (A) diastolic flow wave velocities (E/A ratio), and isovolumetric relaxation time (TRIV).

A Doppler ultrasound (Medmega DV610) was used to assess the SBP according to the standards of the American College of Veterinary Internal Medicine (Brown *et al* 2007). The mean value of five consecutive SBP measurements was used for the statistical analysis. Patients with SBP > 160 mmHg were considered hypertensive.

GraphPad Prism software, version 6.0 (La Jolla, CA, USA) was used for the statistical analysis. Quantitative variables were presented as mean, standard deviation, minimum, and maximum values. The D´Agostino-Pearson test was used to check data distribution and normality. The variables were compared between groups using Student's t test. Pearson's correlation coefficient was used to compare cardiovascular parameters with other cardiovascular and biochemical parameters, insulin dosage, and time of DM diagnosis.

RESULTS

Thirty dogs were evaluated in this study. The DM group included eighteen dogs (one intact male, four neutered males, two intact females, and eleven spayed females), consisting of four mongrel dogs, two Poodles, two Dachshunds, two miniature Schnauzers, one Labrador, one Brazilian Terrier, one Fox Terrier, one Maltese, one Chow Chow, one Pointer, one West Highland White Terrier, and one Scottish Terrier, with a mean age of 10.6 ± 2.9 years (range: 4-14 years) and a mean weight of 10.8 ± 8.1 kg (range: 4.1-35.4 kg). The time since the initial DM diagnosis was 25.6 ± 17.8 months (range: 2-60 months). The healthy control group included twelve dogs (seven spayed females, five neutered males), consisting of six mongrel dogs, two Shih Tzus, one Poodle, one Dachshund, one Border Collie, and one Yorkshire Terrier, with a mean age of 10.4 ± 3.1 years (range: 5-15 years) and a mean weight of 13.0 ± 9.4 kg (range: 3.5-34.1 kg).

Results from the comparisons of cardiovascular and biochemical parameters between healthy and diabetic dogs are presented in table 1. There was no significant difference (P=0.22) in cTnI between the two groups. All diabetic dogs had concentrations of cTnI below the limits of detection of the assay. The serum fructosamine concentration was significantly high (P=0.003) in diabetic dogs. Correlations between cardiovascular parameters, time of DM diagnosis, and insulin dosage are presented in table 2. In addition, there were no significant correlations between time of diagnosis and insulin dosage (r=0.28; P=0.25), insulin dosage and serum fructosamine concentration (-0.15; P=0.54), or time of DM diagnosis and serum fructosamine concentration (r=0.04; P=0.87). The percentage of hypertension was 50% in diabetic dogs (SBP 172 ± 38.0 mmHg, range: 130 to 270 mmHg) and 33.3% in the healthy control group (SBP 150 ± 14.8 mmHg, range: 128 to 170 mmHg). However, there was no significant difference in the mean SBP values between the two groups (P>0.05). In DM group, the SBP was not significantly correlated with left ventricular free wall thickness in diastole (r=-0.37; P=0.15), and interventricular septum thickness in diastole (r =-0.33; P=0.19).

DISCUSSION

Troponin I, but not brain natriuretic peptide, has been shown to be elevated in human patients with DM and coronary disease (Segre *et al* 2015). Therefore, cTnI appears to be the most promising biomarker for assessing the impact of DM on cardiomyocytes. Rabbit hearts subjected to prolonged alloxan-induced DM showed oedema, histiocyte proliferation, and myocarditis (Mir and Darzi 2009). In dogs, however, our results suggest that chronic hyperglycaemia does not seem to be significant to cause cardiomyocyte damage sufficiently to increase cardiac troponin I. One limitation of the present study is the detection limit of the troponin assay. It would be interesting to evaluate the cTnI concentration in diabetic dogs using tests that detect lower values. A comparison of serum cTnI measurements in untreated dogs with DM would be interesting, though ethically questionable.

Table 1. Cardiocirculatory parameters, and blood concentrations of glucose and fructosamine (mean ± standard deviation [minimum-maximum]) in diabetic and healthy dogs.

Parameter (unit)	DM group	Control group	P
SBP (mmHg)	172.28±38.04 [130-270]	150.25±14.80 [128-70]	0.06
HR (bpm)	125.28±32.43 [75-220]	116.25±20.68 [75-40]	0.4
Troponin I (ng/mL)	0.16 [0.16-0.16]	0.19 [0.16-0.49]	0.22
LVWd (cm)	0.57±0.13 [0.38-0.91]	0.61±0.14 [0.42-0.84]	0.4
IVSd (cm)	0.64±0.12 [0.44-0.91]	0.70 ± 0.18 [0.46-0.98]	0.23
FS (%)	38.22±5.87 [30-52]	41.25±5.12 [34-49]	0.15
EF (%)	70.67±6.75 [60-86]	73.75±5.72 [65-82]	0.2
E/A ratio	1.0±0.28 [0.7-1.4]	1.2±0.39 [0.4-1.7]	0.11
TRIV (ms)	64.7±14.4 [35-90]	59.1±9.7 [40-70]	0.3
Glucose (mg/dL)	272.11±161.61 [84-691]	108.44±19.93 [83-145]	0.006*
Fructosamine (mmol/L)	442.52±182.27 [248-1027]	244.8±27.96 [195-281]	0.003*
Cholesterol (mg/dL)	329.24±96.49 [221-517]	218.38±71.32 [112-303]	0.09
Triglycerides (mg/dL)	306.56±292.46 [36-931]	93.0±30.18 [46-122]	0.13

*Indicates statistically significant difference between patients before and after treatment ($P<0.05$).
SBP: systolic blood pressure, HR: heart rate, LVWd: left ventricular free wall thickness in diastole, IVSd: interventricular septum thickness in diastole, FS: fractional shortening, EF: ejection fraction, E: transmitral early diastolic flow, A: transmitral late diastolic flow, TRIV: isovolumetric relaxation time.

Table 2. Pearson's correlation coefficients among cardiovascular parameters, time of diagnosis of DM, insulin dosage, and fructosamine.

Parameter (unit)	Time of diagnosis	Insulin dosage	Fructosamine in diabetic dogs	Fructosamine in all dogs
SBP	0.17 (P=0.47)	0.41 (P=0.08)	0.54 ($P<0.05$)*	0.57 ($P<0.01$) *
FS	0.25 (P=0.31)	–0.24 (P=0.32)	0.05 (P=0.83)	–0.139 (P=0.48)
EF	0.22 (P=0.37)	–0.18 (P=0.45)	–0.02 (P=0.91)	–0.186 (P=0.35)
E/A ratio	–0.28 (P=0.27)	0.24 (P=0.35)	–0.31 (P=0.24)	–0.07 (P=0.78)
TRIV	–0.07 (P=0.8)	0.39 (P=0.12)	0.23 (P=0.4)	0.26 (P=0.24)

*Significant ($P<0.05$);
SBP: systolic blood pressure, FS: fractional shortening, EF: ejection fraction, E: transmitral early diastolic flow, A: transmitral late diastolic flow, TRIV: isovolumetric relaxation time.

The only significant differences between diabetic and healthy dogs in this study were the serum concentrations of fructosamine and glucose, highlighting that the primary difference between the groups was the glycaemic status. The two groups were homogeneous with respect to mean weight and age. Most patients had satisfactory DM control at the time of evaluation. Those patients with fair or poorly controlled DM did not demonstrate significant changes in the evaluated parameters.

The effect of insulin on blood pressure in dogs remains uncertain. Studies have shown antinatriuretic (Baum 1987, Ferailli *et al* 1995) and hypertensive (Edwards and Tipton 1989, Song *et al* 2006) actions of insulin in animal models. However, a previous study that evaluated dogs receiving an insulin infusion did not show an increase in blood pressure (Brands *et al* 1991). Our results demonstrate a tendency toward positive correlation between insulin dosage and SBP. This may reinforce the importance of measuring blood pressure in diabetic dogs during treatment, especially those poorly controlled despite receiving higher insulin dosages. One limitation of this study was the absence of serum electrolytes measurements.

The association of fructosamine with cardiovascular parameters is not well understood in diabetic and non-diabetic dogs. High fructosamine levels appear to be associated with micro- and macro-vascular complications (Selvin *et al* 2011, Selvin *et al* 2014), as well as mortality, in human patients with or without a history of diabetes (Browner *et al* 1999, Shafi *et al* 2013). The results of the present study suggests a positive correlation between serum fructosamine levels and blood pressure in both diabetic and healthy dogs. This suggests that there may be a correlation between fructosamine levels and vascular complications in dogs.

Previous reports show that 35-46% of diabetic dogs have systemic hypertension (Struble *et al* 1998, Mazzi *et al* 2008, Herring *et al* 2014). The percentage of hypertension

in diabetic dogs was higher in the present study than previously reported. However, the values of SBP in diabetic dogs did not show any significant difference compared with healthy dogs in the control group. Although we standardised the SBP measurement, excitement or anxiety may have led to artifactual hypertension (Brown *et al* 2007). A study conducted at the same veterinary hospital showed that the environment may significantly influence blood pressure values (Soares *et al* 2012). The high percentage of hypertension in the group of healthy dogs reinforces this hypothesis.

Cardiovascular complications, including heart failure, coronary disease, and systemic hypertension, lead to death in more than 50% of human diabetic patients (Stamler *et al* 1993). In humans with type I DM, hypertension occurs later in the course of disease and may be associated with nephropathy (Sowers *et al* 2001). A longitudinal study did showed no association between vascular complications and the time of diagnosis in diabetic dogs. In the same study, dogs diagnosed less than 1 year prior to the study were included and followed up for 24 months (Herring *et al* 2014). The present study also did not demonstrate a correlation between cardiovascular parameters and the time of DM diagnosis. Furthermore, dogs that had been diagnosed with DM up to 60 months prior to the study were included, reinforcing that the time of diagnosis is not a risk factor for the development of cardiovascular complications in diabetic dogs.

Induced DM in rats led to systolic dysfunction, as evidenced by a reduction in fractional shortening and the EF (Wichi *et al* 2007). An experimental study in dogs with induced DM suggested depression of myocardial inotropism (Law *et al* 1991). The present study, however, did not find a difference in systolic function, evaluated in terms of fractional shortening and EFs, between diabetic and healthy dogs, or any significant correlation between the results of echocardiography and other parameters. Echocardiographic evaluation was not the primary focus of the present study; therefore, further studies are needed to evaluate the echocardiographic effects of canine DM.

Previous studies evidenced diastolic dysfunction in poor controlled (Liu *et al* 2001) and asymptomatic (Zabalgoitia *et al* 2001) diabetic patients. In some canine diseases, diastolic dysfunction can be an early event detected by a reversal E/A ratio and prolonged TRIV by echocardiography. In our patients, however, there was no significant difference in diastolic function between diabetic and control group.

In conclusion, diabetic dogs receiving insulin treatment, regardless of the time of diagnosis, do not have significantly elevated serum troponin I levels as detected by chemiluminescence. Our findings also suggest that there is a correlation between fructosamine levels and hypertension in dogs. These results highlight the importance of monitoring a complete panel in diabetic dogs, including insulin dose, cardiovascular, and biochemical parameters.

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