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Correlation of malondialdehyde concentration with cardiac parameters of different stages of chronic mitral valve disease in dogs

[Correlação da concentração de malondialdeído com parâmetros cardíacos de diferentes estágios da doença valvar mitral crônica em cães]

W.K. *Almeida*, L.A. *Yonezawa*, D.R. *Soares*, B. *Silva*, T.A. *Santos*, M.E. *Saito*

Universidade do Estado de Santa Catarina, Lages, SC, Brasil

ABSTRACT

Malondialdehyde (MDA) is a biomarker of oxidative metabolism, released under situations such as the stretching of atrial myocytes, a fact related to the development of chronic mitral valve disease (MVD) in dogs. This study aimed to evaluate the serum concentration of MDA in dogs at different stages of MVD and correlate it with echocardiographic and radiographic parameters referring to cardiomegaly signs. Thirty-seven dogs were divided into four stages of the disease: GA (n=10), GB1 (n=10), GB2 (n=9), and GC (n=8), following the criteria of the American College of Veterinary Internal Medicine (ACVIM). Blood was collected by jugular puncture and then the serum was frozen for later measurement of MDA concentration by the spectrophotometry technique. No difference was found in MDA concentration between groups despite the presence of cardiac remodeling in dogs at advanced stages of MVD. Moreover, no significant relationship was observed between MDA values and the studied cardiovascular parameters. Serum MDA showed no variation throughout MVD development even with evidence of its release, with no possibility of demonstrating the relationship between its concentration and the cardiovascular alterations caused by the disease in dogs.

Keywords: congestive heart failure, oxidative metabolism, echocardiogram

RESUMO

Malondialdeído (MDA) é um biomarcador do metabolismo oxidativo, liberado em situações como o estiramento dos miócitos atriais, fato este relacionado ao desenvolvimento da doença valvar mitral crônica (DVM) em cães. O objetivo deste trabalho foi avaliar as concentrações séricas de MDA em cães com diferentes estágios de DVM e correlacionar esses resultados com parâmetros ecocardiográficos e radiográficos referentes aos sinais de cardiomegalia. Foram utilizados 37 cães, divididos em quatro estágios da doença: GA (n=10), GB1 (n=10), GB2 (n=9) e GC (n=8), de acordo com os critérios do Colégio Americano de Medicina Interna Veterinária (ACVIM). O sangue foi colhido por venopunção jugular, sendo então o soro congelado para posterior mensuração da concentração de MDA pela técnica de espectrofotometria. Apesar da presença de remodelamento cardíaco nos cães em estágios avançados de DVM, não houve diferenças entre as concentrações de MDA entre os grupos. Além disso, não houve relação significativa dos valores de MDA com os parâmetros cardiovasculares estudados. Mesmo havendo evidências de suas liberações, o MDA sérico não varia ao longo do desenvolvimento da DVM e suas concentrações não estão relacionadas às alterações cardiovasculares provocadas pela doença em cães.

Palavras-chave: insuficiência cardíaca congestive, metabolismo oxidativo, ecocardiograma

Corresponding author: wkaida@hotmail.com
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INTRODUCTION

Chronic mitral valve disease (MVD) is a valvulopathy that mainly affects male dogs of small breeds such as the Cavalier King Charles Spaniel, Poodle, Chihuahua, Pinscher, Yorkshire, Shih Tzu, Dachshund, and Pekingese (Borgarelli and Buchanan, 2012; Keene *et al.*, 2019; Mattin *et al.*, 2015). Atrial dilation is one of the main alterations among the cardiac repercussions that this disease can cause, and its severity is one of the factors used for staging and prognostic evaluation of dogs (Keene *et al.*, 2019; Vezzosi *et al.*, 2021). Other cardiac effects of MVD include ventricular dilation and eccentric myocardial hypertrophy due to the overload that the ventricle undergoes with the increase in atrial volume (Vatnikov *et al.*, 2020).

Stretching of atrial myocytes can stimulate an increase in the production of reactive oxygen species (ROS) without increasing their antioxidant defenses, thus leading to oxidative stress (Chen *et al.*, 2009; Reimann *et al.*, 2017). Furthermore, ventricular dilation and its hypertrophy can also play an important role in ROS production in humans (Pimentel *et al.*, 2001). The increased presence of ROS can cause deleterious effects on cardiomyocytes and may stimulate hypertrophy or even apoptosis of myocardial cells (Giordano, 2005). ROS can also cause an event in cardiomyocytes called lipid peroxidation, leading to damage to the cell membrane and altering the flow of ion channels, such as calcium channels (Giordano, 2005).

Malondialdehyde (MDA) is the end product of lipid peroxidation, thus being an important biomarker of oxidative metabolism in veterinary medicine (Prasad *et al.*, 1996; Reimann *et al.*, 2017; Verk *et al.*, 2017; Yonezawa *et al.*, 2010). In humans, the relationship between increased serum levels of MDA and unfavorable prognosis has already been established in patients with heart failure (Romuk *et al.*, 2019). According to Reimann *et al.* (2017), plasma MDA levels in dogs do not vary between MVD stages but variables such as sex, body score, and serum cholesterol are related to MDA in these dogs. However, the relationship between serum MDA and cardiovascular variables still needs to be studied for a better understanding of oxidative stress in valvopathies in dogs.

The development of MVD in dogs leads to cardiac and systemic alterations until the emergence of CHF but other mechanisms associated with these changes occur in the organism of animals and may cause the worsening of this disease. In this sense, this study aimed to evaluate the MDA concentration in dogs at different stages of MVD and verify whether there is a relationship between the variables used for the disease staging and phisiopathogenesis.

MATERIAL AND METHODS

This study was approved by the Ethics Committee on Animal Use (CEUA) of the State University of Santa Catarina (UDESC). The study was carried out on the facilities of the Hospital de Clínicas Veterinárias (HCV) at the Centro de Ciências Agroveterinárias – CAV, UDESC, in Lages/SC, with the collaboration of the Small Animal Medical Clinic Services, Veterinary Cardiology, and Laboratory of Veterinary Clinical and Imaging Diagnostic.

Cardiovascular analyses used for staging and comparison were those described by the American College of Veterinary Internal Medicine (ACVIM), namely: thoracic radiography for measuring vertebral heart size (VHS) and presence of pulmonary edema; and echocardiographic evaluation to measure the left atrium-to-aorta ratio (LA:Ao) and the weight-normalized diastolic diameter (WNDD), following the methods described by Keene *et al.* (2019). In addition, parameters indicative of cardiac hypertrophy were also included in the echocardiographic evaluation, such as the measurement of the thickness of the interventricular septum (IVS) and the left ventricular free wall (LVFW) at systole and diastole.

Thirty-seven dogs were allocated into four groups, according to the ACVIM classification for dogs with chronic mitral valve disease (Keene *et al.*, 2019), namely: Group A (GA), composed of adult dogs of breeds at high risk of developing the disease (Borgarelli and Buchanan, 2012; Keene *et al.*, 2019; Mattin *et al.*, 2015) but still did not present cardiac structural alterations; Group B1 (GB1), composed of dogs with cardiac structural alterations (presence of a murmur on

auscultation) but with no signs of remodeling on echocardiography and thoracic radiography (LA:Ao<1.6, WNDD<1.7, and VHS<10.5); Group B2 (GB2), composed of dogs with cardiac structural changes (murmur of grade III or greater on auscultation) and signs of remodeling on echocardiography and thoracic radiography (LA:Ao>1.6, WNDD>1.7, and VHS>10.5); and Group C (CG), composed of dogs with signs of congestive heart failure resulting from structural cardiac alterations with the acute presence of pulmonary edema or stabilized by medications recommended for the treatment.

Venous blood was collected using the jugular vein puncture method, using a 22G needle coupled to a 10mL syringe. The samples were placed in tubes containing clot activator to obtain the serum, followed by freezing at 20°C for subsequent MDA measurement. The determination of serum malondialdehyde was performed by spectrophotometry, according to the technique described by Esterbauer and Cheeseman (1990).

A computerized statistical program was used for statistical analyses. The data were analyzed using the Kolmogorov-Smirnov test and, subsequently, the parametric variables were first evaluated using the one-way analysis of variance (ANOVA) test for repeated measures and the means were compared using the Tukey's test when significant. Non-parametric data were analyzed using the Kruskal-Wallis test. Pearson's correlation analysis was performed when parametric or Spearman's correlation when non-parametric, with MDA results being compared with those obtained by echocardiographic evaluation and thoracic radiography. All analyses were considered significant when $p<0.05$.

RESULTS

Among the 37 dogs accepted to participate, ten were from GA, ten from the GB1 group, nine from GB2, and eight from GC. Twenty-three animals were female (62.16%) and 14 males (37.84%), and the age of the animals was 10.6±3.5 years, with a weight of 6.04±3.13kg.

Regarding breeds, Poodle (40%), Shih-Tzu (30%), Lhasa Apso (10%), Yorkshire (10%), and Dachshund (10%) breeds were used in GA. The other 27 animals in GB1, GB2, and GC groups consisted of mixed-breed dogs (MBD) (33.4%), Poodle (22.2%), Dachshund (11.1%), Pinscher (7.4%), Schnauzer (7.4%), Yorkshire (7.4%), Shih-Tzu (3.7%), Lhasa Apso (3.7%), and Chihuahua (3.7%). Regarding the clinical presentation of symptomatic dogs (GC), three of them had an acute episode of CHF, and five had a stabilized CHF after starting treatment at the time of evaluation.

Table 1 shows the MDA concentration of the different groups. However, no difference was observed between the studied groups.

Table 1. Mean ± standard deviation of serum malondialdehyde (MDA) concentration in dogs at different stages of mitral valve disease

	GA	GB1	GB2	GC	p
MDA (µmol/L)	1.177 ±0.621	0.996 ±0.293	1.147 ±0.477	1.085 ±0.331	0.821

MDA: malondialdehyde; p: significance level.

M-mode echocardiographic evaluation (Table 2) presented a significantly higher value for the left atrium diameter in dogs from GB2 and CG compared to GA. The LA:Ao ratio in groups A and B1 was lower than that found in groups B2 and C. WNDD and left ventricular internal diameter at diastole (LVIDd) were higher in groups B2 and C when compared to GA. All dogs in groups B1 and B2 exhibited LA:Ao and WNDD values within the range mentioned for their classification, according to Keene *et al.* (2019).

Although significant differences between MVD stages were found both in the M-mode evaluation, no correlation was observed between these values and the MDA results (Table 3).

Table 4 shows a comparison between the results of the thorax radiography of dogs at different MVD stages. Groups GB2 and GC had a higher value than GA in the thorax radiography evaluation.

Table 2. Mean ± standard deviation or median (25th percentile; 75th percentile) of M-mode echocardiographic parameters in dogs at different stages of mitral valve disease

	GA	GB1	GB2	GC	p
Aorta (mm)	11.65 ±1.87	12.71 ±3.80	12.02 ±2.32	11.61 ±2.37	0.80
LA (mm)	14.76^{a} ±1.94	18.36^{ab} ±5.44	22.74^{b} ±4.96	23.63^{b} ±6.06	<0.01
LA:Ao	1.25^{a} (1.18; 1.36)	1.49^{a} (1.40; 1.54)	1.92^{b} (1.66; 2.10)	2.05^{b} (1.83; 2.24)	<0.01
IVSd (mm)	6.40 ±0.80	6.40 ±1.10	6.73 ±0.84	6.72 ±1.24	0.89
IVSs (mm)	10.16 ±1.55	11.12 ±2.20	12.73 ±1.74	12.13 ±2.67	0.06
LVFWd (mm)	5.70 (5.30; 8.80)	5.90 (5.40; 7.80)	6.90 (5.50; 7.70)	6.50 (6.20; 7.10)	0.77
LVFWs (mm)	9.75 ±2.26	10.01 ±2.24	11.02 ±2.16	10.68 ±0.81	0.28
WNDD	1.32^{a} ±0.12	1.42^{a} ±0.17	2.05^{b} ±0.23	1.83^{b} ±0.35	<0.01

LA: left atrium; LA:Ao: left atrium-to-aorta ratio; IVS: interventricular septum; d: diastole; s: systole; LVFW: left ventricular free wall; WNDD: weight-normalized diastolic diameter; p: significance level. Means followed by different letters on the same row indicate a significant difference between groups (p<0.05).

Table 3. Correlation between malondialdehyde (MDA) and cardiac chamber sizes on the echocardiographic examination of dogs at different stages of mitral valve disease

	MDA	
	r	p
AE:Ao	0.003	0.982
DDNP	0.014	0.932
SIVd	-0.102	0.549
SIVs	-0.151	0.373
PLVEd	-0.056	0.740
PLVEs	-0.079	0.639

MDA: malondialdehyde; r: correlation coefficient; p: significance level; LA:Ao: left atrium-to-aorta ratio; WNDD: weight-normalized diastolic diameter; IVS: interventricular septum; d: diastole; s: systole, LVFW: left ventricle free wall.

Table 4. Mean ± standard deviation of the vertebral heart size (VHS) of dogs at different stages of mitral valve disease

	GA	GB1	GB2	GC	p
VHS	9.9^{a} ±0.76	10.73^{ab} ±0.83	11.49^{bc} ±0.68	11.98^{c} ±1.04	<0.001

p: significance level. Means followed by different letters on the same row indicate a significant difference between groups (p<0.05).
No significant correlation was found between VHS values and serum MDA concentrations (r= 0.029; p= 0.864).

DISCUSSION

The difference between groups regarding echocardiographic parameters was already expected, as atrial dilation and its relationship with the aorta, left ventricular diastolic diameter are described as factors for disease staging (Keene *et al.*, 2019; Vezzosi *et al.*, 2021). VHS was higher in CG than in GA, reflecting the signs of cardiac remodeling caused by the disease (Keene *et al.*, 2019; Malcolm *et al.*, 2018). However, the value of this parameter found in GB1 was higher than that indicated by Keene *et al.* (2019). It suggests that the VHS analysis should always be followed by an echocardiographic examination for a better assessment of the presence of cardiac dilation. According to Poad *et al.* (2020), a VHS higher than 10.8 has a greater correlation with cardiac dilation found in MVD in dogs, which corroborates the finding of this research.

In this study, no difference was found in the concentration of MDA in the different stages of MVD in dogs. MDA is the result of lipid peroxidation, a process related to the increase in ROS release in situations such as atrial dilation or volume overload (Chen *et al.*, 2009; Reimann *et al.*, 2017). In humans, MDA concentration in patients with heart failure is higher than that in patients with heart disease without heart failure and can even be used as a prognostic tool (Romuk *et al.*, 2019; Serdar *et al.*, 2001). However, there are no studies correlating MDA values in humans with DVM before CHF development, generating uncertainties about its

release during the initial stages (Reimann *et al.*, 2017).

Regarding oxidative metabolism, Lynch *et al.* (2015) verified the occurrence of high oxidative stress in an experimental model of mice with dilated cardiomyopathy induced by a gene mutation. It is an important finding, as therapeutic strategies to attenuate oxidative stress may represent a new way to reduce the development of cardiomyopathy and heart failure. In a study with dogs with MVD or dilated cardiomyopathy, Verk *et al.* (2017) found a difference between the MDA concentration of asymptomatic dogs and those with moderate signs of heart failure, which was random, with no biological significance. However, Svete *et al.* (2021), also studying dogs with MVD or dilated cardiomyopathy, found no difference in MDA concentrations between symptomatic and asymptomatic groups, corroborating the findings of this study, suggesting that the extent of lipid peroxidation may occur regularly in the heart even as the disease progresses. The serum values of MDA found in this research are close to those found by Reimann *et al.* (2017), in which the mean plasma concentration of this marker was 1.04 µmol/L in dogs with stage C MVD, also not differing from the values found in the other groups.

Although a correlation between MDA values and the measurements of the cardiovascular tests has not been demonstrated, a positive relationship between left atrial enlargement and hypertension with the presence of oxidative stress has already been established in humans, in addition to a negative correlation of the ejection fraction with MDA concentration (Chen *et al.*, 2009; Polidori *et al.*, 2002). In dogs with MVD, the already identified correlations with MDA values are related to sex, body score, and serum cholesterol concentration (Reimann *et al.*, 2017). However, a study with tissue MDA from the left ventricle of dogs with mitral regurgitation (Prasad *et al.*, 1996) showed a higher concentration in patients compared to healthy ones, which could be related to the depuration or binding of MDA after its formation (Esterbauer and Cheeseman, 1990). Therefore, MDA can be formed in heart diseases, but it does not seem to be reflected in its serum concentration, except when related to other non-cardiac factors.

The inhibition of antioxidant defenses may have a role in increasing MDA production, as the oxidizing actions allow the inhibition of lipid peroxidation (Michael, 2007). Freeman *et al.* (1999) demonstrated a decrease in the plasma concentration of vitamin E, a fat-soluble antioxidant, in the most severe cases of idiopathic dilated cardiomyopathy in 18 dogs. However, feeding and supplementation with antioxidant agents in dogs under circumstances that stimulate stress may decrease ROS production in these animals (Baskin *et al.*, 2000). Furthermore, Hetyey *et al.* (2007) found no differences in total antioxidant activity between healthy dogs and those diagnosed with MVD, suggesting that antioxidant activity may remain preserved in this disease. Therefore, extrinsic and intrinsic factors may be related to ROS production in MVD through antioxidant actions.

CONCLUSION

Serum MDA concentration had no variation between MVD stages even though its release is related to the development of the disease. The findings on the echocardiographic and thorax radiographic examinations changed with the disease evolution due to the enlargement of the heart chambers, but no correlation was observed with the serum MDA concentration.

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