RESEARCH ARTICLE

Creatine Kinase-MB as a Cardiac Biomarker in Canine Cardiac Disorders

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Abstract

Cardiovascular diseases are the most common causes of death in dogs. Estimation of Creatine Kinase-MB is used for diagnostic and prognostic information in dogs' case of congestive heart failure. The present study was undertaken to determine the serum concentrations of the Creatine Kinase-MB isoenzyme in apparently healthy dogs and dogs affected with congestive heart failure and also to distinguish between the stages and duration of congestive heart failure. Creatine Kinase-MB was measured in dogs with cardiac diseases and in apparently healthy dogs. There was a significant increase ($p \leq 0.01$) in the concentration of Creatine Kinase-MB in cardiac disorder in dogs compared to the apparently healthy dogs. Creatine Kinase-MB concentration reached a peak in per-acute, acute and sub-acute stages of the cardiac disorders as compared to the apparently healthy dogs. In CHIEF system, there was a significant ($p \leq 0.01$) increase in the Creatine Kinase-MB in stages B, C1, and C2, and C3 as compared with the apparently healthy dogs and decrease ($p \leq 0.01$) in the Creatine Kinase-MB in stage D as compared to other stages.

Keywords: Cardiac disorders, Creatine Kinase-MB, Dogs.

Ind J Vet Sci and Biotech (2021): 10.21887/ijvsbt.17.3.6

Introduction

Cardiovascular diseases risk assessment has alluded the markers that assist and anticipate long haul morbidity and mortality for heart diseases. Circulatory markers like low-density lipoproteins, high-density lipoproteins, triglycerides, total cholesterol, high-sensitivity C-reactive protein (hs-CRP), atrial natriuretic peptide, brain natriuretic peptide, and many others are used in veterinary medical practice. However, in clinical cases of suspected myocardial injury, the cardiovascular assessment relies on a different class of serum biomarkers (Saenger, 2010). Measurement of Creatine Kinase-MB, Cardiac Troponin - T, and Cardiac Troponin - I, are used to diagnosing myocardial injury. Clinicians rely on Creatine Kinase-MB and troponin markers for diagnostic and prognostic information in cases of congestive heart failure in dogs (Blake, 2018). Creatine Kinase is a dimer enzyme composed of two monomer subunits termed as "M" to denote muscle origin and "B" to denote brain origin. These two subunits combine to form three distinct cytosolic isoenzymes: two homodimers, CK-MM and CK-BB, and one heterodimer, CK-MB. The CK-MM is mostly found in muscle cells, CK-BB exists mainly in the brain cells, CK-MB is found in higher concentration in the myocardium and to a lesser extent in skeletal muscle. Increased circulating levels of the CK-MB isoform will occur if the myocardium is damaged. The isoenzyme CK-MB is more cardiac-specific and hence helps to distinguish myocardial damage from skeletal muscle damage (Saenger, 2010).

The present study was undertaken with the objective to determine the serum concentrations of the Creatine Kinase-MB isoenzyme in apparently healthy dogs and that affected with congestive heart failure due to cardiac disorders and also to distinguish between the stages and duration of congestive heart failure.

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How to cite this article: Sagar, R.S., Mudraje, N.B., Chikmagalur, A.K., Siddaraju, N.K., Appaiah, K.M., Sahadev, A., Rao, S., & Panchaxarayya, K.G. (2021). Creatine Kinase-MB as a Cardiac Biomarker in Canine Cardiac Disorders. Ind J Vet Sci and Biotech, 17(3): 27-30.

Source of support: Nil

Conflict of interest: None.

Submitted: 12/02/2021 **Accepted:** 24/06/2021 **Published:** 16/08/2021

Materials and Methods

One hundred dogs with the clinical signs suggestive of cardiac diseases *viz*. lethargy, weakness, dyspnoea, cough, syncope, exercise intolerance, ascites, oedema of dependant parts, anorexia, weight loss and depression brought to the Small Animal Medicine Unit, Department of Veterinary Medicine, Veterinary Hospital, Veterinary College, Hebbal, Bengaluru were included in the present study. Twenty-five apparently healthy dogs were kept as control. To diagnose cardiac disorders, all affected dogs were subjected to detailed

clinical examination, electrocardiography, radiography, echocardiography, and haemato-biochemical assays. Cardiac diseases were confirmed by echocardiography and thoracic radiography.

The dogs with cardiac disease were categorized based on the duration of illness into per-acute (1 day), acute (2-5 days), sub-acute (5-7 days) and chronic (more than 7 days).

The dogs with cardiac disease or signs of weakness or collapse indicating congestive heart failure were classified as per the Canine Heart failure International Expert Forum (CHIEF) system (Wolf *et al.,* 2012). Class A: Patients at risk for heart disease; Class B: Patients with structural heart disease, but no signs of CHF; Class C: Past or current signs of CHF; C1: Patients with no clinical signs of CHF; C2: Patients with mild to moderate CHF; C3: Patients with severe to life-threatening CHF; Class D: Patients with refractory heart failure.

Estimation of Creatine Kinase–MB (CK-MB)

Blood was collected in BD serum vacutainer. Serum was stored at 2°C to 8°C if the assay was performed on the same day or frozen at -20°C and assayed later. Total CK activity was quantified by using spectrophotometric enzymatic assay using ERBA kit (ERBA PATH, Cat. No. BLT00081 and XSYS0029). Specific antibodies against CK-M inhibit the complete CKMM activity and the CK-M subunit of CKMB and only CK-B activity is measured (Gulia and Goswami, 2020).

The CKMB values obtained were compared with the apparently healthy dogs and correlated with the stages of congestive heart failure classified as per the Canine Heart failure International Expert Forum (CHIEF) system. Results were interpreted by using statistical software package – IBM SPSS Statistics v24 for Macbook.

Results and Discussion

In the present study, among hundred dogs, seven types of cardiac disorders were diagnosed *viz.* Dilated Cardiomyopathy (48/100, 48%) followed by Mitral Valve Disease (40/100, 40%), Idiopathic Pericardial Effusion (6/100, 6%), Tricuspid Valve Disease (4/100, 4%), Hypertrophic Cardiomyopathy (2/100, 2%) and Aortic Stenosis (1/100, 1%).

The occurrence of per-acute, acute, sub-acute and chronic forms of cardiac disorders were 7, 17, 42 and 34 per cent, respectively.

One hundred dogs with cardiac disorders were classified into various categories with Canine Heart failure International Expert Forum (CHIEF) system. Dogs categorized had six groups, Class A: Patients at risk for heart disease (0/100, 0%), Class B: Patients with structural heart disease, but no signs of CHF (13/100, 13 per cent), Class C1: Patients have no current signs of CHF (10/100, 10 per cent), Class C2: Dogs with mild to moderate signs of CHF (37/100, 37 per cent), Class C3: Dogs with severe to life-threatening heart failure (32/100, 32 per cent), Class D: Dogs with refractory failure (8/100, 8 per cent).

Creatine Kinase-MB

There was a significant ($p \leq 0.01$) increase in the Creatine Kinase-MB of dogs with cardiac disorder as compared to healthy dogs (Table 1). A significant ($p \leq 0.01$) increase was observed in the Creatine Kinase-MB in dogs with dilated Cardiomyopathy, mitral valve disease, idiopathic pericardial effusion, tricuspid valve disease, and Hypertrophic Cardiomyopathy. This might be due to the myocardial injury in cardiac disorders as suggested by Ishikawa *et al.* (1997).

There was significant ($p \leq 0.01$) increase in the Creatine Kinase-MB in dogs affected with per-acute (1 day), acute (2-5 days) and sub-acute (5-7 days) cardiac illness as compared to apparently healthy dogs (Table 2). The concentration of Creatine Kinase-MB plotted on Y axis to duration of illness in days on X axis, there was decrease in the concentration of Creatine Kinase-MB as the time lapses *i.e.,* in chronic cardiac cases, there concentration of Creatine Kinase-MB would come to normal (Fig. 1). Creatine Kinase-MB is primarily a cytosolic protein that is released into the lymphatics and rapidly cleared from the circulation. Serum Creatine Kinase-MB activity rises and falls over 72 hours following coronary artery occlusion (Aktas *et al.,* 1993; Voss *et al.,* 1995). The plasma half-life of Creatine Kinase in the dog has been reported as 2 hours and 24 minutes (Billadello *et al.,* 1989).

Creatine Kinase-MB analyzed in normal dogs, and dogs classified w.r.t. Canine Heart failure International Expert Forum system for congestive heart failure due to cardiac

Table 1: Mean ± SE values of Creatine Kinase – MB in healthy and cardiac disorders dogs

Sl No.	*Cardiac Disorders*	*Number of Dogs*	*CK-MB of Cardiac Disorders*	*T-Value*	*P-Value*
1.	Cardiac Disorders	100	24.98 ± 1.15	6.48**	0.000
2.	Dilated Cardiomyopathy (DCM)	48	27.54 ± 1.42	8.86**	0.000
3.	Mitral Valve Disease (MVD)	40	20.27 ± 1.53	5.34**	0.000
4.	Idiopathic Pericardial Effusion (IPE)	6	40.15 ± 4.66	12.87**	0.000
5.	Tricuspid Valve Disease (TVD)	3	18.70 ± 7.12	3.60**	0.001
6.	Hypertrophic Cardiomyopathy(HCM)	2	29.65 ± 22.45	4.02**	0.000
7.	Aortic Stenosis	1	9.1	2.34	0.817
8.	Healthy Dogs	25	9.66 ± 0.47	-	-

NS: Non-Significant at $P > 0.05$ level;* Significant at $P \leq 0.05$ level;** Significant at $P \leq 0.01$ level

Table 2: Mean ± SE values of Creatine Kinase – MB in cardiac disorders dogs classified by duration of illness

Sl No.	*Duration of Illness*	*Number of Dogs*	*CK-MB*	*T-Value*	*P-Value*
1.	Per acute (1 day)	7	40.67 ± 3.20	16.72**	0.000
2.	Acute (2-5 days)	17	35.10 ± 1.05	25.68**	0.000
3.	Sub-acute (5-7 days)	42	29.45 ± 1.90	16.04**	0.000
4.	Chronic (>7 days)	34	11.16 ± 0.56	1.95	0.056

NS: Non-Significant at P > 0.05 level;* Significant at P ≤ 0.05 level;** Significant at P ≤ 0.01 level

Table 3: Mean ± SE values of Creatine Kinase – MB in cardiac disorders dogs classified by CHIEF system

Class	*Description*		*Number of Dogs*	*CK-MB*	*T-Value*	*P-Value*
Class A	Patients at risk for heart disease		0	-	-	-
Class B	Patients with structural heart disease, but no signs of CHF		13	34.62 ± 0.82	28.32**	0.000
	C1	Patients have no current signs of CHF	10	29.21 ± 2.33	11.99**	0.000
Class C	C2	Patients have mild to moderate CHF	37	25.16 ± 1.45	8.53**	0.000
	C3	Patients have severe to life-threatening CHF	32	23.13 ± 2.58	4.54**	0.000
Class D	Patients with refractory heart failure		8	10.58 ± 0.95	0.93	0.359

NS: Non-Significant at P > 0.05 level;*Significant at P ≤ 0.05 level;** Significant at P ≤ 0.01 level

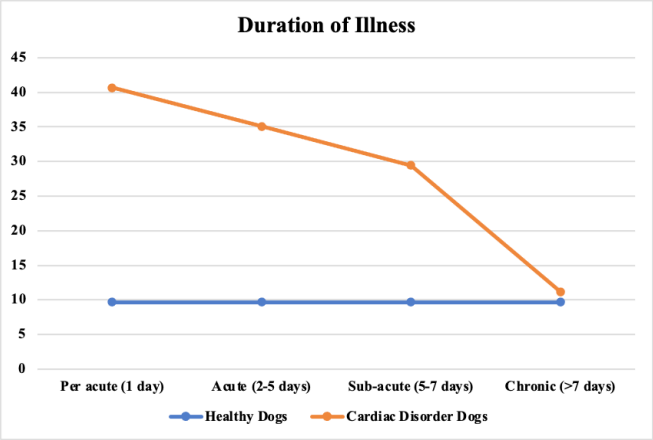

Fig. 1: Line Graph showing mean ± SE values of Creatine Kinase – MB in healthy and cardiac disorders dogs classified by duration of illness

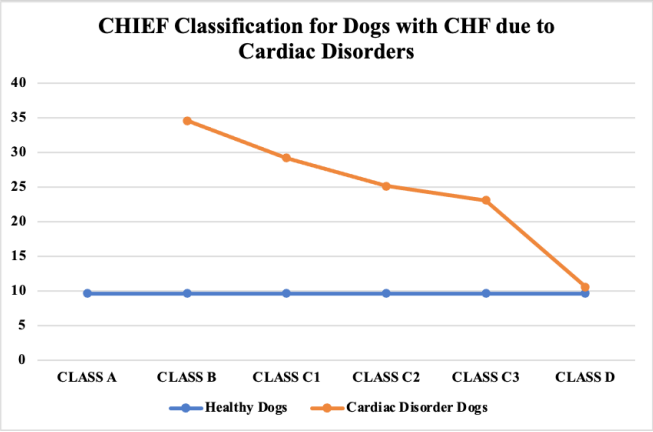

Fig. 2: Line Graph showing mean ± SE values of Creatine Kinase – MB in healthy and cardiac disorders dogs classified by CHIEF system

disorder showed significant (p ≤ 0.01) increase in Class B, C1, C2, and C3, and there was a non-significant difference in the concentration of the Creatine Kinase-MB in Class D: patients as compared to healthy dogs (Table 3). The concentration of Creatine Kinase-MB plotted on Y axis to stages of congestive heart failure on X-axis, there was a decrease in the concentration of Creatine Kinase-MB in advanced stages of congestive heart failure (Fig. 2). The short serum half-life of Creatine Kinase-MB is likely to be a reason for enzyme's poor diagnostic utility in dogs with cardiac disease.

Conclusion

This study concludes that, serial serum sampling of Creatine Kinase-MB and estimation of Creatine Kinase-MB at 8-to-12-hour intervals for 60 hours is required to observe the characteristic increase, peak and disappearance of Creatine Kinase-MB. Hence, single reading is insufficient, and serial readings of Creatine Kinase-MB are necessary to diagnose and stage the cardiac disorder. Creatine Kinase-MB is not a suitable indicator of cardiac failure or disease in dogs as it occurs in the natural setting. The lack of diagnostic utility likely relates to the difficulty in timing blood sampling to bouts of significant myocardial injury and shorter half-life of Creatine Kinase-MB. Measuring Creatine Kinase-MB may therefore have little clinical utility in the clinical setup.

Acknowledgment

The authors are thankful to Veterinary College, Hebbal, Bengaluru, and Karnataka Veterinary Animal and Fisheries Sciences University, Bidar, Karnataka for financial and material support.

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