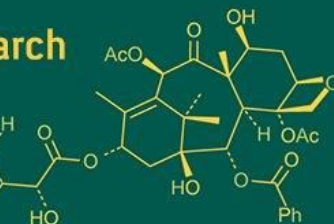
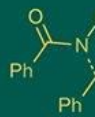


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Diagnostic significance of hematobiochemical and cardiac biomarkers in canine CHF associated with MVD and DCM

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Abstract

The present study aimed to evaluate the diagnostic significance of hematobiochemical parameters and cardiac biomarkers in dogs suffering from congestive heart failure (CHF) associated with dilated cardiomyopathy (DCM) and mitral valve disease (MVD). A total of 37 dogs diagnosed with CHF (DCM: 25; MVD: 12) were included based on clinical and diagnostic criteria. Blood samples were collected for hematological and biochemical analysis using automated analyzers, while cardiac biomarkers, namely cardiac troponin-I (cTnI) and N-terminal pro-B-type natriuretic peptide (NT-proBNP), were estimated using ELISA kits. Statistical analysis was performed using Student's t-test. The results revealed no significant differences in hematological and biochemical parameters between DCM and MVD groups, although mild alterations such as increased leukocyte count, elevated RDW, hypoalbuminemia and increased BUN were observed, indicating systemic involvement. In contrast, cardiac biomarkers showed marked elevation in both groups, reflecting myocardial injury and cardiac stress, though differences between groups were statistically non-significant. The study concludes that while hematobiochemical parameters provide supportive information, cardiac biomarkers such as cTnI and NT-proBNP serve as more sensitive and reliable indicators for the diagnosis and assessment of CHF in dogs, irrespective of underlying etiology.

Keywords: Congestive heart failure, dilated cardiomyopathy, mitral valve disease

Introduction

Cardiovascular diseases are among the major causes of morbidity in dogs and are increasingly recognized in veterinary practice with the rising companion animal population (Goutal *et al.*, 2010) [7]. Congestive heart failure (CHF) is a clinical syndrome characterized by impaired cardiac function and neurohormonal activation resulting in pulmonary or systemic fluid accumulation (Atkins *et al.*, 2009; Erling and Mazzaferro, 2008) [1, 5]. The most common underlying causes of CHF in dogs are Myxomatous Mitral Valve Disease (MMVD) and Dilated Cardiomyopathy (DCM), which lead to progressive cardiac remodelling, decreased cardiac output and eventual heart failure (Keene *et al.*, 2019) [8]. The diagnosis of CHF traditionally relies on clinical examination and imaging techniques such as thoracic radiography, electrocardiography and echocardiography. (Nelson and Couto, 2008) [10]. However, these techniques may not always detect early myocardial injury or reflect systemic alterations associated with disease progression. In addition to imaging, hematobiochemical parameters provide important supportive information regarding systemic involvement in dogs with CHF. Routine hematological findings such as alterations in hemoglobin concentration, packed cell volume, and leukocyte count may reflect anemia, inflammation, or stress responses associated with chronic cardiac disease. Similarly, biochemical parameters including liver enzymes, renal function markers and electrolyte imbalances may indicate secondary organ dysfunction resulting from reduced cardiac output and venous congestion (Shah *et al.*, 2021) [15]. These changes are particularly relevant in advanced stages of CHF, where multi-organ involvement is commonly observed. In recent years, cardiac biomarkers have emerged as valuable tools in the diagnosis and prognosis of cardiac diseases in dogs. These biomarkers reflect underlying pathophysiological changes within the myocardium and facilitate early detection of cardiac

injury and heart failure. Among these, cardiac Troponin I is considered a highly sensitive and specific indicator of myocardial cell injury, while N-terminal pro-B-type natriuretic peptide (NT-proBNP) is released in response to myocardial wall stress and ventricular stretching (Oyama *et al.*, 2007) [12]. Elevated concentrations of NT-proBNP and cardiac troponins have been consistently reported in dogs with CHF and are strongly associated with disease severity and prognosis. Measurement of these biomarkers provides important diagnostic and prognostic information, aiding in differentiation of cardiac from non-cardiac causes of clinical signs such as respiratory distress and allowing better assessment of disease progression.

Therefore, the combined evaluation of cardiac biomarkers along with hematobiochemical parameters can enhance diagnostic accuracy and provide a more comprehensive understanding of the pathophysiological status in dogs affected with CHF secondary to MMVD and DCM.

Materials and Methods

Study Location and Selection of Animals

The present study was conducted at the Department of Veterinary Medicine in collaboration with the Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari, Gujarat. A total of 37 dogs of different breeds, ages and sexes diagnosed with congestive heart failure (CHF) secondary to Dilated Cardiomyopathy (DCM) (n = 25) and Mitral Valve Disease (MVD) (n = 12) were included based on clinical history and diagnostic evaluation. Cases were included according to ACVIM (American College of Veterinary Internal Medicine) Stage C and D guidelines, while dogs with non-cardiac systemic conditions affecting cardiovascular dynamics were excluded.

Hematobiochemical Analysis

Approximately 5 mL of blood was aseptically collected from the cephalic or saphenous vein and aliquoted into K₃EDTA containing tubes for hematological analysis and clot activator tubes for serum separation. Hematological parameters including hemoglobin concentration, total erythrocyte count, total leukocyte count, packed cell volume, platelet count and differential leukocyte count were analyzed using a fully automatic blood cell counter (MEK-6420P, Nihon Kohden India). Biochemical analysis was performed using a semi-automated analyzer (Microlab 300) with commercially available diagnostic kits to estimate alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum creatinine, blood urea nitrogen (BUN), total protein and albumin.

Cardiac Biomarker Estimation

Serum concentrations of N-terminal pro-B-type natriuretic peptide (NT-proBNP) and cardiac troponin I (cTnI) were determined using commercially available ELISA kits FINETEST Canine NT proBNP and FINETEST Canine hs-cTnI ELISA kits. The analysis was performed using an ELISA reader (I Gene Lab serve). These biomarkers were evaluated to assess myocardial stress and injury in dogs with CHF.



Fig 1: Fully automatic blood cell counter (MEK-6420P, Nihon Kohden India)



Fig 2: Semi-automated biochemical analyzer (Microlab 300)



Fig 3: Enzyme-Linked Immunosorbent assay Kit for cTnI



Fig 4: Enzyme-Linked Immunosorbent assay Kit for NT-proBNP

Statistical Analysis

Data were analyzed using descriptive statistics and expressed as mean ± standard error (SE). Differences between DCM and MVD groups were evaluated using Student’s t-test, with significance set at $p \leq 0.05$. Statistical analysis was performed using IBM SPSS software (version 27.0).

Results and discussion

During the study period, out of 840 clinical cases presented at the Veterinary Clinical Complex, Navsari, 37 dogs were diagnosed with congestive heart failure, including 25 cases of dilated cardiomyopathy and 12 cases of mitral valve disease.

Hematobiochemical Findings

Hematological evaluation revealed no statistically significant differences ($p>0.05$) between DCM and MVD groups, indicating a comparable hematological response in both conditions (Table 1). Most parameters remained within normal reference ranges with only mild deviations. A slight increase in total leukocyte count and neutrophils suggested a stress leukogram or low-grade inflammatory response, which is commonly associated with chronic cardiac disease. These findings are consistent with previous reports (Brown, 2004; Ristic, 2004; Bomassi, 2007) [3, 13, 2] indicating that hematological changes in CHF are often subtle and non-specific.

A notable finding of the present study was the mild elevation in Red Cell Distribution Width (RDW) in affected dogs. Although the difference between DCM and MVD groups was not statistically significant, increased RDW may reflect early erythropoietic stress, tissue hypoxia and chronic systemic inflammation associated with heart failure. Similar observations have been reported by Tangmahakul *et al.* (2023) [16] and Sathyabama *et al.* (2022) [14], who highlighted RDW as a potential early indicator of disease progression and prognostic marker in canine cardiac disorders. Furthermore, recent findings by Kocaturk *et al.* (2024) [9] suggest that RDW-related indices may increase with worsening heart failure, supporting its clinical relevance even in the absence of marked hematological alterations. Thus, RDW represents an important supportive parameter in evaluating systemic involvement in CHF.

Biochemical analysis also showed no significant difference ($p>0.05$) between DCM and MVD groups (Table 2). However, mild alterations were observed, including slightly increased total protein, mild hypoalbuminemia and elevated BUN levels than the reference range. These changes may be attributed to chronic inflammation, hepatic congestion and reduced renal perfusion secondary to decreased cardiac output. Creatinine levels remained within normal limits but showed a mild increasing trend suggesting early renal involvement. Liver enzyme levels (ALT) were within normal limits indicating absence of significant hepatocellular injury despite possible passive congestion. These findings are in agreement with previous studies (Freeman *et al.*, 2012) [6], which reported that biochemical changes in CHF are generally mild and become more pronounced with disease progression.

Table 1: Means of hematological parameters in CHF dogs (n = 37)

Sr. No.	Parameters	DCM (n = 25)	MVD (n = 12)	p value
1	Hb (g/dL)	10.98±0.86	10.8±0.71	0.86
2	PCV (%)	35.98±0.87	35.9±0.43	0.98
3	RBC ($10^6/\mu\text{L}$)	06.42±0.67	06.63±0.38	0.67
4	WBC ($10^3/\mu\text{L}$)	17.3±0.28	14.24±0.86	0.28
5	Neutrophils (%)	76.11±0.33	73.10±0.02	0.33
6	Lymphocytes (%)	18.13±0.53	20.08±0.17	0.53
7	Eosinophils (%)	02.21±0.81	02.08±0.48	0.81
8	Monocyte (%)	03.50±0.42	04.50±0.37	0.05
9	Platelet ($10^5/\mu\text{L}$)	02.60±0.25	02.40±0.31	0.62
10	RDW (%)	16.63±0.38	15.60±0.42	0.99

Table 2: Means of biochemical parameters in CHF dogs (n = 37)

Sr. No.	Parameters	DCM (n = 25)	MVD (n = 12)	p values
1	ALT (IU/L)	42.17±8.30	50.92±11.45	0.54
2	Total protein (gm/dl)	07.85±0.38	07.51±0.30	0.49
3	Albumin (gm/dl)	02.18±0.13	02.21±0.13	0.83
4	BUN (mg/dl)	35.60±3.46	33.67±1.67	0.61
5	Creatinine (mg/dl)	01.05±0.06	01.10±0.07	0.60

Cardiac Biomarkers

In contrast to hematobiochemical parameters, cardiac biomarkers showed marked elevation in dogs with CHF. Both cardiac troponin-I (cTnI) and NT-proBNP levels were significantly higher compared to normal reference values, although no statistically significant difference was observed between DCM and MVD groups ($p>0.05$) (Table 3). This indicates that despite differences in etiology, both conditions produce a similar degree of myocardial injury and hemodynamic stress in advanced stages of CHF.

Table 3: Means of cardiac biomarkers in CHF dogs (n = 37)

Sr. No.	Parameters	DCM (n = 25)	MVD (n = 12)	p values
1	Cardiac troponin-I (ng/ml)	0.72±0.07	0.91±0.11	0.58
2	NT-proBNP (pmol/L)	2758±348.2	2832±593.12	0.78

The increased cTnI levels observed in the present study reflect ongoing cardiomyocyte damage, consistent with findings of Oyama and Sisson (2004) [11], who described cTnI as a highly sensitive marker of myocardial injury. Similarly, elevated NT-proBNP levels indicate ventricular stretch and volume overload, supporting its role as a reliable marker of cardiac dysfunction (Oyama *et al.*, 2007) [12]. The simultaneous elevation of both biomarkers highlights their complementary diagnostic value. While cTnI indicates

structural myocardial damage, NT-proBNP reflects functional cardiac stress and volume overload. Previous studies (Chanmongkolpanit *et al.*, 2024) ^[4] have also reported increased levels of these biomarkers in dogs with advanced cardiac disease, correlating with disease severity and prognosis.

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