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Research Article

HOSPITAL PREVALENCE OF CONGESTIVE HEART FAILURE IN DOGS AT AIZAWL AND ITS EFFECT ON HEMATO-BIOCHEMICAL AND OXIDANT-ANTIOXIDANT STATUS

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ABSTRACT

Objectives of the present study were to gauge the prevalence of canine congestive heart failure among dog population presented to Veterinary Clinical Complex, Aizawl and to find out the effect of the disease on hemato-biochemical and serum oxidant-antioxidant status in the affected dogs. The diagnosis was based on clinical examination, electrocardiography, thoracic radiography and echocardiography. Hospital prevalence of congestive heart failure was found to be 0.63%. Hemogram, plasma biochemical profile and oxidant/anti-oxidant status were assessed among the affected dogs and compared with healthy control. Hemogram revealed significant ($p<0.05$) reduction in lymphocyte count. Plasma biochemistry revealed significant ($p<0.05$) reduction in the levels of calcium, sodium, chloride and cholesterol. Serum oxidant-antioxidant levels of the affected dogs revealed significant ($p<0.05$) reduction in glutathione level with insignificant alterations in the levels of superoxide dismutase, lipid hydroperoxide and total antioxidants.

Keywords: Congestive heart failure, Echocardiography, Hemato-biochemistry, Serum oxidant-antioxidant status

Canine cardiac diseases are common, complex and often silent killers. Hence, any cardiac abnormality requires to be dealt with priority to avoid morbidity and mortality (Kumar *et al.*, 2014). The diagnosis of congestive heart failure (CHF) is generally accomplished by characteristic clinical signs, electrocardiography (ECG), radiography and echocardiography (Tilley *et al.*, 2008).

Oxidants are formed as a normal product of aerobic metabolism and are produced at elevated rates under pathophysiological conditions. An imbalance between production of reactive oxygen species and their neutralization leads to oxidative stress (Park *et al.*, 2017). Recent studies have shown that estimation of oxidant-antioxidant status have good diagnostic and prognostic value in humans and animals with chronic heart failure (Park *et al.*, 2017). Several studies have evaluated survival, diagnostic and the prognostic value of hemato-biochemical parameters in dogs with heart disease of varying severity (Farabaugh *et al.*, 2004). However, role of oxidative stress in the pathogenesis of naturally occurring CHF in dogs is quite scarce and hence, the present study was conceptualized with an aim to find out their role in mediating the disease pathogenesis.

MATERIALS AND METHODS

Study area and animals: Total 108 dogs were found to be suspected for cardiac insufficiency out of 2,040 animals screened at Teaching Veterinary Clinical Complex (TVCC) of the college over a period of 1.5 years (January, 2017 - July, 2018). Those 108 dogs were subjected to detail clinical and laboratory examinations for confirmation of CHF. Classification of CHF patients was done according to American College of Veterinary Internal Medicine (ACVIM) consensus classification system.

Seven healthy dogs were categorized as healthy group (Group I) for comparative study and dogs confirmed for CHF (stage B2) were categorized as diseased group (Group II; n=8).

Sampling: All the 8 dogs suffering from CHF (stage B2) were recruited for sampling. Hematology was done in blood containing EDTA as anticoagulant. Biochemical evaluation was carried out in plasma and serum was used for estimation of oxidant-antioxidant parameters.

Diagnosis of CHF: Diagnosis of CHF was based on physical examination (cardiopulmonary auscultation, evaluation of pulse and heart rate per minute, hepatojugular reflux, body condition score (BCS), capillary refill time (CRT), skin turgor test (STT) followed by confirmation with the help of ECG (BPL Cardiart 9108, Kerala, India), thoracic radiography (lateral and ventrodorsal view; Siemens, India) and echocardiography (My Lab 40 Vet, Esaote, The Netherlands).

Hemato-biochemistry: Hematology was done using MS4e automated haematology analyzer (France). Plasma

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Table 1
Classification of patients with heart failure as per ACVIM Consensus classification system (Atkins *et al.*, 2009)

State	Inclusion Criteria
A	Patients at high risk for developing heart disease, but with no current identifiable lesions.
B	Patients with structural heart disease, who have never had clinical signs of heart failure. This stage is further subdivided into Stage B1 and B2.
B1	Patients without radiographic or echocardiographic evidence of cardiac remodeling in response to chronic valvular disease
B2	Patients with radiographic and/or echocardiographic evidence of left-sided heart enlargement
C	Patients with past or current clinical signs of congestive heart failure.
D	Patients with end-stage disease and clinical signs of congestive heart failure that are refractory to standard therapy, and require specialized treatment to remain comfortable.

biochemical parameters viz. blood urea nitrogen (BUN), creatinine (CRT), triglyceride (TG), cholesterol, enzymes viz. alanine transaminase (ALT), alkaline phosphatase (ALP) and creatine kinase - MB (CK-MB) and electrolytes viz. calcium (Ca++), sodium (Na+), potassium (K+) and chloride (Cl-) were estimated by automated dry chemistry analyzer (Fujifilm DriChem 4000i).

Serum oxidant-antioxidant status: Serum oxidant-antioxidant parameters viz. lipid hydroperoxides (LPO), superoxide dismutase (SOD), glutathione (GSH) and total antioxidant (TA) were estimated using commercial kit (Cayman, USA) as per the manufacturer's instructions.

Statistical Analysis: Independent sample t test was applied between healthy and diseased group to see the significant changes. Results are presented as mean ± SE and differences were considered significant when $P < 0.05$. The data obtained were analysed using statistical package SPSS version 20.

RESULTS AND DISCUSSION

Out of 2,040 dogs screened for cardiac insufficiency, 108 dogs were found to be suspected for CHF with 13 confirmed cases and prevalence was calculated to be 0.63%. Out of total 13 confirmed cases, 8 cases (61.53%) belonged to stage B2, 3 cases (23.07%) belonged to stage D and 1 case (7.6%) each to stage B1 and C indicating that majority of the dogs had radiographic or echocardiographic evidence of cardiac remodelling (Deepti and Yathiraj, 2015).

In general, TVCC of the college is considered as one of the major referral hospitals for dogs and the current study was first of its kind from the area. Clinical, radiographic and echocardiographic findings of the dogs with CHF are described in tables 2, 3 and 4. The clinical findings observed among the affected dogs varied widely (Table 3) and were in agreement with Behera *et al.* (2019) and Shah *et al.* (2020).

Thoracic radiography showed increase in vertebral heart score (VHS) in 67.67% cases whereas 33.33% cases showed no increase as compared to normal values published by various authors (Table 4). Right atrial enlargement was seen in 11.11% cases. Pleural effusion in a patient (11.11%) with CHF was noted that obscured the cardiac silhouette (Deepti and Yathiraj, 2015). Bronchial calcification was seen in 22.22% senior dogs. The use of breed specific VHS values has a high specificity for normal heart size (Lamb *et al.*, 2001). Variations in the normal canine heart occur more than in any other organ due to size because of its contractility during the cardiac cycle (Kumar *et al.*, 2014).

Findings of echocardiographic examination (2D and M-mode) are mentioned in table 4. The reduction in the sphericity index (SI) in the present study was indicative of ongoing cardiac remodelling that occurs in heart failure (Boon, 2016). In the current study, fractional shortening (FS%) was much lower than the normal values in 75% animals. A reduced FS% is secondary to poor preload, increased afterload, or decreased contractility. This is seen in cases of dilated cardiomyopathy (DCM) and Myxomatous Mitral Valve Degeneration (Boon, 2016; Shah *et al.*, 2020). 37.5% dogs having CHF showed a reduced Ejection fraction (EF%). Decreased EF is one of the major criteria for diagnosis of DCM along with decreased FS (Shah *et al.*, 2020). There was marked mitral valve regurgitation noted in 61.53% of the affected cases which was indicative of cardiac insufficiency. So, in conclusion, LV dilation, increased EPSS, normal or thin wall and septum with a poor FS are consistent with DCM (except in boxers) which was the diagnosis in 50% cases (Boon, 2016; Behera *et al.*, 2019).

Out of the 13 dogs with CHF, 84.61% dogs were purebred and only 15.38% were mixed bred. German shepherd (10.18%) was the most commonly reported dog followed by other purebreds. The findings were in accordance with Kumar *et al.* (2014). In any geographical area, the frequency of cardiomyopathy, according to

Table 2

Signalment and vital indices of animals with CHF (n=13) on the day of presentation (day 0)

Sl. No	Breed	Age (year)	Sex	Muscle condition score	Body condition score (1-5)	Temp. (°F)	MM	Pulse Rate (per min)	CRT (sec.)	STT (sec.)	HR (Bpm)
1.	St. Bernard	5	M	Moderate	2	10.0	Pink	220	2	3	220
2.	Boxer	5	M	Normal	3	101.5	Pink	144	1	1	144
3.	GSD	11	M	Marked	1	102.5	Pale	77	2	1	77
4.	Great Dane	2	M	Marked	2	102.4	Pink	80	1	1	80
5.	Labrador	9	M	Normal	4	100.0	Pink	108	1	1	108
6.	Rottweiler	5	M	Marked	1	102.3	Pink	186	2	1	186
7.	Mixed	7	M	Moderate	2	102.9	Pink	185	1	1	185
8.	Boxer	2	M	Mild	2	101.5	Pink	160	2	1	169
9.	Mixed	1	F	Normal	4	102.6	Pink	169	2	1	170
10.	Pitbull	0.5	F	Normal	3	102.6	Pink	157	1	1	157
11.	Lhasa Apso	7	F	Marked	2	100.0	Pale	200	1	1	200
12.	GSD	1	F	Marked	1	102.0	Pink	246	1	1	246
13.	GSD	8	M	Moderate	2	101.5	Pink	178	2	2	176
	Mean ± SD	4.6±3.2			2.2±0.9	95.32±0.9		159.28± 47.92	1.42± 0.49	1.21± 0.55	160.14± 48.05

breeds, may be affected by the preference of specific breeds by the owners of those areas. As per Alex and Alison (2004), purebred dogs suffered from this condition at a prevalence rate of about 0.65% compared to only 0.16% for mixed breed dogs. They suggested that majority of cases were genetic, familial or of unknown etiology.

The mean and standard error for haematological, biochemical and oxidant-antioxidant parameters for CHF dogs are given in table 5 and 6. Hemogram revealed non-significant ($p>0.05$) derangements except in lymphocyte count which was in agreement with Vishnurahav *et al.* (2017). Inflammatory cells, including monocytes, lymphocytes, eosinophils and neutrophils have been implicated in cardiac diseases (Farabaugh *et al.*, 2004). Lymphopenia was correlated to a worsening prognosis in dogs (Farabaugh *et al.*, 2004). In the present study, affected dogs had higher neutrophil counts and reduction in PCV than healthy dogs. Lymphopenia, reduced PCV and neutrophilia might be due to enhanced corticosteroid production or other neurohormonal alterations that occur in heart failure (Farabaugh *et al.*, 2004).

Plasma biochemistry revealed non-significant ($p > 0.05$) alterations except cholesterol, calcium, sodium and chloride which were found significantly ($p<0.05$) reduced. Hypocholesterolemia could be due to malabsorption and protein losing enteropathy that occurs in dogs with cardiac insufficiency (Tilley *et al.*, 2008). Significant decrease in calcium levels of CHF dogs was in agreement with Tilley *et al.* (2008) hampering contractile function of cardiomyocytes. Significant reduction in sodium and chloride levels in the present study was in agreement with Boswood and Murphy (2006). Hypochloremia is an useful marker for heart disease refractory to diuretic therapy. In the present study, non-significant hypokalemia was in agreement with earlier report (Deepti and Yathiraj, 2015).

Table 3

Clinical and auscultatory findings in animals with CHF (n=13) on the day of presentation (day 0)

Sl. No	Clinical signs	No. of animals	Percentage
1.	Dyspnoea	12	92.30
2.	Exercise intolerance	11	84.61
3.	Inappetence	9	62.23
4.	Cough	8	61.53
5.	Ascites	7	53.84
6.	Tachycardia	6	46.15
7.	Cardiac cachexia	5	38.46
8.	Holosystolic murmurs	4	30.76
9.	Peripheral edema	2	15.38
10.	Wheezing	2	15.38
11.	Hepatojugular reflux	1	7.69
12.	S2 splitting	1	7.69
13.	Pulse deficit	1	7.69

Table 4

Echocardiographic and radiographic findings of the dogs with CHF on the day of presentation (day 0))

Sl.No.	Breed	BW	EF%	FS %	LA:Ao	SI	EPSS (mm)	Vertebral Heart Score on Day 0
1.	Great Dane	45.0	3.0	7.90	1.52	1.32	12.5	11.0
2.	Labrador	33.0	61.0	14.00	1.40	1.40	7.40	12.5
3.	Rottweiler	24.0	32.0	1.98	0.95	1.20	22.60	14.5
4.	Mixed	11.0	51.0	26.30	1.18	1.85	6.50	9.5
5.	Mixed	15.0	79.6	35.35	1.17	1.50	7.30	9.5
6.	Pitbull	13.5	56.6	56.50	1.02	1.30	6.20	12.0
7.	Boxer	14.5	45.0	31.35	1.91	1.30	6.00	12.0
8.	Lhasa Apso	7.8	20.0	2.78	0.80	2.60	9.00	11.5
9.	Boxer	NA	NA	NA	NA	NA	NA	10.5
	Mean ± SD	43.525 ±22.81	22.02± 17.70	1.2±0.33	1.38±0.24	9.6±3.26		
	Reference Indices	50-65	33-46	0.83-1.13	>1.6	<7.7		

Note: Echocardiographic reference indices adapted from Boon (2016); Vertebral Heart Score reference indices adapted from Nelson and Couto (2014); NA- Not applicable.

Table 5

Hemogram (Mean± S.E) of dogs with CHF (n=8) in comparison with healthy control animals (n=7)

Sl. No.	Parameter	Group I (Healthy Control)	Group II (CHF)	Significance (T-Test; $p<0.05$)
1	Hb (g/dl)	12.70± 1.60 (8.0-15.0)	12.92± 0.78 (8.5-15.6)	NS
2	PCV (%)	33.50± 4.41 (22.2-53.4)	37.40 ± 2.95 (26.2-50.6)	NS
3	TEC ($\times 10^6$/µl)	5.66 ± 0.95 (3.18-7.8)	5.34± 0.39 (3.5-6.69)	NS
4	TLC ($\times 10^3$/µl)	10.65 ± 1.10 (7.8-14.7)	9.90 ± 1.02 (3.45-13.01)	NS
5	THR ($\times 10^5$/µl)	3.02 ± 0.47 (1.9-5.0)	3.03± 0.35 (1.9-4.9)	NS
6	N (%)	58.25 ± 5.51 (46.0-69.0)	67.56 ± 4.09 (48.0-84.0)	NS
7	L (%)	27.50 ± 2.25 (14.0-31.0)	18.00 ± 3.55 (3.0-31.0)	S
8	M (%)	5.00 ± 2.44 (1.0-11.0)	6.93 ± 0.84 (4.0-11.0)	NS
9	E (%)	8.25 ± 3.27 (3.0-16.0)	6.87 ± 1.67 (0-5.0)	NS

Note: NS: Non-significant difference ($p>0.05$); S: Significant difference ($p<0.05$). Values in the parentheses are indicative of range.

Electrolyte derangements are common complications of CHF due to renal dysfunction, elevation of neurohormonal substances, activation of the RAAS system and diuretic therapy (Tilley *et al.*, 2008). In the present study, there was mild decrease in mean creatinine values of affected dogs which was contradicting with Deepti and Yathiraj (2015). Mild elevations of mean ALT and ALP levels in the present study might be due to hepatic congestion (Jan *et al.*, 2018). In the present study, there was mild increase in TG level. As per Senturk *et al.* (2002) elevated TG level was associated with higher risk of developing ischemic heart disease. Mild increase in the mean value of CPK-MB of the diseased animals was in agreement with earlier report (Vishnurahav *et al.*, 2017). Variations in hemato-biochemical parameters in the diseased animals might be due to variations amongst the animals with respect to their age, breed, sex, environment, physiological status, population size or the stage of the disease (Farabaugh *et al.*, 2004) and found to be non specific in diagnosing rather may be used for ruling out concurrent disease (Vishnurahav *et al.*, 2017).

Serum oxidant-antioxidant parameters revealed non-significant alterations except GSH which was reduced significantly ($p<0.05$) and found to be in agreement with Viviano *et al.* (2009). Decreased GSH concentrations may result from decreased intracellular GSH synthesis and increased GSH utilization (Viviano *et al.*, 2009). There was mild increase in the levels of LPO which was in agreement with Verk *et al.* (2017). Mild decrease in the levels of SOD was in agreement with Freeman *et al.* (2005). Mild decrease in TA level was in agreement with

Table 6

Plasma biochemistry and serum oxidant-antioxidant status (Mean± S.E) of dogs with CHF (n=8) in comparison with healthy control animals (n=7)

Sl.No.	Parameter	Group I (Healthy Control)	Group II (CHF)	Significance (T-Test; p<0.05)
1.	BUN (mg/dl)	12.15 ± 2.99 (8.4-21.0)	18.07 ± 3.24 (7.7-64.0)	NS
2.	Creat (mg/dl)	1.22 ± 0.17 (1.2-1.6)	1.03 ± 0.09 (0.7-1.5)	NS
3.	ALT (IU/L)	34.0 ± 6.25 (20.0-46.0)	48.42 ± 8.89 (18.0-91.0)	NS
4.	ALP (IU/L)	75.25 ± 25.24 (1.0-106.0)	146.62 ± 70.96 (1.0-460.0)	NS
5.	Triglyceride (mg/dl)	61.25 ± 11.98 (35.0-91.0)	142.25 ± 43.5 (33.0-412.0)	NS
6.	Cholesterol (mg/dl)	266.25 ± 11.10 (243.0-259.0)	202.62 ± 16.76 (128.0-258.0)	S
7.	CPK-MB (IU/L)	137.75 ± 20.64 (78.0-173.0)	188.12 ± 35.39 (60.0-300.0)	NS
8.	Ca++ (mg/dl)	9.00 ± 1.80 (3.6-10.8)	6.78±1.07 (3.2-10.4)	S
9.	Na+ (mmol/L)	142.25 ± 2.71 (138.0-142.0)	124.75 ± 3.49 (115.0-137.0)	S
10.	K+ (mmol/L)	4.18± 0.17 (3.8-4.4)	3.57 ± 0.16 (2.7-4.3)	NS
11.	Cl- (mmol/L)	107.00 ± 0.91 (105.0-108.0)	94.62 ± 4.48 (83.0-118.0)	S
Serum oxidant-antioxidant status				
12.	LPO (nm)	0.29 ± 0.01 (0.02-0.06)	0.40±0.01 (0.08-0.48)	NS
13.	SOD (U/ml)	0.91± 0.18 (0.44-1.23)	0.61± 0.14 (0.11-1.11)	NS
14.	GSH (µm)	2.52±1.18 (1.58-5.1)	0.70 ± 0.03 (0.64-1.07)	S
15.	TA (mM)	2.03± 0.36 (1.23-2.85)	1.92± 0.22 (1.22-2.54)	NS

Note: NS: Non-significant difference (p>0.05); S: Significant difference (p<0.05). Values in the parentheses are indicative of range.

earlier report and its role has been proven in both cardiac and distal gastric cancer (Hetyey *et al.*, 2007). So, increased LPO levels with a concurrent decrease in SOD, GSH and TA levels in CHF dogs were indicative of their role in mediating the disease pathogenesis.

The current study underscored the changes in hemato-biochemical and oxidant-antioxidant markers in dogs with CHF. Hemato-biochemical parameters are important in ruling out concurrent disease processes and to assess the patient's prognosis. Clinical implications of all these parameters to be used as diagnostic and/or prognostic biomarkers need to be further validated in a larger sample size. Nevertheless, this study is one of the few existing literatures where the role of oxidant-antioxidants in the pathogenesis of naturally occurring heart diseases has been evaluated in dogs.

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