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Studies on hemato-biochemical, radiological and echocardiographic changes in geriatric canine heart failure

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Abstract
The present study was conducted to evaluate hemato-biochemical, radiological and ultrasonographic changes in dogs suffering from cardiac failure. A through cardiovascular check-up comprising of haematology, clinical biochemistry, radiology and ECG was performed. There was a non significant decrease in haemoglobin, TEC and PCV compared to healthy geriatric dogs of same age. There was a significantly (P<0.05) increased level of CPK (236.54±27.30 U/L), LDH (1365±182.5 U/L), creatinine (3.01+1.29 mg/dl), nitric oxide (30.84±4.44 U/L) and other parameters. The thoracic radiography review cardiomegaly, increase in chest depth and increase in chest width. A-V block, congestive heart failure and atrial enlargement were the significant electrocardiographic findings in geriatric dogs.

Keywords: heart failure, canine, echocardiographic, radiological

1. Introduction
The concept of biomarkers of age and age-related disorders began to appear in the gerontologic literature in the early 1980s. In early days, the interest was in negating the confounding influence of research on aging so that indicators of underlying processes of aging could be predicted [1]. A Biomarker of Aging must possess the ideal quality to accurately predict capability of organs at some late age [2].

Elevated levels of plasma Low Density Lipid fraction are associated with an increased risk for and coronary heart disease [3]. There are studies which have reported total and LDL cholesterol levels increase with age [4], and gradual decline in the fractional clearance of LDL from the circulation [5, 6]. There are also reports of the reduced expression of hepatic LDL receptors (LDLRs) with advancement of age [7]. Many studies have indicated age related vascular events are closely related to the progression of endocrine and metabolic syndrome. Obesity, hyperlipidemia, hyperinsulinemia, hyperglycaemia, and hypertension are known as risk factors for cardiovascular and cerebrovascular events. It is well known that high LDL- with low HDL-cholesterol is one of the risks of acute cardiovascular events.

2. Material and Methods
2.1 Study animals
In present study total number of 957 Dogs of different age group and different breeds presented to Referral Veterinary Polyclinic were used for study. Out of which 251 dogs were more than 5 years old and selected as geriatric dogs. Dogs showing heart failure were categorized as diseased group and 20 normal healthy dogs presented for routine vaccination and deworming were categorized as healthy group for comparative study.

2.2 Inclusion and exclusion criteria
1. Animals above five years of age (human equivalent) showing signs of heart failure were included in study.
2. Animals which have not undergone any medication which affects cardiovascular function were included while those dogs which have history of cardiovascular medication were excluded from study.
3. Animals which have showed presence of other disease condition associated with heart failure were excluded from the study, with the sole purpose to include only those canines which were exclusively affected with cardiac failure.

2.3 Sampling
Blood (5.0 ml) was collected by venipuncture from the cephalic and/or radial vein using a

disposable syringe after taking consent of the owners. Blood samples were centrifuged for 15 minutes at 3000 rpm to separate serum. Resulting sera were transferred to eppendorf micro tubes and frozen at -20°C till further use. This was used for estimation of various biochemical and endocrine parameters.

2.4 Haematology
The haematological parameters were estimated within 8 hrs of collection of blood. Haematological indices Packed cell volume (PCV %) in whole blood was determined by capillary microhaematocrit method by centrifugation at 10000 r.p.m for 15 min. The hemoglobin was estimated according to method of Drabkin (Frankel *et al*., 1970). Total erythrocyte and total leukocyte count were performed using by haemocytometer (Jain, 1986). 3.4.2.3 Differential leukocyte count Blood smears were prepared from fresh blood. Smear was stained using Geimsa stain (1:9 Dilutions for 45 min) for differential leukocyte count. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated as described earlier [8].

2.5 Blood biochemistry
Creatine phosphokinase CPK was estimated as per the method of [9] and results were expressed as U/L. Alkaline phosphatase Alkaline phosphatase was estimated following the method of [10] and results were expressed as KA units. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) enzyme was measured following the method of [11] and Values were expressed as U/L. Serum total protein (g/dl) and albumin (g/dl) were determined by the modified Biuret and Dumas method [12]. Globulin was estimated by subtracting albumin from total protein. Urea concentration in serum (mg/dl) was measured by di-acetyl monoxime (DAM) Method [13] and urea nitrogen concentration was calculated by multiplying the urea concentration with factor 0.467 as indicated in the protocol of diagnostic kit. Serum Creatinine was estimated following alkaline picrate method [14]. Serum glucose concentration was determined following the method of [15] and results were expressed in mg/dl. Serum cholesterol was determined following the method of [16]. Serum Total bilirubin (mg/dl) was measured as per method described by [17]. Serum lactate dehydrogenase activity was determined by optimised DGKC, Kinetic assay method [18]. The values of LDH were expressed in IU/L. Phosphorus Phosphorus concentration in serum was estimated as per [19] UV Molybdate end point assay method. The values of phosphorus were expressed in mg/dl. Calcium in serum was estimated as per [19] UV Molybdate end point assay method. The values of phosphorus were expressed in mg/dl.

2.6 Electrocardiogram
ECG was also performed to refute/confirm the changes in haematobiochemical markers in appropriate cases using standard techniques mentioned in the instruments manual.

2.7 Radiographic parameters
Dogs found to have signs dyspnea and respiratory problem during physical examination were subjected to radiography. Radiographic Views included right to left lateral (RL) and left to right lateral (LL), and ventrodorsal (VD) radiographs. Radiographs were taken at full inspiration, if possible, with focus to film distance of 100 cm and a tabletop technique. Measurements of different radiographic parameters were recorded following the method of [20].

2.8 Statistical analysis
Data was analyzed statistically using SPSS software, Statistical Analysis. Data were subjected to statistical analysis.

3. Result
Abnormal cardiovascular function was recorded in 22 geriatric dogs during screening. A through cardiovascular check-up comprising of haematology, clinical biochemistry, radiology and ECG was performed. The detailed haematology is presented in (Table 1). There was a non significant decrease in haemoglobin, TEC and PCV compared to healthy geriatric dogs of same age. The biochemical changes suggestive of cardiovascular disorders are presented in (Table 2). There was a significantly (P<0.05) increased level of CPK (236.54±27.30 U/L), LDH (1365±182.5 U/L), creatinine (3.01+1.29 mg/dl), nitric oxide (30.84±4.44 U/L) and other parameters. The thoracic radiography review cardiomegaly, increase in chest depth and increase in chest width (Fig. 3-4). A-V block, congestive heart failure and atrial enlargement were the significant electrocardiographic findings in geriatric dogs (Fig. 1-2).

Table 1: Haematological changes in geriatric dogs with cardiovascular disorders.

Parameters	Group 1 (Healthy control)	Group 2 (Heart failure)
RBC (×106 cells/μl)	6.10±0.45	5.80±0.86
Hemoglobin (g/dl)	13.45±0.95	10.68±1.27
PCV (%)	45.22±2.44	34.19±4.69
MCV(fl)	69.28±2.50	60.97±6.89
MCH (pg)	35.15±1.90	21.73±2.78 *
MCHC (%)	23.77±1.30	32.30±2.75 **

Values with superscript * differ significantly (P<0.05) between the group.
Values with superscript ** differ significantly (P<0.01) between the group.

Table 2: Serum biochemistry in geriatric dogs with cardiovascular disorders.

Parameters	Group 1 (Healthy control)	Group 2 (Heart failure)
CPK (IU/L)	86.31±9.01	236.54±27.30**
LDH (IU/L)	208.00±26.89	1365.00±182.59**
Creatinine (mg/dl)	1.10±0.24	3.01±1.29**
NO (μmol/L)	9.36±1.29	30.84±4.44**
GGT (IU/L)	5.58±1.75	13.00±1.12**
BUN (mg/dl)	17.56±1.65	29.58±5.44
ALP (IU/L)	126.22±18.26	139.09±20.41
ALT (IU/L)	49.68±5.27	70.18±8.23*
AST (IU/L)	58.95±5.54	99.31±18.57*

Values with superscript * differ significantly (P<0.05) between the group.
Values with superscript ** differ significantly (P<0.01) between the group
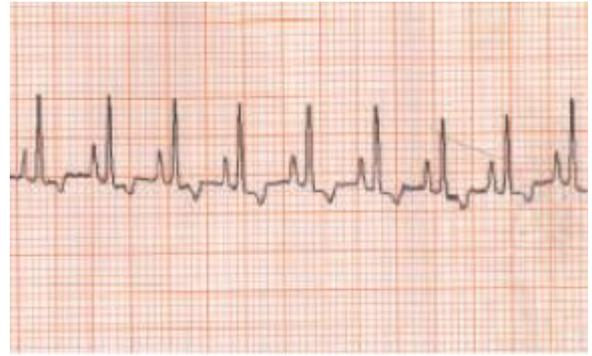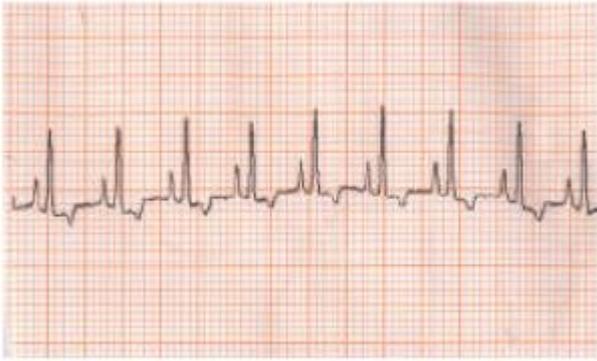
Fig 1: Tachycardia with tall P wave=0.4mv and QRS complex=1.2 mv suggesting right atrial enlargement
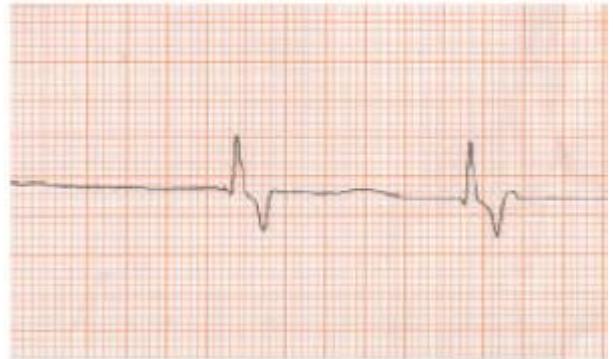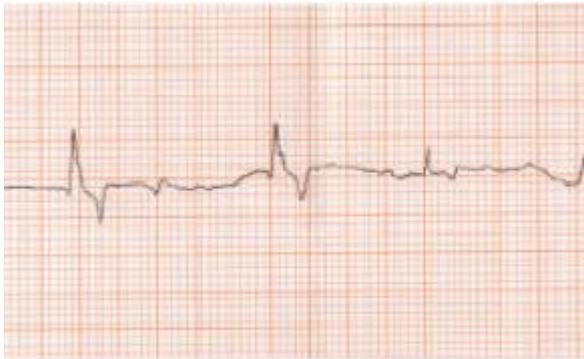
Fig 2: Missing P wave before every QRS complex suggesting congestive heart failure
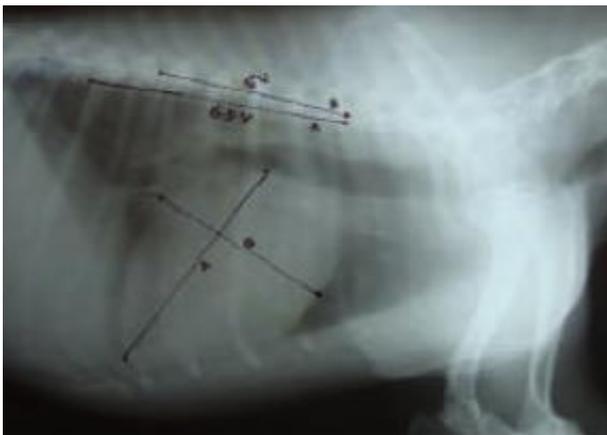
Fig 3: Right lateral thoracic radiographic in 8 year old dog suffering from cardiomegaly displaying longitudinal, transverse axis and vertebral axis
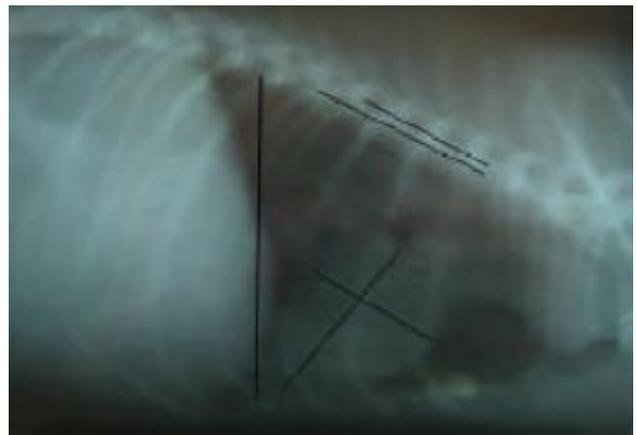
Fig 4: Left lateral thoracic radiographic in 6 year old dog suffering from acute heart failure displaying longitudinal, transverse axis and vertebral axis

4. Discussion

In our study 22 dogs were found to be affected with different cardiac disorder of which majority of cases were dialated cardiomyopathy (DCM). There are earlier studies which have suggested the prevalence of DCM increases in middle-aged or older dogs, especially in males [21]. The clinical signs observed in these dogs were weakness, exercise intolerance, anorexia, and coughing. The present observation could nor elucidate any significant difference in red blood cell count, hematocrit, Hb% and MCV, while MCH was found significantly lower (P<0.05) in dogs with heart failure. In present study Serum creatinine, LDH, CPK, ALT and AST values were found to be significantly (P<0.01) higher in dogs with cardiac failure compared to dogs with normal cardiac function. Increased creatinine probably reflects a reduction in renal blood flow and hence glomerular filtration. The BUN concentration was found within physiological limits. Our observation is not in agreement with finding of [21, 22] who have reported increased BUN levels in dogs with heart failure. The GGT, ALT and AST activities of 6- to 11-year-old canines with CHF were significantly (P<0.05) higher than in healthy control dogs of the same age group. The increase in the blood serum LDH and CK activities in CHF were especially significant (P<0.01). Similarly Nitric oxide was significantly higher in dogs with heart failure. Our observations, for above biochemical parameters is in accordance with [23]. Further it is supported by the fact that increased serum LDH is typically present in the first available blood sample obtained from patients hospitalized for acute myocardial infarction in geriatric dogs [24]. LDH, is typically increased when congestive failure occurs without infarction [12]. Many workers have also reported that Incidence and prevalence of congestive heart failure increases with advancement of age [24, 25].

The vertebral heart size (VHS) is a method for objectively determining the size of the canine cardiac silhouette on

thoracic radiographs [19]. This technique is useful for examining the heart enlargement associated with eccentric hypertrophy because of volume overload [9]. It is easy to perform, and the measurements are independent of both patient related (i.e., thoracic conformation, gender and side of lateral recumbency) and operator-related variables i.e., level of experience [19]. The mean VHS of canines with cardiac abnormalities were significantly higher compared with the mean VHS of normal healthy dogs. In our study we found short left axis measurement in cardaic failure were significantly (p<0.05) higher than normal healthy dogs. The left VHS and RVS were found significantly (P<0.05) higher in cardiac failure group. DV VHS in cardiac failure group was significantly higher (P<0.01). Earlier studies have found VHS over 10.7 moderately accurate sign of cardiac disease [9]. The majority of diseased canines in current study had a VHS above the general limit of normality (i.e., 10.5). Combined right and left sided cardiac enlargement was likely responsible for the increased VHS in these patients.

5. Conclusion
From present study it may be concluded that with advancement of age there is progression of cardiac malfunction. Early marker of geriatrics should be identified and periodic organ functions should be evaluated to access the organ dysfunction.

6. References

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