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Analysis of certain blood biochemical parameters in relation to oxidative stress in chronic mitral valve insufficiency of dogs with heart failure

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ABSTRACT

Chronic mitral valve insufficiency (CMVI) is the most common acquired heart disease in dogs. In heart failure, the cellular oxygenation and metabolism are affected, which leads to the production of free radicals. Free radicals damage DNA, lipid and protein molecules in cells. In the present experiment, blood samples were collected from CMVI dogs with heart failure and were compared with the results obtained from healthy dogs. A significant increase in the levels of xanthine oxidase, AST, LDH and CK and decrease in the activity of catalase were noticed in CMVI dogs when compared to healthy dogs, which revealed overall cardiac and skeletal muscle damage in CMVI dogs. Results of biochemical parameters revealed an increase in urea level and decrease in sodium, potassium and calcium levels in CMVI dogs as compared to control dogs, all of which indicate cardiac damage in dogs. Study on hematological parameters revealed a significant decrease in Hb, PCV, RBC and platelet counts and an increase in total WBC counts and percentage of neutrophils, decrease in percentage of the lymphocyte and monocyte in CMVI dogs than control. These results indicate secondary phenomenon to heart failure. The present research data indicates the usefulness of these biomarkers in the diagnosis and prognosis of CMVI with heart failure in dogs.

Key words: Cardiac enzymes, Chronic mitral valve insufficiency, Heart failure, Oxidative stress, Xanthine oxidase.

INTRODUCTION

Congestive heart failure (CHF) refers to the heart unable to pump blood to the body at the designed volume and pressure. In dogs, CHF is mainly caused by chronic mitral valve insufficiency (CMVI) and dilated cardiomyopathy (DCM) (Besche *et al*., 2007). CMVI is commonly noticed in smaller breed dogs (Haggstrom *et al*., 1992). CMVI is characterized by regurgitation of blood from left ventricle (LV) to the left atrium (LA), which causes volume overload that leads to left sided CHF with risk of remodeling and myocardial alterations (Hyun and Layulo, 2011).

Oxidative stress describes imbalance between formation of reactive oxygen species (ROS) and antioxidant defences in the body. In human patients with CMVI, oxidative stress can be a factor for development of remodelling of myocytes and dysfunctions in heart (Ahmed *et al*., 2010). In vitro studies have reported that ROS level increases with overstretching and cyclic stretch of myocytes. Due to this process, contractile function is impaired and myocytes can undergo apoptosis (Cheng *et al*., 1995). Studies on animals have indicated altered antioxidant status and biomarkers in oxidative stress in dogs with both spontaneous and experimentally induced cardiovascular disease. Experimentally induced heart failure in guinea pigs also has shown increased oxidative stress (Dhalla *et al*., 1996). Induced chronic volume overload in canine model also shown to increase oxidative stress resulting in impaired contractile function of heart (Prasad *et al*., 1996).

The enzyme xanthine oxidase (XO) is highly expressed in the failing heart and it impairs mitochondrial energy production in cardiac myocytes. Xanthine oxidase has been shown to be a major source of free radical generation leading to oxidative stress under ischemic conditions (Xia *et al*., 1996; Pandey *et al*., 2000). These oxygen free radicals generated by xanthine oxidase in turn oxidize macromolecules like DNA, proteins and lipid resulting in myocardial cellular injury (Gorman and Zweier, 1990). As a consequence of damage, malondialdehyde (MDA) a secondary byproduct of lipid peroxidation, becomes useful for assessing the oxidative damage in most of the tissues (Ayala *et al*., 2014). The normal myocardium contains antioxidant proteins that act to scavenge H_2O_2 mainly catalase, glutathione peroxidase, and peroxiredoxin. Catalase accounts for nearly 80% of all peroxidase activity in cardiomyocytes (Li *et al*., 1997).

In this study the oxidative stress markers like Xanthine oxidase (XO), Malondialdehyde (MDA) and catalase along with cardiac enzymes like total Creatine kinase (CK), Lactate dehydrogenase (LDH), Lactate dehy

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drogenase- 1 (LDH-1), Aspartate transaminase (AST) and other routine biochemical and hematological parameters for the prognosis of CMVI with heart failure dogs were assessed.

MATERIALS AND METHODS

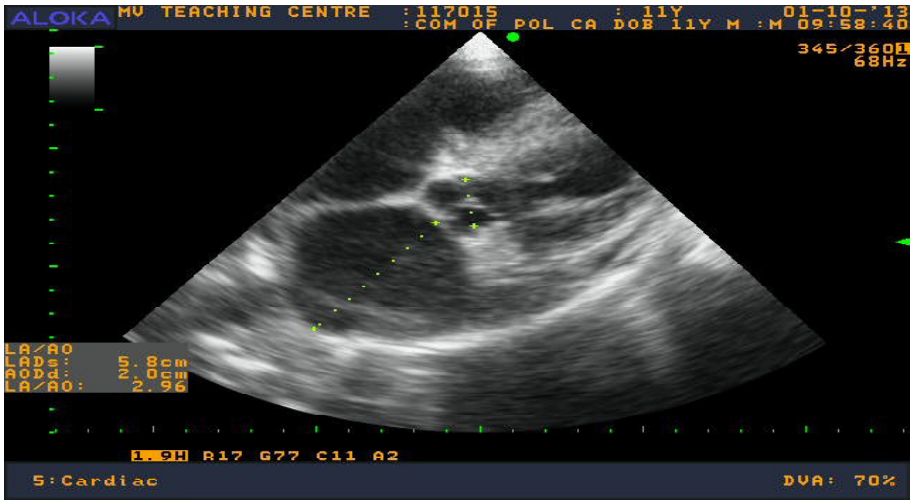
Blood samples were collected from the dogs attending the OP ward of Madras Veterinary College Teaching Hospital, Chennai. Ten dogs diagnosed with CMVI were included in the present study after confirmation with ECG, Chest Radiography and Doppler echocardiography. Chronic mitral valve disease was confirmed based on the following observation in colour Doppler echocardiography, *viz*. degenerative changes in the mitral valve leaflets, dilated left atrium in comparison with aorta, dilated left ventricle and regurgitation at the level of mitral valve (Plate 1).

A total of 30 other dogs were examined clinically to serve as healthy control. About 5 ml of blood sample was collected from cephalic vein from all healthy and diseased dogs for estimation of blood, enzyme and biochemical parameters.

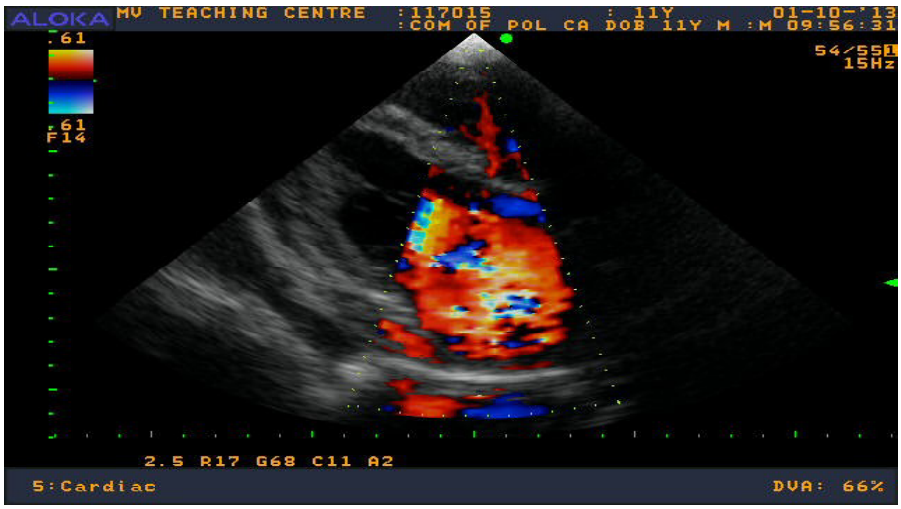
XO activity was assayed by the modified method of Bergmeyer *et al*., (1974). MDA level was measured by the method of Satho, (1978). Catalase activity was determined by the method of Beers and Sizer (1952). All the enzymatic and biochemical parameters were analyzed, using diagnostic kits (M/S. Agappe Diagnostics Ltd., Ernakulum, and Kerala, India) as per the manufacturer's protocol except LDH-1 isoenzyme, which was measured as per the method of Welshman and Carol Rixon (1967). All the chemicals used in the assay were of analytical grade (Merck or equivalent). Hematological parameters were analysed using auto-analyzer (Biosystems, A15).

The results are expressed as mean ± SE for all parameters. The data was analyzed by "unpaired t test" (Snedecor and Cochran, 1994) and the level of significance of the test falling less than 0.01 ($p < 0.01$) was considered statistically significant.

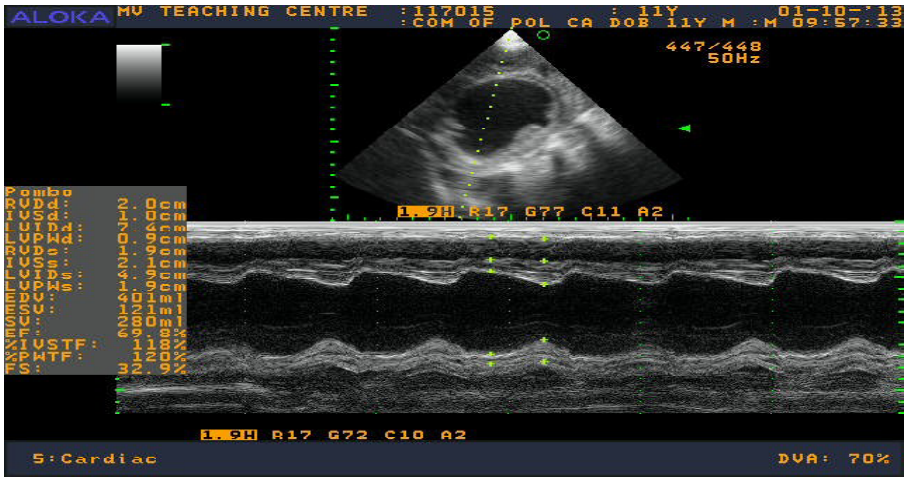
Plate 1: Echocardiographic findings with cmvi.



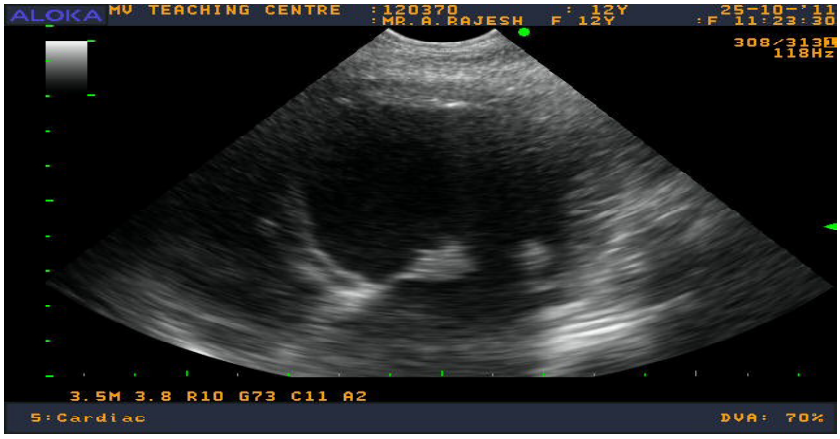
A) 2-D Echocardiogram showing severely dilated left atrium and highly increased LA/AO Ratio.



B) 2-D Echocardiogram and color flow doppler showing severe mitral regurgitation.



C) M-Mode echocardiogram showing severely dilated left ventricles.



D) 2D Echocardiogram showing degenerative changes in mitral valve leaflets.

RESULTS AND DISCUSSION

A. Oxidative stress markers: The results of the oxidative stress markers for control and CMVI groups are presented in the Table 1.

In the present study, a highly significant increase in the activity of xanthine oxidase (XO) was observed in dogs with CMVI, much in concurrence with the findings of Sesh *et al*., (2015) who noticed a significantly increased activity of XO in dogs with dilated cardiomyopathy, when compared to healthy dogs. Berry and Hare (2004) opined that circulating XO levels localized in blood and myocyte are up-regulated in congestive heart failure. The XO enzyme expression is increased in the failing heart (Ekelund *et al*., 1999; Jong *et al*., 2000; Cappola *et al*., 2001; Amado *et al*., 2005) as much as four fold, recorded in the failing dog heart. Amado *et al*., (2005) showed that XO in total heart extracts is upregulated at the transcriptional level. A similar mechanism presumptively underlines the observations of the present study. Although Ayala *et al*., (2014) reported elevation of MDA in acute myocardial injury, in the present study there was no significant difference of MDA level between the control and CMVI group which may be due to chronic nature of the disease.

Similar findings were expressed by Freeman *et al*., (2005) in their investigations on Chronic Valvular Disease in dogs (CVD) who opined that MDA is a less precise indicator of lipid peroxidation and Reiman *et al*., (2017) in their investigations on Myxomatous Mitral Valve Disease (MMVD) in dogs, opined that the increase in MDA concentration may be noted in cardiac tissues but may not be reflected in the circulating plasma. Hence, the above reasons also hold good for the results observed for MDA concentrations in the present study and plasma estimation of MDA may not serve as a reliable indicator for CMVI.

There was a highly significant decrease in catalase activity in CMVI dogs than control. Baumer *et al*., (2000) in their studies on Idiopathic Dilated Cardiomyopathy in humans reported that the mRNA and protein levels of catalase remained unchanged but the catalase activity decreased, which may be due to the post-translational modification of the enzyme leading to inactivation. Endogenous inhibition of catalase activity in vivo may be a mechanism with potential

Table 1: Serum and hematological markers in healthy and chronic mitral valve insufficient (CMVI) dogs (values expressed in Mean ± SE).

Parameters	Control	CMVI	P-value	Result
Oxidative stress markers				
Xanthine oxidase (U/mL)	6.57 ± 0.41	11.40 ± 0.61	0.000	***
MDA (µmol/L)	1.61 ± 0.16	2.08 ± 0.24	0.135	NS
Catalase (U/mg Hb)	0.25 ± 0.005	0.22 ± 0.008	0.004	***
Cardiac marker enzymes				
AST (U/L)	30.26±1.53	41.22±2.29	0.001	***
CK (U/L)	29.48±2.19	75.55±5.96	0.000	***
LDH (WU/L)	309.46± 23.83	401.98±30.44	0.047	**
LDH-1 (WU/L)	26.47±2.40	34.70±3.2	0.080	NS
Biochemical parameters				
Glucose (mg/dL)	88.57 ± 3.96	81.60 ± 4.26	0.347	NS
Total protein (g/dL)	5.41 ± 0.18	4.72 ± 0.35	0.071	NS
Total-Cholesterol (mg/dL)	169.72 ± 8.14	161.49 ± 14.17	0.110	NS
HDL – cholesterol (mg/dL)	58.40 ± 3.87	53.35 ± 3.82	3.771	NS
Sodium (mEq/L)	151.79 ± 4.05	101.70 ± 8.36	0.000	***
Potassium (mEq/L)	5.25 ± 0.17	4.37 ± 0.38	0.021	**
Calcium (mg/dL)	9.98 ± 0.28	7.81 ± 0.48	0.000	***
Urea (mg/dL)	28.60 ± 2.12	54.89 ± 6.05	0.000	***
Creatinine (mg/dL)	0.99 ± 0.05	1.26 ± 0.18	0.054	NS
Hematological parameters				
Hemoglobin (g/dL)	12.77 ± 0.34	9.66 ± 0.66	0.000	***
PCV (%)	41.08 ± 0.99	26.52 ± 1.41	0.000	***
RBC (millions/mm3)	6.28 ± 0.12	4.12 ± 0.43	0.000	***
Platelets (lakhs/mm3)	267440.00 ± 7580.32	157700.00 ± 26373.83	0.000	***
WBC (millions/mm3)	8968.33 ± 651.16	14945.00 ± 1089.20	0.000	***
Neutrophils (%)	69.57 ± 0.77	78.30 ± 1.09	0.000	***
Lymphocytes (%)	23.33 ± 0.74	17.60 ± 0.95	0.000	***
Monocytes (%)	5.03 ± 0.22	2.60 ± 0.43	0.000	***
Eosinophils (%)	1.97 ± 0.24	1.50 ± 0.27	0.297	NS
Basophils (%)	0.10 ± 0.05	0.00 ± 0.00	0.311	NS

NS = Not Significant; ** = P<0.05; *** = P<0.01

consequences for the intracellular redox balance. Khaper *et al*., (2003) reported that in myocardial infarction, catalase level, while stable initially, decreased over time, a plausible reason again justifying the present findings. As most of the peroxidase activity in myocytes is from catalase, this significant change can drastically alter redox balance in the myocardium (Pendergrass *et al*., 2011).

B. Cardiac enzymes: The results of the cardiac enzymes for control and CMVI group are presented in the Table 1. The level of total Creatine kinase (CK), Aspartate transaminase (AST) and Lactate dehydrogenase (LDH) significantly increased in CMVI group than control. Skeletal muscle blood flow is limited in patients with chronic heart failure, due to a combination of low cardiac output and increased peripheral resistance, leading to muscle degeneration. Structural or metabolic changes in skeletal muscle could also be of importance (Schaufelberger *et al*., 1996). It is opined that the elevated levels of total CK, AST, and LDH observed in our study is a consequence of skeletal muscle damage in CMVI dogs. Further it is also confirmed by the result as obtained for LDH-1 activity, wherein no significant difference was observed between control and CMVI groups.

C. Biochemical parameters: The results of the biochemical parameters for control and CMVI groups are presented in the Table 1.

There was no significant difference with respect to levels of glucose, protein, total cholesterol, HDL- cholesterol and creatinine between the groups.

A highly significant reduction of sodium levels were observed in CMVI group than control. A similar observation was also noticed by Sesh *et al*., (2013) in Dilated Cardiomyopathy cases of dogs, who suggested that the decrease in the serum sodium levels may be due to sodium influx into cardiac tissue for depolarization and excitation. Bosswood and Murphy (2006) observed that sodium was seen to vary significantly in dogs with heart failure suggesting hyponatremia as a marker of severe or end-stage heart failure.

Calcium levels in CMVI group were significantly reduced when compared to control. It is well established that secondary parathyroidism is a covariant of CHF. The aldosteronism of CHF predisposes the patient to secondary hyperparathyroidism because of chronic increase in Ca^{2+}

losses in urine and feces, with fall in their serum ionized Ca^{2+} level. Hyperparathyroidism stimulates excessive accumulation Ca^{2+} in heart, skeletal muscle leading to low level of calcium in blood (Alsafwah *et al*., 2007). The reduced serum calcium levels observed in our study concurs with the above reports.

The level of serum potassium in CMVI group was significantly reduced than control. The data from experimental models of cardiac hypertrophy or failure and terminal heart failure in man, indicate that down-regulation of potassium currents, particularly transient outward potassium current may cause abnormalities in repolarization and contribute to the increased electrical instability of failing hearts (Tomaselli *et al*., 1994) which correlates with our findings.

In the present study a highly significant increase in the level of urea was observed in dogs with CMVI when compared to control. But no significant difference in the level of creatinine was observed among the groups. Atkins *et al*., (2002) have found that serum urea nitrogen concentration increased over time in dogs with severe mitral regurgitation. An increase in plasma urea without concomitant changes in plasma creatinine could be due to a decrease in renal blood flow with relatively smaller reductions in glomerular filteration rate as shown in experimental canine CHF (Lohmeier *et al*., 2000). Patients with congestive heart failure and intact kidneys commonly present with an elevation of blood urea nitrogen level and without increase in the creatinine level (Hosten, 1990). The increase in urea level observed in the present study may be due to heart failure, which may decrease the renal blood flow.

D. Hematological parameters: The results of the hematological parameters for control and CMVI groups are presented in the Table 1.

In the present study highly significant decrease in Hb, PCV, RBC, platelet count was observed in dogs with CMVI as compared to control group. Anemia in people with CHF is common and has been correlated with the severity of heart failure. Low hematocrit and hemoglobin have both been shown to be the predictors of mortality in human patients and dogs with heart failure (Tanner *et al*., 2002 ; Farabaugh *et al*., 2004). The decreased hematocrit and hemoglobin concentrations observed in dogs with CMVI in the present work may be a secondary phenomenon to heart failure.

A highly significant increase in total leukocyte count and percentage of neutrophils was observed in CMVI when compared to control. No significant difference was observed in the percentage of eosinophils and basophils between the groups. Kyne *et al*., (2000) and Farabaugh *et al*., (2004) have reported that total leukocyte and neutrophil count was increased in heart failure in humans and dogs, which concurs with the result of the present study.

A highly significant decrease in percentage of lymphocytes and monocytes was observed in dogs with CMVI. A decrease in the percentage of lymphocyte has been observed in different cardiovascular diseases. It was interpreted as a marker of the physiological stress response, mediated by an increased release of endogenous catecholamines or cortisol as suggested by Rudiger *et al*., (2006). Similarly, Horne *et al*., (2005) have demonstrated that in cardiovascular failure there is an increase in circulating neutrophils and decrease in total mononuclear cells (lymphocytes plus monocytes).

CONCLUSION

In addition to cardiac enzymes, oxidative stress markers like xanthine oxidase and catalase are also useful to assess the prognosis of CMVI dogs with heart failure along with ECG / Doppler echocardiography observations. Among the biochemical parameters the levels of calcium, sodium and urea are also useful to assess the severity of the condition. The assessment of hematological parameters also support to evaluate the severity of the condition.

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REFERENCES

Ahmed, M.I, Gladden, J.D, Litovsky, S.H., *et al*. (2010). Increased oxidative stress and cardiomyocyte myofibrillar degeneration in patients with chronic isolated mitral regurgitation and ejection fraction >60%. *J Am Coll Cardiol*., **55:** 671–679.

Alsafwah, S., LaGuardia, S.P and Newman, K.P. (2007). Congestive heart failure is a systemic illness: a role for minerals and micronutrients. *Clin. Med. Res*., **5**(4): 238-243.

Amado, L. C., Saliaris , A.P., Shubha, V.Y., Lehrke, S., John, M.S., Xie, J., Stewart, G., *et al* . (2005). Xanthine oxidase inhibition ameliorates cardiovascular dysfunction in dogs with pacing-induced heart failure. *J. Mol. Cell. Cardiol*., **39:** 531–536.

Atkins, C. E., Brown, W.A and Coats., J.R. (2002). Effects of long-term administration of enalapril on clinical indicators of renal function in dogs with compensated mitral regurgitation. *J. Am. Vet. Med. Assoc*., **221:** 654–658.

Ayala, A., Munoz, M.F. and Arguelles, S. (2014). Lipid peroxidation: production, metabolism and signaling mecanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Medicine and Cellular Longevity*. 1-31.

Baumer, A.T., Flesch, M., Wang, X., Shen, Q., Feuerstein, G.Z. and Bohm. M. (2016). Antioxidative enzymes in human hearts with idiopathic dilated cardiomyopathy *J. Mol. Cell. Cardiol*. **97**: 93–105.

Beers, R.F. and Sizer I.W. (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem*., **95:** 133- 140.

Bergmeyer, H.I., Gawehn, K. and Grassl, M. (1974). In Methods of Enzymatic analysis (Bergmeyer, H.U. ed.) Second edition, Volume I, 521-522, Academic Press Inc., New York, NY.

Berry, C. E and Hare, J.M. (2004). Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. *Journal of Physiology*, **555** (3): 589–606.

Besche, B., Chetboul, V., Lefay, M.P.L. and Grandemange, E. (2007). Clinical evaluation of imidapril in congestive heart failure in dogs: results of the EFFIC study. *J. Small. Anim. Pract*., **48:** 265-270.

Boswood, M. A and Murphy, A. (2006). The effect of heart disease, heart failure and diuresis on selected laboratory and electrocardiographic parameters in dog. *J.Vet.Cardiol*., **8:** 1-9.

Cappola, T.P., Kass, D.A and Nelson, G.S. (2001). Allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy. *Circulation*, **104:** 2407–2411.

Cheng, W., Li, B., Kajstura J., *et al*. (1995). Stretch-induced programmed myocyte cell death. *J Clin Invest*., **96:** 2247–2259.

Dhalla, A., Hill, M. and Singal, P. (1996). Role of oxidative stress in transition of hypertrophy to heart failure. *J Am Coll Cardiol*., **28:** 506–514.

Ekelund, U. E., Harrison, R.W., Shokek, O., Thakkar, R.N., Tunin, R.S., Senzaki, H., Kass, D.A.,Marban, E. and Hare. J.M. (1999). Intravenous allopurinol decreases myocardial oxygen consumption and increases mechanical efficiency in dogs with pacing-induced heart failure. *Circ Res*., **85:** 437– 445.

Farabaugh, A.E., Freeman, L.M., Rush, J.E. and George, K.L. (2004). Lymphocyte subpopulations and hematologic variables in dogs with congestive heart failure. *J. Vet. Intern. Med*., **18:** 505–509.

Freeman, L. M., Rush, J.E., Milbury, P.E. and Blumberg, J.B. (2005). Antioxidant status and biomarkers of oxidative stress in dogs with congestive heart failure. *J. Vet. Intern. Med*., **19:** 537–541.

Gorman, S.L.T. and Zweier, J.L. (1990). Evaluation of the role of xanthine oxidase in myocardial repurfusion injury, *J.Biol.Chem.*, **265:**6656-6663.

Haggstrom, J., Hansson, K., Kvart, C and Swenson, L. (1992). Chronic valvular disease in the Cavalier King Charles Spaniel in Sweden. *Vet Rec*., **131:** 549-53.

Horne, B.D., Anderson, J. L., John, J.M., Weaver, A., Bair, T.L., Jensen, K.R., Renlund, D.G. and Muhlestein. J.B. (2005). Which white blood cell subtypes predict increased cardiovascular risk? *J. Am. Coll. Cardiol*., **45:** 1638-1643.

Hosten, A.O. (1990). Clinical Methods: The history, physical and laboratory examinations. 3rd edition.Walker, H.K., W.D. Hall and J.W. Hurst. Pp: 258-267.

Hyun, C. and Layulo, L (2011). Calcium related genes in dogs as potential cardiac biomarkers for the detection of chronic mitral valve disease. *Recent Patents on Biomarkers*. **1:** 68-80.

Jong, D. J. W., Schoemaker, R.G., De Jonge, R., Bernocchi, P., Keijzer, E., Harrison, R., Sharma, H.S. and Ceconi, C. (2000). Enhanced expression and activity of xanthine oxidoreductase in the failing heart. *J. Mol. Cell. Cardiol*., **32**(11): 2083–2089.

Khaper, N., Kaur, K., Li, T., Farahmand, F. and Singal, P.K. (2003). Antioxidant enzyme gene expression in congestive heart failure following myocardial infarction. *Mol Cell Biochem.*, **251** (1-2): 9-15.

Kyne, L., Hausdorff, J and Knight, E. (2000). Neutrophilia and congestive heart failure after acute myocardial infarction. *Am. Heart. J*., **139:** 94–100.

Li, G., Chen, Y., Saari, J.T. and Kang, Y.J. (1997). Catalase-overexpressing transgenic mouse heart is resistant to ischemia-reperfusion injury. *Am J Physiol*., **273:** H1090–1095.

Lohmeier, T.E., Mizelle, H.L., Reinhart, G.A and Montani, J.P (2000). Influence of angiotensin on the early progression of heart failure. *Am. J. Physiol*., **278:** 74–86.

Pandey, N.R., Kaur, G., Chandra, M., Sanwal, G.G and Misra, M.K. (2000). Enzymatic oxidants and antioxidants of human blood platelets in unstable angina and myocardial infarction. *Int. J. Cardiol*., **76:** 33-38.

Pendergrass, K.D., Varghese, S.T., Rafferty, K.M., Brown, M.E., Taylor, W.R and Davis, M.E (2011). Temporal effects of catalase overexpression on healing following myocardial infarction. *Circ Heart Fail*., **4**(1):98-106.

Prasad, K., Gupta, J., Kalra, J., *et al*. (1996). Oxidative stress as a mechanism of cardiac failure in chronic volume overload in canine model. *J Mol Cell Cardiol*., **28:** 375–385.

Reimann, M. J., Haggstrom, J., Moller, J, E., Lykkesfeldt, J., Falk, T and Olsen, L.H. (2017). Markers of oxidative stress in dogs with myxomatous mitral valve disease are influenced by sex, neuter status, and serum cholesterol concentration. *J Vet Intern Med*., **31**(2): 295-302.

Rudiger, A., Burckhardt, O.A., Harpes, S.P., Muller, A. and Follath, F. (2006). The relative lymphocyte count on hospital admission is a risk factor for long-term mortality in patients with acute heart failure. *Am.J. Emer. Med*., **24:** 451-454.

Satho, K. (1978). Serum lipid peroxidation in cerebrovascular disorders determined by a new colorimetric method. *Clin. Chim. Acta*., **90:** 37- 43.

Schaufelberger, M., Andersson, G., Erikssonj, B.O., Grimbyt, G., Held, P and Swedberg, K. (1996). Skeletal muscle changes in patients with chronic heart failure before and after treatment with enalapril. *European Heart Journ*., **17:** 1678-1685.

Sesh, P. S. L., Venkatesan, P., Jeyaraja, K., Chandrasekar, M. and Pandiyan, V. (2013). Blood biochemical, enzymatic and hematological status of dogs affected with dilated cardiomyopathy. *International Journal of Advanced Veterinary Science and Technology*, **2**(1): 47-51.

Sesh, P.S.L., Venkatesan, P., Jeyaraja, K., Chandrasekar, M. and Pandiyan,V. (2015). Xanthine oxidase as a biochemical marker of dilated cardiomyopathy in dogs. *Indian J. Anim. Res.*, **49** (2): 187-190.

Snedecor, G.W. and Cochran. W.G (1994). Statistical Method 9th edition, Iowa State University Press, Ames, U.S.A.

Tanner, H., Moschovitis, G and Kuster, G (2002). The prevalence of anemia in chronic heart failure. *Int. J. Cardiol*., **86:** 115–121.

Tomaselli, G.F., Beuckelmann, D.J., Calkins, H.G., Berger, R.D., Kessler, P.D., Lawrence, L.H., Kass, D., Feldman, A.M and Marban, E (1994). Sudden cardiac death in heart failure. The role of abnormal repolarization. *Circulation*. **90:** 2534–2539.

Welshman, S.E. and Carol Rixon, E (1967). Colorimetric estimation of lactate dehydrogenase isoenzymes by urea inhibition. *Clin. Chim. Acta*., **19:** 121-123.

Xia,Y., Khatchikian,G. and Zweier,J. (1996). Adenosine deaminase inhibition prevents free radical mediated injury in the post ischaemic heart. *J. Biol. Chem*., **271:** 10096-10102.