Xanthine oxidase as a biochemical marker of dilated cardiomyopathy in dogs

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**ABSTRACT**

Dilated cardiomyopathy occurs more frequently in large breed of dogs and the common form is the Idiopathic Dilated Cardiomyopathy. Xanthine oxidase is considered as one of the potent enzyme causing oxidative stress in the failing heart, and it uncouples cardiac energy consumption from cardiac contraction resulting in heart failure. In the present study xanthine oxidase activity was estimated in the serum of Dilated cardiomyopathy affected dogs and compared with that of healthy dogs serving as control. Similarly, other known cardiac enzyme markers like creatine kinase, aspartate transaminase and lactate dehydrogenase activities were also estimated. The results showed a 300% increase in the specific activity of xanthine oxidase and a 200% increase in the total activity of xanthine oxidase. This indicates that measurement of xanthine oxidase activity can serve as an early biochemical marker for diagnosis of Dilated cardiomyopathy in canines.

**Key words:** Cardiology, Cardiac markers, Dilated cardiomyopathy, Dogs, Xanthine oxidase.

**INTRODUCTION**

The usefulness of biochemical markers in the diagnosis and prognosis of heart disease is well documented in humans. This diagnostic approach has also been extended towards the detection of heart failure in companion animals. Dilated Cardiomyopathy (DCM) characterized by chamber dilatation and myocardial systolic and diastolic dysfunction is one of the most common heart diseases in dogs (Tidholm, et al., 2001). DCM occurs more frequently in large and giant breed dogs (Doberman Pinschers, Boxers, etc) and rarely in small breeds (cocker spaniels) or mixed breeds (Mc Evan, 2000). The most common form is Idiopathic DCM, as many processes could lead to myocardial injury in dogs – viz. genetic, nutritional, metabolic, inflammatory, infectious agents or drug or toxin induced (Borgarelli, et al., 2001, Petric and Tomsic, 2008).

The veterinary biochemical markers of cardiac dysfunction on date are of two types: The first group comprises of biochemical markers of myocardial injury and necrosis like cardiac enzymes - creatine kinase (CK-MB), aspartate transaminase (AST), lactate dehydrogenase (LDH), and the recently evaluated structural myocardial proteins – troponins. The second group of biochemical markers is used to assess the degree of cardiac dysfunction and is represented by the plasma neurohormones. The most commonly used indicators of neuroendocrine activation are plasma norepinephrine (NE), the atrial natriuretic peptide (ANP), the B-type peptide (BNP), peptides of the renin - angiotensin system (RAS); – vasopressin and aldosterone, and plasma big endothelin –I (Petric and Tomsic, 2008). But each marker has their own limitation and hence search for new markers are continuing (Kemp et al., 2004).

Oxidative stress, an imbalance between the formation and degradation of free radicals within the myocardium, contributes to metabolic derangements in patients with DCM. The enzyme xanthine oxidase (XO) is expressed in the failing heart, and it uncouples cardiac energy consumption from cardiac contraction in the setting of chronic heart failure. Xanthine oxidase has been shown to be a major source of free radical generation leading to oxidative stress under ischaemic conditions (Xia et al., 1996; Pandey et al., 2000). These oxygen free radicals generated by xanthine oxidase in turn oxidize cellular proteins and membranes resulting in myocardial cellular injury (Gorman and Zweier, 1990). The clinical trial on the intravenous injection of allopurinol (Xanthine oxidase inhibitor) for the treatment of Idiopathic DCM in human beings is under progress since 2006 at National Heart Lung and Blood Institute (NHLBI), USA (Internet source: [http://clinicaltrials.gov/ct2/show/study/NCT00281255](http://clinicaltrials.gov/ct2/show/study/NCT00281255)). In India, the role of xanthine oxidase (XO)
as a biochemical marker in myocardial infarction (MI) in human beings has been reported by Raghuvanshi et al., 2007. In the present study xanthine oxidase activity was estimated along with total CK, ALT, LDH and LDH-1 activities in the blood of canine patients with DCM and compared with that of healthy canine subjects serving as control.

MATERIALS AND METHODS

The blood samples were collected from the dogs attending the OP ward of Madras Veterinary College Teaching Hospital, Chennai. Fifty blood samples were collected from healthy dogs that served as control (Group I) after routine clinical examination and another fifty blood samples were collected from DCM diagnosed dogs (Group-II) after screening for cardiac dysfunction tests by ECG, Chest Radiography and Doppler echocardiography.

From each dog about 5 mL of blood was collected from the cephalic vein and serum was separated and maintained at 4°C till analysis. All the enzyme assays were carried out on the same day.

The activity of creatine kinase (total), lactate dehydrogenase (total), aspartate transaminase and total protein concentration were assayed using the diagnostic kits (M/S. Agappe Diagnostics Ltd., Ernakulum, Kerala, India) as per the manufacturer’s protocol. LDH-1 activity was determined by the method of Welshman et al., (1967) and Xanthine oxidase activity by the modified method of Bergmeyer, et al., (1974) as described below. All the chemicals used in the assay were of Analytical grade (Merck or equivalent).

Assay of LDH-1 activity: LDH-1 activity was determined by the method of Welshman et al., (1967). To 10 ml of urea phosphate buffer, 1 mg of NADH was added and mixed well. 2.8ml of the above solution was taken and 0.1ml of serum was added to it. The contents were mixed well and incubated for 20 min at 25°C. After incubation, 0.1ml of sodium pyruvate solution was added and transferred immediately to cuvette. Then the absorbance was read at 340 nm for 5 minutes with an interval of 1 minute. The difference in the optical density was noted and expressed as Wroblewski units/ml of urea stable LDH (LDH-1).

Calculation: Wroblewski units/ml of urea stable
LDH = Δ OD / min x 10⁶

Conversion: 1 U/L x 2.07 = 1 Wroblewski unit/ml

Assay of xanthine oxidase activity: Assay of xanthine oxidase was carried out essentially according to the modified method of Bergmeyer, et al., (1974). The assay mixture, in final volume of 3.0 ml, consisted of 0.3ml of Tris-HCl buffer, 50mM pH 7.4; 0.3ml of CuSO₄, 10mM; 0.05ml Xanthine, 2.58mM per ml in 0.05M glycine buffer, pH 7.4; 0.1ml of diluted blood (10µl in 900µl buffer). Change in absorbance was recorded at 290 nm at 15 seconds interval for one minute. Sample blank (control) was also run simultaneously. One unit of enzyme activity is defined as the amount of enzyme that will convert 1.0 µmole of xanthine to uric acid per minute at pH 7.5 at 25°C.

Calculation
Total activity:
Units/ml enzyme =
(Δ A290nm/min Test - Δ A290nm/min Blank) (3) (df)

Where, 3 = Total volume (in millilitres) of assay
df = dilution factor
12.2 = Millimolar extinction coefficient of Uric acid at 290nm.
0.1 = Volume (in millilitres) of blood

Specific activity:

         Units/ml enzyme
mg protein/ml enzyme

Statistical analysis: The results are expressed as mean ± SE for all the 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.

RESULTS AND DISCUSSION

The results of established cardiac marker enzymes are presented in Table 1 and that of xanthine oxidase activity is presented in Table 2.

Creatine kinase (CK) activity had a significant increase in DCM affected serum samples, while Aspartate transaminase (AST), Lactate dehydrogenase (LDH) and LDH-1 activity were well within the normal range and no significant difference was observed between the groups (Table 1). The tissue specificity of CK-MB in dogs has been shown not to be absolute due to its presence in skeletal muscle besides myocardial muscle, both types being released into blood stream following trauma (Swaanenburg et al., 1998; Apple, 1999). Moreover, by measuring the total CK activity itself, it is possible to assess cardiac damage in dogs (Aktas, et al., 1993) and hence in the present study measurement was restricted to total CK activity only (CK-MB was not measured). These results indicate that the affected dogs were presented at acute / per acute stage of illness, as the CK enzyme activity is the first to rise in any cardiac dysfunction i.e. within 3-6 hrs (Hossein –Nia, et al., 1997) and the activity of AST and LDH enzymes rises in serum only after 4-5 days and 2-3 weeks respectively (Kaneko, 2008).
TABLE 1: Cardiac marker enzymes in Healthy and DCM dogs

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control(n=50)</th>
<th>DCM(n=50)</th>
<th>Reference range</th>
<th>t - test</th>
<th>P - value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL - CK (U/L)</td>
<td>20.23±1.0387</td>
<td>107.89±23.3291</td>
<td>1.15-28.40</td>
<td>3.75</td>
<td>0.0009</td>
<td>***</td>
</tr>
<tr>
<td>LDH (WU/ml)</td>
<td>352.98±17.51</td>
<td>388.12±40.9138</td>
<td>93.15-482.31</td>
<td>0.79</td>
<td>0.4337</td>
<td>NS</td>
</tr>
<tr>
<td>LDH – 1(WU /ml)</td>
<td>31.31±3.1691</td>
<td>28±6.7178</td>
<td>3.5-51.34</td>
<td>0.45</td>
<td>0.6601</td>
<td>NS</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>45.91±1.9208</td>
<td>59.26±7.4831</td>
<td>23.0-66.0</td>
<td>1.73</td>
<td>0.0901</td>
<td>NS</td>
</tr>
</tbody>
</table>

U/L - Units/Litre; WU/ml - Wroblewski units/millilitre; *** - p < 0.01 (Highly significant); NS – Not significant.

TABLE 2: Xanthine oxidase activity in Healthy and DCM dogs

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control(n=50)</th>
<th>DCM(n=50)</th>
<th>t - test</th>
<th>P - value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL ACTIVITY - (U/ml)</td>
<td>6.26 ± 0.3703</td>
<td>13.83 ± 0.9653</td>
<td>7.32</td>
<td>0.0000</td>
<td>***</td>
</tr>
<tr>
<td>XANTHINE OXIDASE SPECIFIC ACTIVITY</td>
<td>0.01 ± 0.009</td>
<td>0.03 ± 0.0027</td>
<td>5.40</td>
<td>0.0001</td>
<td>***</td>
</tr>
</tbody>
</table>

U/ml – Units/millilitre; *** - p < 0.01 (Highly significant).

As far as Xanthine oxidase (XO) activity is concerned (Table-2) there is a highly significant difference between the two groups, with DCM cases showing higher values. The total activity of XO was found to be 6.26 ± 0.3703 U/ml in control group whereas it increased to 13.83 ± 0.9653 (200% increase) in DCM cases. Similarly, a 300% increase in the specific activity of XO was observed in DCM (0.01 ± 0.0027 U/ml) cases when compared to the control (0.01 ± 0.009 U/ml) group. These results suggest the potent role and expression of XO during myocardial injury leading to oxidative stress, resulting in heart failure (Raghuvanshi et al., 2007). As the results of other cardiac enzyme markers have suggested that the samples analysed were from per acute DCM cases, it is evident that there is an early expression of XO in DCM.

Therefore, it may be concluded that measurement of Xanthine Oxidase activity may be used as a biochemical marker of Dilated Cardiomyopathy in dogs, at acute stage of illness along with ECG / Doppler echocardiography observations.

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REFERENCES


