HAEMATO-BIOCHEMICAL ANALYSIS OF DILATED CARDIOMYOPATHIC DOGS TREATED WITH ENALAPRIL AND SPIRONOLACTONE

S.S. Sreenesh1, Usha Narayana Pillai2, P.C. Alex3, S. Ajith Kumar4 and C.B. Devanand5
1P.G. Student, 2Professor, 3Ex. Professor, 4Professor and Head, Department of Clinical Veterinary Medicine, 5Professor and Head, Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651(Kerala), India.
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Dilated cardiomyopathy (DCM) is a primary heart disease of dogs with cardinal signs of myocardial systolic dysfunction which eventually progress to the congestive heart failure and death. The haemato-biochemical parameters of DCM patients treated with enalapril at the dose rate of 0.5 mg/kg body weight b.i.d and spironolactone at the dose rate of 1.0 mg/ kg body weight b.i.d. for three months as neurohormonal modification therapy were statistically analysed. The cardiac biomarker- brain natriuretic peptide (BNP) levels of DCM dogs were elevated (20.17 ± 2.19pg/ml). The treatment could not significantly modify the BNP levels after three months. Slight stress leukogram was observed in the initial presentation.A reversal of granulocytosis pattern was observed after the treatment. No statistically significant differences were noticed in serum sodium, potassium, creatinine and blood urea nitrogen before and after the treatment.Creatine phosphokinase concentrations were inconclusive. Brain natriuretic peptide was excellently discriminated cardiac and non-cardiac dyspnoea in the present study while screening the DCM dogs and was identified as the most specific biochemical parameter for the cardiac disease in dogs. The neuro-hormonal modification therapy alone without using an inotropic agent for DCM is not effective.

Key Words: Dilated cardiomyopathy, enalapril, spironolactone, neurohormone, brain natriuretic peptide.

Dilated cardiomyopathy (DCM) is a primary myocardial abnormality which is having suspected relationship with some genetic disorders. Preclinical cardiomyopathy progresses to clinical DCM, to the congestive heart failure and death eventually. The exact aetiology is unknown and supposed to be multifactorial. So, the diagnosis and therapy is difficult in these patients. The recent studies confirm the role of neurohormonal changes in these cardiac patients. A neurohormonal modulatory treatment in DCM patients was conducted by using enalapril and spironolactone and various haemato-biochemical parameters were analysed.

Materials and Methods

Dogs presented to the Teaching Veterinary Clinical Complex, Mannuthy and University Veterinary Hospital, Kokkala, Kerala, India, with confirmed dilated cardiomyopathies were used during the present study. The Blood BNP levels of dogs with dyspnoea were analysed before going to the detailed cardiac examination as it is a quick test. Complete physical examination in specific to cardiovascular system, ECG, Blood Pressure, Haemotobiochemical analysis, Echocardiography, and Thoracic Radiographic examinations were utilized to confirm the DCM. Major changes observed in the DCM dogs were cardiac arrhythmia, increased vetebral heart score, increased E-point to septal separation with central regurgitant jet, reduced ejection fraction and fractional shortening and elevated end diastolic volume. Six DCM dogs were subjected to detailed haematobiochemical examination at the initial visit, then at one month intervals up to three months according to the evaluation schedule.

Haematological parameters [Complete blood count - Haemoglobin (Hb) in gm per cent, Red blood cell count (RBC) in 106/μl, Volume of packed red cells (VPRC) in per cent, White blood count (WBC) in 1000/μl, Differential blood count (DLC) in 1000/μl and Platelet count (PLT) in 1000/μl] and

*Part of M.V.Sc. thesis of first author
biochemical parameters [Sodium, Potassium, Blood Urea Nitrogen, Creatinine, Creatine phosphokinase and B-type natriuretic peptide] were recorded.

Two ml whole blood was collected into Nex Gen vacutainer tubes with K2 EDTA by following the standard sample collection techniques. ORPHEE Mythic 18 Vet CBC Machine was used for the analysis.

Blood was drawn into a four ml capacity m-tube vacutainers coated with clot activator. Serum was separated from the clotted blood. Sera thus separated were stored at -20°C until further analysis. Biochemical analysis was performed with HOSPITEX DIAGNOSTICS, MASTER T machine. A kinetic enzymatic method based CORMAY Liquick Cor-UREA mini kit was used for blood urea nitrogen estimation. Modified Jaffé’s method based CORMAY Liquick Cor- CREATININE mini kit was used for creatinine estimation. An optimized kinetic method based COMRAY Liquick Cor- CK mini kit was used for CK-MB estimation.

One step sandwich immunoassay based BNP Alere heart check system was utilized for BNP estimation. It was a patient bed side test.

The six DCM dogs were treated with Angiotensin-converting enzyme Inhibitor - Tab. enalapril at the dose rate of 0.5 mg/kg b.i.d. PO and Tab. Spiranolactone at the dose rate of 1.0 mg/kg b.i.d. PO for the three months of study period.

Haemato-biochemical responses in different parameters on day 0, 30, 60 and 90 were analysed with Rpeated measures ANOVA by using IBM SPSS Statistics, Version 20 software. The paired parameter like BNP, before and after treatment was statistically analysed by using the paired t-test.

**Results**

The results of various haematobiochemical parameters at initial presentation, 30th, 60th and 90th days are presented in the tables below.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(n)</th>
<th>Baseline (0th Day) (Mean ± S.F.)</th>
<th>30th Day (Mean ± S.F.)</th>
<th>60th Day (Mean ± S.F.)</th>
<th>90th Day (Mean ± S.F.)</th>
<th>Reference interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (1000/µl)</td>
<td>6</td>
<td>21.87 ± 4.09</td>
<td>18.45 ± 2.49</td>
<td>16.52 ± 2.35</td>
<td>16.15 ± 0.86</td>
<td>6.0-17.0</td>
</tr>
<tr>
<td>LYM (1000/µl)</td>
<td>6</td>
<td>2.82 ± 1.30</td>
<td>1.86 ± 0.38</td>
<td>1.74 ± 0.15</td>
<td>2.38 ± 0.40</td>
<td>0.7-5.1</td>
</tr>
<tr>
<td>MON (1000/µl)</td>
<td>6</td>
<td>0.84 ± 0.54</td>
<td>0.46 ± 0.08</td>
<td>0.54 ± 0.02</td>
<td>0.54 ± 0.12</td>
<td>0.2-1.7</td>
</tr>
<tr>
<td>GRA (1000/µl)</td>
<td>6</td>
<td>18.22 ± 3.34</td>
<td>14.90 ± 3.66</td>
<td>13.56 ± 0.52</td>
<td>13.36 ± 0.64</td>
<td>4.4-12.6</td>
</tr>
<tr>
<td>LYM (%)</td>
<td>6</td>
<td>11.22 ± 1.62</td>
<td>10.27 ± 1.78</td>
<td>15.38 ± 5.22</td>
<td>12.82 ± 1.84</td>
<td>12.0-30.0</td>
</tr>
<tr>
<td>MON (%)</td>
<td>6</td>
<td>2.78 ± 1.08</td>
<td>2.34 ± 0.36</td>
<td>3.18 ± 0.35</td>
<td>3.40 ± 0.63</td>
<td>3.0-10.0</td>
</tr>
<tr>
<td>GRA (%)</td>
<td>6</td>
<td>86.02 ± 3.06</td>
<td>87.14 ± 2.48</td>
<td>86.56 ± 1.43</td>
<td>82.44 ± 1.79</td>
<td>40.0-74.0</td>
</tr>
<tr>
<td>RBC (10⁹/µl)</td>
<td>6</td>
<td>5.00 ± 0.45</td>
<td>5.72 ± 0.48</td>
<td>5.59 ± 0.19</td>
<td>5.99 ± 0.32</td>
<td>5.50-8.50</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>6</td>
<td>10.55 ± 1.50</td>
<td>10.48 ± 0.84</td>
<td>9.85 ± 0.39</td>
<td>11.35 ± 1.01</td>
<td>12.0-18.0</td>
</tr>
<tr>
<td>VPRC (%)</td>
<td>6</td>
<td>32.90 ± 3.99</td>
<td>33.90 ± 2.23</td>
<td>33.65 ± 1.26</td>
<td>35.98 ± 2.06</td>
<td>37.0-55.0</td>
</tr>
<tr>
<td>MCV (µm³)</td>
<td>6</td>
<td>64.57 ± 4.26</td>
<td>60.23 ± 3.91</td>
<td>60.12 ± 1.46</td>
<td>60.63 ± 3.55</td>
<td>60.0-77.0</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>6</td>
<td>20.55 ± 2.11</td>
<td>18.53 ± 1.47</td>
<td>17.57 ± 0.50</td>
<td>19.32 ± 1.41</td>
<td>19.0-25.0</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>6</td>
<td>31.38 ± 1.38</td>
<td>30.80 ± 0.81</td>
<td>29.55 ± 0.95</td>
<td>31.83 ± 0.99</td>
<td>32.0-36.0</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>6</td>
<td>17.20 ± 0.88</td>
<td>18.80 ± 1.42</td>
<td>17.84 ± 0.68</td>
<td>17.16 ± 1.05</td>
<td>11.0-14.0</td>
</tr>
<tr>
<td>PLT (1000/µl)</td>
<td>6</td>
<td>409.50 ± 74.86</td>
<td>341.33 ± 58.83</td>
<td>426.67 ± 56.95</td>
<td>379.00 ± 78.22</td>
<td>160-525</td>
</tr>
</tbody>
</table>
Table 2. Blood BNP values of diseased dogs under treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(n)</th>
<th>Before treatment-0th Day (Mean ± S.E)</th>
<th>After treatment-90th Day (Mean ± S.E)</th>
<th>Reference interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain Natriuretic Peptide (pg/ml)</td>
<td>(6)</td>
<td>20.17 ± 2.19</td>
<td>21.00 ± 2.34</td>
<td>&lt; 18</td>
</tr>
</tbody>
</table>

Table 3. Clinical biochemistry parameters of DCM dogs in treatment trial

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(n)</th>
<th>Baseline (0th Day) (Mean ± S.E)</th>
<th>30th Day (Mean ± S.E)</th>
<th>60th Day (Mean ± S.E)</th>
<th>90th Day (Mean ± S.E)</th>
<th>Reference interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (mEq/L)</td>
<td>(6)</td>
<td>145.50 ± 0.67</td>
<td>144.83 ± 1.45</td>
<td>143.50 ± 1.82</td>
<td>140.17 ± 2.33</td>
<td>145-154</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>(6)</td>
<td>4.40 ± 0.21</td>
<td>4.32 ± 0.09</td>
<td>4.17 ± 0.09</td>
<td>4.25 ± 0.21</td>
<td>3.6-5.3</td>
</tr>
<tr>
<td>Creatine Phosphokinase (IU/L)</td>
<td>(6)</td>
<td>201.10 ± 82.78</td>
<td>297.81 ± 91.26</td>
<td>191.40 ± 27.32</td>
<td>198.45 ± 17.84</td>
<td>51-399</td>
</tr>
<tr>
<td>Blood UreaNitrogen (mg/dL)</td>
<td>(6)</td>
<td>26.73 ± 4.95</td>
<td>22.78 ± 3.30</td>
<td>24.78 ± 7.07</td>
<td>28.45 ± 5.75</td>
<td>10.7-53.5</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>(6)</td>
<td>1.30 ± 0.131</td>
<td>1.20 ± 0.12</td>
<td>1.29 ± 0.03</td>
<td>1.37 ± 0.09</td>
<td>0.3-1.2</td>
</tr>
</tbody>
</table>

Discussion

None of the haematological parameters were statistically significant. Marginal granulocytosis was observed in the initial presentation. This is similar to the findings of Peddle and Sleeper (2009) and James (2011), which reported that stress leukogram as the major haematological changes in DCM dogs. It is assumed that, the increased cortisol level released from the adrenal gland is a reason for the stress leukogram in DCM dogs even though there is no cortisol assay in the present study. The pain, distress and the dehydration in congested patients are assumed as the cause of stress leukogram in these animals.

Lombard (2011) reported mild anaemia and rarely stress leukograms and other haemogram abnormalities in dogs with primary myocardial diseases, contrary to that the author reports severe stress leukogram in secondary myocardial diseases.

The reversal of stress leukogram pattern was observed in animals. The angiotensin-II had no effect on catecholamine secretion in Ca2+-free medium as also reported by Kuwashima et al. (2003). So, possible reasons in the reversal of stress leukogram are reduced respiratory distress, rehydration and better tissue oxygenation in these animals treated with enalapril and spironolactone.

No statistically significant differences were noticed in serum sodium, potassium, creatinine and blood urea nitrogen before and after the treatment. The mean serum sodium levels slightly reduced after the treatment with enalapril and spironolactone. Thomason et al. (2014) reported similar findings that the combination therapy with ACEI and aldosterone receptor antagonist could lower serum sodium concentrations.

The relatively higher serum potassium level as also recorded by James (2011) in untreated DCM dogs could have been reduced after the medical attention. The expected increase in serum potassium level proposed by Thomason et al. (2014) in animals treated with an ACEI and aldosterone receptor antagonist could not be observed in the current study. This is in accordance with the findings of Schuller et al. (2011).

The analyses of serum CPK concentrations were not conclusive. Even though, the elevation in CPK level in cardiac patients as was also reported by some of the authors like James (2011), the present treatment protocol produced variable
concentrations of CPK in these animals. As CPK is one of the earliest blood biomarker of cardiac injury, its sensitivity and specificity is relatively lower as also reported by Solter (2007).

There was no significant difference between brain natriuretic peptide levels before and after treatment. But the blood levels were found to be elevated in the animals diagnosed with DCM. While screening the animals for DCM, the dogs with primary lung diseases showed normal blood BNP levels. But dogs presented with cardiogenic dyspnoea showed elevated blood BNP levels. Thus, BNP excellently discriminated cardiac and non-cardiac dyspnoea in the present study. These findings agree with the reports of Oyama, (2009) and Unny (2014). The expected myocardial stretch due to the global dilatation of heart in DCM patients and the myocardial damage is assumed as the result for an elevated BNP levels. The concentration was still found to be elevated even after the treatment. Decreased systolic functions of these dogs towards the end to third month of treatment could result in an elevation in BNP level. During development of left ventricular dysfunction, gene expression of atrial ANP and BNP gradually increases as also reported by Haggstrom, et al. (2013). Further studies in the prognosis and median survival time of DCM dogs in relation with the BNP level is warranted.

Conclusion
In conclusion, brain natriuretic peptide was excellently discriminated cardiac and non-cardiac dyspnoea in the present study and was identified as the most specific parameter for the cardiac disease in dogs. The neurohormonal modification therapy alone without using an inotropic agnet for DCM is not effective.

References