Cardiac Troponin I Levels in Dogs with Dirofilariosis and without Dirofilariosis

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ABSTRACT

Differences about Cardiac Troponin I (cTnl) levels between the dogs without dirofilariais were investigated in this study. There were statistically significant differences about cTnl levels between the groups (For Group 1: 5.55 ng mL⁻¹ (2.1-10.0); for Group 2: 0.05 ng mL⁻¹ (0.03-0.06) p<0.001). As well as heart rate, respiration rate and temperature, cTnl levels were also found as statistically significantly important (p<0.001, p = 0.008 and p = 0.012, respectively). There was increased relative risk ratio for the body temperature group 2 (Relative risk ratio: 6.667 (2.827-15.724, Confidence interval: %95, p<0.001). There are statistically significant differences between the dogs with and without dirofilariais about cTnl levels. It was speculated that cTnl level might be important indicator for evaluation of myocardial injury in the dogs with dirofilariais.

Key words: Dirofilariais, troponin I, heart rate, respiration, body temperature

INTRODUCTION

Canine heartworm appears to be common in the world. Canine heartworm infection has been reported in many parts of United States (Tzipory et al., 2010), Iran (Gholami et al., 2011), Japan (Nogami and Sato, 1997) and Turkey (Borku et al., 1995; Icen et al., 2011). It is known that approximately 40 species of Dirofilaria spp. can be found in dogs and cats that common occured species D. immitis and D. repens (Burgu et al., 2004).

Heartworm (Dirofilaria immitis) infection is a mosquito-borne disease of dogs and wild canids, which also serve as reservoirs. Adult worms are located in the pulmonary arteries and in the right cardiac chambers. Dirofilaria immitis is a zoonotic diseases, causes respiratory and systemic disorders, transmitted by female flies to dog, a parasite agent (Schober et al., 1999; Oyama and Sisson, 2001; Ranjbar-Bahadori et al., 2007). Dirofilariais is generally common seen between 3-15 ages in dogs (Jackson et al., 1992). It may cause pulmoner nodules in human (Shah, 1999).

Adult worms of Dirofilaria immitis causes endocarditis, arteritis, circulation defeat and hypertension in definitive hosts. Hypertrophy of heart, liver congestion, cirrhosis and ascites are commonly seen as symptoms of heartworm infection in dogs (Icen et al., 2011).

The troponins exhibit myocardial tissue specificity and high sensitivity. The level of Tn-I remains elevated for a much longer period of time (6-10 days), thus providing for a longer window of detection of cardiac injury (Oyama and Sisson, 2004).
Unexpected deaths might be contributed by myocardial injury due to dirofilariaasis. To detect myocardial damage, measurement of cardiac troponin I (cTnI) is currently the most sensitive and specific method. Range of cTnI is smaller than 0.03-0.07 ng mL⁻¹ in the dogs (Sleeper et al., 2001). cTnI levels were analyzed in dogs with canine dirofilariaasis and the levels of cTnI were compared with healthy dogs in the present study.

MATERIALS AND METHODS

Animal selection: Many dogs were examined by the clinically and blood smears with dyspnea, cough and exercise intolerance in an official dog kennel club province Ankara in Turkey during the period of 2003-2006. Eventually, dirofilariaasis was diagnosed in 11 dogs [8 crossbreed, 2 anatolian shepherd (domestic breed) and 1 german shepherd of both sexes (mean age: 4.6 years; min-max age: 3.0-6.0] by using physical examination and blood smear analyses methods.

The group of dogs with dirofilariaasis compared with eleven clinically healthy crossbred dogs according to vital parameters and Cardiac troponin I levels. Dogs with myocarditis associated with infections ruled out by laboratory investigations included Babesia spp. (Breitschwerdt et al., 1999), Borrelia burgdorferi (Levy and Duray, 1988) bacteraemia, disseminated fungal infection, Angiostrongylus spp. Toxoplasma gondii or granulocytic ehrlichiosis (Montoya et al., 1997). Myocarditis has been reported in canine Systemic Lupus Erythematosus (SLE) (Shah and McGowan, 2003). The dogs which were detected SLE were also excluded the study.

Blood serum samples: Each dog was examined clinically and then 5 mL blood without anticoagulant were taken from V. cephalica parva for c Tn I tests. To get serum, the samples were stored for an hour at room temperature and centrifuged at 1400 g for 10 min.

Cardiac troponin I (cTnI) analyses: cTnI was measured from each dog blood by based on a chemiluminescent enzyme immunoassay method (CLEIA) (PATHFAST, Mitsubishi Kagaku Iatron Inc, Tokyo, Japan). In briefly, alkaline phosphatase labeled anti cTnI monoclonal antibody and anti cTnI monoclonal antibody coated magnetic particles are mixed in sample. cTnI contained in the specimen binds to the anti cTnI antibodies forming an immunocomplex with enzyme labeled antibody and antibody coated magnetic particles. After removing the unbound enzyme labeled antibody, a chemiluminescent substrate is added to the immunocomplex. After a short incubation, the luminescence generated by enzyme reaction is detected. The intensity of the measured luminescence is in relationship with the cTnI concentration in the specimen which is calculated by means of a standard curve.

Statistical analysis: All statistical analysis was performed using SPSS version 15 (SPSS, Chicago, IL, USA). Data were expressed as median value (25-75% percentile). For the purpose of statistical comparison between the groups, animals were categorized into two groups as follows:

- **Group 1**: The dogs with dirofilariaasis
- **Group 2**: The dogs without dirofilariaasis (Control group)

For the purpose of revealing the statistical relationships between Troponin I (TPI) levels and vital parameters:
Patients were categorized into three groups according to TPI levels as follows:

- TPI group 1 (TPI-1): 0.03-0.06 ng mL\(^{-1}\) (Normal)
- TPI group 2 (TPI-1): 0.07-4999 ng mL\(^{-1}\)
- TPI group 3 (TPI-1): ≥5000 ng mL\(^{-1}\)

For the purpose of evaluation of statistical differences among TPI levels, Kruskal-Wallis test (Riffenburgh, 2006) was used for statistical analysis. Afterwards, relationships among vital parameters and TPI levels were showed by the correlation analysis (Spearman) (Ozdamar, 2003).

On the other hand, for the purpose of evaluating the relative risk ratios for body temperature groups about TPI levels; patients were categorized into two groups according to body temperature levels as follows:

- Body temperature group 1: <39.0°C
- Body temperature group 2: ≥39.0°C

Statistical evaluation was performed for determination of differences about vital parameters. Non-parametric statistical analyses were performed. Mann-Whitney U test (Ozdamar, 2003) was used for statistical evaluation of the differences between the groups. Besides, Spearman test was used to evaluate the correlations among the data.

A p-value of <0.05 was accepted as statistically significant.

RESULTS

In the clinical examination; exercise intolerance (n = 11), cough (n = 11), dyspnea (n = 11) were occurred in all dogs with dirofilariaisis. Ventral edema was observed in one case (n = 1). The clinical characteristics of Group 1 and 2 were shown in Table 1 and Comparison about vital parameters and Troponin I levels between patient (2.12-10.00 ng mL\(^{-1}\)) and control groups (0.03-0.06 ng mL\(^{-1}\)) shown in Table 2. There was no statistically significant difference about age and sex between patient and control groups (p>0.05). There were statistically significant differences about measured vital parameters [Heart rate (group I: 124-140 beats min\(^{-1}\); group II: 98-120 beats min\(^{-1}\)], respiration rate (group I: 28-36 breaths min\(^{-1}\); group II: 22-28 breaths min\(^{-1}\)], temperature (group I: 38.8-39.9°C group II: 38.5-38.8°C) and Troponin I levels) between patient and control groups (Table 2). On the other hand, we also detected that there were statistically significant differences among the TPI groups about vital parameters (Table 3).

There were statistically significant correlations between troponin I levels and vital parameters. On the other hand, heart rate did not found as correlated with temperature and respiration rate. It is calculated the relative risk ratios for the body temperature groups about TPI levels. There was increased relative risk ratio for the body temperature group 2 (Relative risk ratio: 6.667 (2.827-15.724, Confidence interval: %95, p<0.001).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Dirofilariaised group (Group 1, N=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise intolerance</td>
<td>11</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>11</td>
</tr>
<tr>
<td>Cough</td>
<td>11</td>
</tr>
<tr>
<td>Ventral edema</td>
<td>1</td>
</tr>
</tbody>
</table>

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Table 2: Comparison about vital parameters and troponin I levels between patient and control groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 N: 11 median (25-75%)</th>
<th>Group 2 N:11 median (25-75%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>5.0 (3.0-6.0)</td>
<td>4.0 (3.0-5.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate (beats min⁻¹)</td>
<td>130 (124-140)</td>
<td>110 (98-120)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Respiration rate (1 min⁻¹)</td>
<td>32 (28-36)</td>
<td>26 (22-28)</td>
<td>0.007</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>39 (38.8-39.9)</td>
<td>38.8 (38.5-38.8)</td>
<td>0.021</td>
</tr>
<tr>
<td>Troponin I (ng mL⁻¹)</td>
<td>5.55 (2.12-10.00)</td>
<td>0.05 (0.03-0.06)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Mann-whitney U test, p<0.05. NS: Statistically nonsignificant

Table 3: Correlations among vital parameters and troponin I levels

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Heart rate (1 min⁻¹)</th>
<th>Respiration rate (ng mL⁻¹)</th>
<th>Temperature (°C)</th>
<th>Troponin I (1 min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (1 min⁻¹)</td>
<td>r: 0.297</td>
<td>r: 0.278</td>
<td>r: 0.835</td>
<td>r: 0.835</td>
</tr>
<tr>
<td></td>
<td>p: NS</td>
<td>p: NS</td>
<td>p: &lt;0.001</td>
<td>p: &lt;0.001</td>
</tr>
<tr>
<td>Respiration rate (1 min⁻¹)</td>
<td>r: 0.297</td>
<td>r: 0.546</td>
<td>p: 0.009</td>
<td>p: 0.008</td>
</tr>
<tr>
<td></td>
<td>p: NS</td>
<td>p: NS</td>
<td>r: 0.546</td>
<td>r: 0.547</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>r: 0.278</td>
<td>r: 0.009</td>
<td>p: 0.009</td>
<td>p: 0.012</td>
</tr>
<tr>
<td>Troponin I (ng mL⁻¹)</td>
<td>r: 0.835</td>
<td>r: 0.547</td>
<td>r: 0.527</td>
<td>p: 0.012</td>
</tr>
<tr>
<td></td>
<td>p: &lt;0.001</td>
<td>p: 0.008</td>
<td>p: 0.012</td>
<td></td>
</tr>
</tbody>
</table>

Spearman test, r: Correlation coefficient. p<0.05. NS: Statistically nonsignificant.

DISCUSSION

There were statistically significant differences about Cardiac troponin I (cTnI) levels between the dogs with dirofilariasis (Group 1) versus without dirofilariasis (Group 2). This finding shows that there may significant myocardial injury depending on myocarditis caused by dirofilariasis in Group 1.

It is well known that cTnI analysis is a highly sensitive and specific method for the detection of myocyte injury which has been used to diagnose myocardial damage in dogs and cats as well as human (Diniz et al., 2007). Measurement of cardiac troponin can be useful as a non-invasive method to determine suspected myocardial injury, for example in cases presented with cardiac arrhythmia or multi organ dysfunctions (Spratt et al., 2005). Cardiac Troponin I is one of the intracellular contractile-regulating proteins of heart muscle. The plasma levels of cTnI have been shown to be associated with the severity of myocardial injury and survival in humans and dogs (Collinson et al., 2001).

In the present study, there were significant differences between TPI groups about heart rate, respiration rate and body temperature. On the other hand there were also significant correlations between TPI levels and heart rate and respiration rate. These findings are considered that increase in myocardial injury can cause heart failure in dogs. Because of TPI levels closely correlated with width of injured myocardial mass (Ranjbar-Bahadori et al., 2010), heart failure could be predicted by increased TPI levels in the dogs with myocarditis. On the other hand, TPI levels were also found as closely correlated with body temperature. According to these results, in group 1, the dogs with ≥39°C body temperature have increased risk for ≥5000 ng mL⁻¹ TPI levels. At this point of view, this finding was considered us, in the dogs with dirofilariasis, having ≥39°C body temperature might be accepted as poor prognostic criteria. On the other hand, increase in temperature can be a systemic response to inflammatory condition in any localization of the body. Moreover increased body temperature is not obligatory symptom for myocarditis (Liu and Schultheiss, 2008). Although the width of injured and inflammatory area may also cause increase in the response of body
temperature (Londono et al., 2005), this response are closely related with age and the proficiency of immune defense (Gardner, 1980). In this study, the mean body temperature was higher in group 1 than group 2 but these values were between the normal limits. At that point, further and large-scale studies are required for determination of the relationships between body temperature and myocardial injury in the dogs with dirofilariala.

Measurement of cTnI may in the future be used for these diagnostic and prognostic purposes even in the canine species. In dogs, increased plasma levels of cTnI have been demonstrated in cases of toxemia, babesiosis, myocarditis, dirofilariala and pericardial effusion (Spratt et al., 2005).

With regard to the veterinary literature, cardiac markers in Veterinary Cardiology have been commonly used in the last few years because of cost-effective cardiac analyses and the significance of early diagnosis in cardiological diseases from the therapeutic and prognostic perspectives (Sleeper et al., 2001; Herndon et al., 2002; Diniz et al., 2007). In Turkey, according to the authors' knowledge, these markers have not been used very commonly for research purposes; therefore, this study describes the importance of analyzing cTnI for the clinical diagnosis.

CONCLUSION
Cardiac troponin I is not specific parameter for diagnosis of dirofilariala. However, this new diagnostic method can play the critical role for making the diagnosis more clear and also may help to prediction of the prognosis in the patient with dirofilariala. This is a small-scale and pilot study for investigation of the diagnostic values of cTnI in the dogs with dirofilariala. Echocardiographic parameters of the dogs were not evaluated because of technical limitations. Further and large-scale studies can reveal real diagnostic and prognostic values of cTnI for the dogs with dirofilariala.

REFERENCES

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