

High sensitivity Cardiac Troponin T (hs-cTnT) and I (hs-cTnI) levels in dogs with *Dirofilaria immitis*

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Abstract: *Dirofilaria immitis* (*D. immitis*); is a parasitic zoonosis with a prevalence of 53.8%, whose intermediate host is mosquitoes (*Anopheles*, *Aedes*, *Culex*, *Taeniorhynchus*, *Mansonia* and *Armigenes*), final hosts are carnivores (dog, cat, fox, etc.) and humans. The causative agent settles in the pulmonary arteries and causes pulmonary hypertension, which can progress to congestive heart failure. Cardiac troponins (cTn), which have recently been used in veterinary cardiology, are considered the gold standard because of their high sensitivity and specificity for myocardial injury. In the study, high-sensitivity cardiac troponin T (hs-cTnT) and high-sensitivity cardiac troponin I (hs-cTnI) levels in healthy dogs were determined as 0.092 ± 0.009 and 0.135 ± 0.024 ng/mL, respectively. In dogs with *D. immitis*, hs-cTnT and hs-cTnI levels in healthy dogs were determined as 0.164 ± 0.035 and 0.234 ± 0.052 , respectively. As a result; it was determined that there was an increase in hs-cTnT and hs-cTnI levels in dogs with *D. immitis*, although it was not statistically significant ($p > 0.05$). Considering that even the slightest change in the levels of troponins is prognostically important, it can be said that *D. immitis* causes myocardial damage in dogs.

Key words: Canine, *Dirofilaria*, Troponin, vector induced diseases

1. Introduction

Dirofilaria immitis (*D. immitis*); is a parasitic zoonosis in the Filarioidea superfamily of the nematode class, whose intermediate host is mosquitoes (*Anopheles*, *Aedes*, *Culex*, *Taeniorhynchus*, *Mansonia* and *Armigenes*) the final host is carnivores (dog, cat, fox, etc.) and humans [1,2]. *Dirofilaria immitis*, the prevalence of which varies between 0.4%– 53.8% in the world [3–7] is one of the most important vector-borne agents among nematode species that infects dogs [8]. The agent settles in the right atrium and ventricle of the heart, pulmonary arteries, and camra oculi anterior in animals and humans, causing pulmonary hypertension which can progress to congestive heart failure characterized by endarteritis, embolization, and thromboembolism [9,10].

Cardiac troponins (cTn), which have recently been used in veterinary cardiology are accepted as the gold standard due to their high sensitivity and specificity for myocardial damage [11–13]. Troponins are globular proteins that have a role in the contraction

and relaxation of myofibrils [14] and they are included in the circulation when cardiac myocytes are destroyed [15]. It has been reported that cardiac troponin I (cTn-I) and cardiac troponin T (cTn-T) are specific for the heart and show a high sensitivity to myocardial necrosis [16,17]. High-sensitivity cTn-T (hs-cTnT) and high-sensitivity cTn-I (hs-cTnI) levels allow the determination of lower levels compared to cTn-T and cTn-I values, making it possible to detect mild myocardial damage that may occur in the early stages of diseases [18].

It has been reported that even small changes in the levels of troponins, which are frequently used in human studies, have prognostic significance [19–21]. For this reason, more and more sensitive analyses have been used in recent years [15]. In this study, it was aimed to determine in the *D. immitis*, which is one of the vector-borne zoonotic diseases (VBZD), by measuring with high sensitivity with the commercial Elisa kit of cTn-I, which have just started to be used in the veterinary field, in dogs according to age, sex and healthy ones.

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2. Materials and methods

2.1. Animal material

The material of the study consisted of 15 dogs diagnosed with *D. immitis* and 15 healthy dogs which were brought to Iğdır University Tuzluca Animal Hospital. Diagnosis of *D. immitis* was made with the rapid diagnosis kit (SensPERT® One Step Rapid Test Kit, VetAll Laboratories, Korea). Five mL blood was collected from *V. cephalica* of sick and healthy animals into serum tubes. After these bloods were centrifuged at 3000 rpm for 10 min, their serums were separated. The serums were stored at -20 °C until the hs-cTnI levels were measured. Although troponin concentrations can remain stable for a long time at -70/-80 °C, it has been reported that they remain stable at -20 °C for a maximum of 3 months [15,22,23]. For this reason, the serum samples used in the study are 60 days old.

2.2. Measurement of cardiac markers

Levels of serum hs-cTnT and hs-cTnI, were determined by ELISA equipment (Thermo Scientific Multiscan GO, TYPE: 1510) and calculated with commercial test kit (ng/mL) as instructed by the manufacturer (Canine High Sensitivity Cardiac Troponin T and I Kit, BT Lab, Korea).

2.3. Statistical analysis

SPSS 20 software was used for statistical analysis of the obtained data. Independent t-test and General Linear Model (GLM) procedure were followed to reveal the differences in hs-cTnT and I levels of healthy dogs and the dogs with *D. immitis* according to age and sex. The results were given as mean ± SE and mean ± SD.

3. Results

The dogs participating in the study; 47% (14/30) were females and 53% (16/30) were males. The changes in hs-cTnT and hs-cTnI in healthy dogs and dogs with *D. immitis* are given in Table 1.

When Table 1 was examined, although hs-cTnT levels were found to be 0.164 ± 0.136 ng/mL in dogs with *D. immitis* and 0.092 ± 0.035 ng/mL in healthy dogs, no statistically significant difference was found between the groups in the analysis (T = 1.961, p > 0.05). Although hs-cTnI level was found to be 0.234 ± 0.200 ng/mL in dogs with *D. immitis* and 0.135 ± 0.092 ng/mL in healthy dogs, no statistically significant difference was found between the groups in the analysis (T = 1.739, p > 0.05).

When Table 2 is examined, hs-cTnT and hs-cTnI levels were determined to be 0.098 ± 0.051, 0.159 ± 0.089 ng/mL in female dogs and 0.015 ± 0.132, 0.208 ± 0.206 ng/mL in male dogs, respectively, and no statistically significant difference was found between the groups in the analysis performed (p > 0.05).

The dogs were included into the study; 60% were 4 years old and below, 40% were 5 years old and above, and

Table 1. The hs-cTnT and hs-cTnI changes in healthy dogs and dogs with *D. immitis*.

Parameters	Group	N	Mean ± SE (Min-max)	T/P
hs-cTnT (ng/mL)	<i>D.immitis</i>	15	0.164 ± 0.035 (0.027-0.603)	T = 1.961 p > 0.05
	Healthy	15	0.092 ± 0.009 (0.021-0.160)	
hs-cTnI (ng/mL)	<i>D.immitis</i>	15	0.234 ± 0.052 (0.077-0.919)	T = 1.739 p > 0.05
	Healthy	15	0.135 ± 0.024 (0.070-0.377)	

Table 2. The changes of hs-cTnT and hs-cTnI in dogs by sex.

Parameters	Sex	N	Mean ± SE (Min-max)	T/P
hs-cTnT (ng/mL)	Female	14	0.098 ± 0.014 (0.021-0.161)	T = 1.452 p > 0.05
	Male	16	0.015 ± 0.033 (0.034-0.603)	
hs-cTnI (ng/mL)	Female	14	0.159 ± 0.024 (0.071-0.377)	T = 0.830 p > 0.05
	Male	16	0.208 ± 0.051 (0.076-0.919)	

there was no statistically significant difference between the groups in the analysis (p > 0.05; Table 3).

General Linear Model (GLM) was applied to reveal the effect of age, sex and presence of *D. immitis* on hs-cTnT and hs-cTnI, and the data obtained are given in Table 4.

When Table 4 is examined, although hs-cTnT and hs-cTnI values in sick animals have a higher average than healthy animals, it is seen that the interactive effects of sex, age and *D. immitis* on hs-cTnT and hs-cTnI are not statistically significant in the GLM analysis (p > 0.05).

4. Discussion

Vector-borne zoonotic diseases have importance increasing day by day due to factors such as climate changes, unconscious drug use, genetic changes in pathogens, uncontrolled human and animal movements [24]. Among the nematode species, one of the most important vector-borne agents infecting dogs is *D. immitis* [8]. The agent causes pulmonary hypertension that can progress to

Table 3. The changes of hs-cTnT and hs-cTnI according to age in dogs.

Parameters	Age	N	Mean ± SE (Min-max)	T/P
hs-cTnT (ng/mL)	≤ 4	18	0.151 ± 0.029 (0.027-0.272)	T = 1.515 p > 0.05
	≥ 5	12	0.093 ± 0.013 (0.021-0.176)	
hs-cTnI (ng/mL)	≤ 4	18	0.213 ± 0.045 (0.071-0.919)	T = 1.209 p > 0.05
	≥ 5	12	0.141 ± 0.023 (0.077-0.186)	

congestive heart failure [9,10]. In addition, worms that die naturally or are killed as a result of treatment can cause pulmonary thromboembolism, granulomatous inflammation, arterial occlusion and vasoconstriction [21].

Troponins are structural proteins that are a part of the contractile apparatus of skeletal and cardiac muscle, responsible for regulating the interaction of actin and myosin, and are composed of three subunits called troponin I (inhibitory component), troponin T (tropomyosin binding) and troponin C (calcium binding) [15]. When heart damage occurs, cTn-I and cTn-T begin to be secreted within 3–8 h, reach the highest level in 6–12 h and remain at a high level in blood for 14–21 days [12,25,26].

Cardiac troponin T has been reported to be a specific biomarker of cardiac muscle and minimally secreted in skeletal muscle [15,27,28]. In different studies, it has been reported that the cTn-T level in healthy dogs ranges from <0.01 to <0.05 ng/mL [29,30]. In the study, hs-cTnT level, which was stated to be more sensitive than cTn-T in different studies, was evaluated and hs-cTnT level was determined as 0.092 ± 0.009 ng/mL in our control group. The reason why the hs-cTnT level was numerically higher than the cTn-T values mentioned above is thought to be due to the high sensitivity of the measurement. In addition, it has been reported in different studies that cTn-T has increased in cardiac injuries in dogs [31,32]. In two different studies conducted on dogs with *D. immitis*, no change was found in the cTn-T level [33,34]. In our study, although the hs-cTnT levels were found to be 0.164 ± 0.136 ng/mL in dogs with *D. immitis* and 0.092 ± 0.035 ng/mL in healthy dogs, no statistically significant difference was found between the groups (p > 0.05).

In a study, it has been reported that cTn-I is more specific than cTn-T [35]. The fact that the molecular weight of cTn-I is lower has been demonstrated as the reason for this [36]. Therefore, cTn-I is considered to be a more

Table 4. The effect of age, sex, and presence of *D. immitis* on hs-cTnT and hs-cTnI according to the GLM procedure.

Parameters			hs-cTnT	hs-cTnI
Group	Sex	Age	Mean ± SD	Mean ± SD
<i>D.immitis</i>	Female	≤4	0.104 ± 0.066	0.205 ± 0.052
		≥5	0.121 ± 0.009	0.144 ± 0.059
		Total	0.109 ± 0.055	0.187 ± 0.057
	Male	≤4	0.268 ± 0.194	0.353 ± 0.328
		≥5	0.115 ± 0.070	0.144 ± 0.025
		Total	0.211 ± 0.171	0.275 ± 0.271
	Total	≤4	0.186 ± 0.162	0.279 ± 0.235
		≥5	0.118 ± 0.050	0.144 ± 0.034
		Total	0.163 ± 0.136	0.234 ± 0.200
Healthy	Female	≤4	0.127 ^a ± 0.020	0.086 ± 0.026
		≥5	0.058 ^b ± 0.041	0.161 ± 0.144
		Total	0.088 ± 0.048	0.129 ± 0.110
	Male	≤4	0.094 ± 0.016	0.158 ± 0.100
		≥5	0.097 ± 0.031	0.110 ± 0.034
		Total	0.095 ± 0.020	0.140 ± 0.082
	Total	≤4	0.106 ± 0.023	0.131 ± 0.085
		≥5	0.075 ± 0.040	0.139 ± 0.107
		Total	0.092 ± 0.350	0.135 ± 0.093
Total	Female	≤4	0.113 ± 0.052	0.160 ± 0.074
≥5		0.079 ± 0.045	0.155 ± 0.115	
Total		0.098 ± 0.051	0.158 ± 0.090	
	Male	≤4	0.181 ± 0.159	0.256 ± 0.250
		≥5	0.106 ± 0.050	0.127 ± 0.033
		Total	0.153 ± 0.132	0.207 ± 0.206
	Total	≤4	0.151 ± 0.125	0.213 ± 0.195
		≥5	0.093 ± 0.047	0.141 ± 0.082
		Total	0.127 ± 0.104	0.184 ± 0.162
<i>D. immitis</i> *Sex			-p > 0.05	-p > 0.05
<i>D. immitis</i> *Age			-p > 0.05	-p > 0.05
<i>D. immitis</i> *Sex*Age			-p > 0.05	-p > 0.05

sensitive and specific marker than cTn-T for the detection of minor myocardial damage [15,21]. In different studies, it has been reported that the level of cTn-I in healthy dogs varies between 0.004 and 0.136 ng/mL [37,38]. In our study, in parallel with previous studies, hs-cTnI level in healthy dogs was determined as 0.135 ± 0.024 ng/mL. In different studies on dogs, it has been reported that cTn-I levels increase in cardiac damage [39–45]. In the studies on dogs with *D. immitis*, it has been also reported that

cTn-I levels increase significantly [33,46]. In our study, although the hs-cTnI level was found to be 0.234 ± 0.200 ng/mL in dogs with *D. immitis* and 0.135 ± 0.092 ng/mL in healthy dogs, no statistically significant difference was found between the groups ($p > 0.05$). The lack of statistical significance among the groups may have resulted from the low population size due to ethical scope.

In different studies on humans, it has been reported that the level of cTn-I is higher in men [47–49]. However, although hs-cTnT and hs-cTnI levels were higher in male dogs in our study, no statistically significant difference was found between the groups in the analysis ($p > 0.05$).

In a human study, slight increases in cTn-I levels were reported due to increased myocardial reformation with possible cardiomyocyte loss characterized by aging [50]. In a study conducted on dogs, it has been reported that there is a significant correlation between age and cTn-I level [39]. Although hs-cTnT and hs-cTnI levels were higher in $4 \geq$ dogs in our study, no statistically significant difference was found between the groups in the analysis performed ($p > 0.05$).

5. Conclusion

As a result, it was determined that there was an increase in hs-cTnT and hs-cTnI levels in dogs with *D. immitis*, although it was not statistically significant. Considering that even the slightest change on the levels of troponins is prognostically important [19,21,37], it can be said that *D. immitis* causes myocardial damage in dogs. In addition, it

has been reported that cTn-I may not increase in the early stages of heart damage in some patients [25,51].

It can be said that hs-cTnT and hs-cTnI levels will increase as the prognosis of the disease worsens in dogs infected with *D. immitis*. In this point, the lack of statistical significance among the groups may have resulted from the low population size due to ethical scope. Therefore, it is thought that statistical differences may be significant if a larger population study is reconstructed. In the literature review on hs-cTnT and hs-cTnI levels in dogs, it was observed that there were not enough studies/findings and our study is the first data. In addition, considering different age and sex groups in dogs, we think that investigating different stages of *D. immitis* (early-mid-late period) by evaluating more dogs will provide more comprehensive data on this subject.

Acknowledgment/disclaimers/conflict of interest

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Conflict of interest

The authors declare that there is no conflict of interest for this study.

Informed consent

Ethical approve: The study was conducted with the decision of Kafkas University Scientific Publication and Ethics Committee dated KAÜ-HADYEK/2022-055.

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