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Araştırma Makalesi

P WAVE DISPERSION AND CARDIAC TROPONIN I CONCENTRATION IN CANINE VISCERAL LEISHMANIASIS*

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ABSTRACT

In the present study, the aim was to clinically evaluate the probable cardiac damage in dogs with CVL according to its stage via measuring atrial conduction time by use of Pd determined within computerized electrocardiography and cardiac troponin I (CTnI) level.

A total of 18 diseased dogs, of both sexes and various ages, referred with one or more of the clinical findings such as hypertrichosis, periocular alopecia, weight loss, onychogryphosis, skin lesions (severe scaling, exfoliative dermatitis compatible with alopecia) and/or anorexia, lymphadenopathy, hepatosplenomegaly were involved. In the healthy control group for comparison and in dogs with CVL to determine presence, nature and level of cardiac damage, the evaluations were performed with computerized 12-lead ECG device [(1 mV/cm amplitude in resting and 50 mm/sec) (Pd measurement)]. Serum CTnI concentrations were measured by using species specific commercial test kit.

High levels of cTnI concentration were detected in 10 of 18 dogs infected CVL with all polysymptomatic dogs. In all cases of the control group, cTnI levels were in the reference range [<0.03 ng/dL]. Even the comparison of each groups, no statistically significance ($p>0.05$) was found between CVL positive and control dogs. Mean ± standard deviation of Pd values were 22.76±3.12, 22.03±0.80, 22.73±0.80 and 25.67±1.41 in the control group, asymptomatic group, oligosymptomatic group, and polysymptomatic group, respectively. In comparison between groups, polysymptomatic group was significantly different than control ($p=0.026$), asymptomatic ($p=0.012$) and oligosymptomatic ($p=0.027$) groups.

Although a statistically significant difference was not found between CVL positive and control dogs in the present study, it was suggested that the individual increase may be associated with myocarditis due to disease. Besides, it may be claimed that the mean Pd values determined in especially polysymptomatic dogs was higher compared to the control group, whereas this may be accepted in the reference ranges based on mean Pd values reported in healthy dogs. However considering infected dogs population as 6 in each group, it may be safely claimed that further investigations regarding greater number of cases the may be warranted.

Keywords: Canine Visceral Leishmaniasis, Cardiac Troponin I, Dog, P Wave Dispersion, ECG

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CANINE VISCERAL LEISHMANIASIS'TE P DALGA DISPERSIYONU VE KARDIYAK TROPONIN I KONSANTRASYONU

ÖZ

Bu çalışmada, CVL'li köpeklerde evrelerine göre muhtemel kalp hasarının bilgisayarlı elektrokardiyografi ile atriyal iletim süresinin ölçülmesi yoluyla Pd'nin kullanımı ve kardiyak troponin I (CTnI) seviyelerinin ölçülmesiyle, klinik olarak değerlendirilmesi amaçlanmıştır.

Hipertrikozis, perioküler alopesi, kilo kaybı, onikogripozis, lenfadenopati, hepatosplenomegali ve deri lezyonları (şiddetli kabuklanma, alopesi ile uyumlu eksfoliatif dermatitis) ve/veya anoreksiya gibi bir veya daha fazla klinik bulgunun bulunduğu her iki cinsiyetten ve farklı yaşlardan toplam 18 hasta köpek çalışma kapsamına alındı. Kardiyak hasarın varlığı, niteliği ve seviyesini belirlemek için CVL'li köpeklerde, karşılaştırma için sağlıklı kontrol grubunda ve, bilgisayarlı 12 kanallı EKG cihazı [dinlenme ve 50 mm/sn'de 1 mV/cm amplitüd] (Pd ölçümü)] kullanıldı. Serum CTnI konsantrasyonları, türe özgü ticari test kiti kullanılarak ölçüldü.

CVL ile polisemptomatik olarak enfekte 18 köpekten 10'unda yüksek düzeyde cTnI konsantrasyonu tespit edildi. Kontrol grubunun tüm olgularında cTnI seviyeleri referans aralıkta [<0.03 ng / dL] idi. Her bir grubun karşılaştırılmasında, CVL pozitif ve kontrol köpekleri arasında istatistiksel olarak anlamlı bir fark bulunamadı (p> 0.05). Pd değerlerinin ortalama ± standart sapması sırasıyla kontrol grubu, asemptomatik grup, oligosemptomatik grup ve polisemptomatik grupta 22.76±3.12, 22.03±0.80, 22.73±0.80 ve 25.67±1.41 idi. Gruplar arasında karşılaştırıldığında, polisemptomatik grup kontrol grubundan (p=0.026), asemptomatik (p=0.012) ve oligosemptomatik (p=0.027) gruptan anlamlı farklılık gösterdi.

Bu çalışmada CVL pozitif ve kontrol olgular arasında cTnI seviyeleri açısından istatistiksel olarak anlamlı fark bulunmasa da, bireysel artışlar hastalığa bağlı olarak miyokardit ile ilişkili olabileceğini göstermektedir. Ayrıca, özellikle polisemptomatik köpeklerde saptanan ortalama Pd değerlerinin kontrol grubuna göre daha yüksek olduğu ortaya konulsa da bu durum sağlıklı köpeklerde bildirilen ortalama Pd değerlerine dayalı referans aralıklarında kabul edilebilmektedir. Buna karşın, enfekte köpeklere ait her bir grupta 6 olgu olduğu düşünülürse, daha fazla sayıdaki olgunun araştırılmasının faydalı olabileceği öne sürülebilir.

Anahtar Kelimeler: Canine Visceral Leishmaniasis, Kardiyak Troponin I, Köpek, P dalga dispersiyonu, EKG

INTRODUCTION

Canine Visceral Leishmaniasis (CVL), as a well recognized parasitic and zoonotic disease, is caused by protozoan of the genus Leishmania. The etiological agent may be transmitted to the dog via Phlebotomus species bite. Among Mediterranean location, *L. infantum* is the species usually implicated in CVL, a systemic form of the disease presenting involvement of multiple organs such as kidneys, liver, lymph nodes, bone marrow, spleen and skin (1, 2). Although clinical signs related to cardiac disease were not frequently reported (3, 4), myocaridal lesion might exist (5-7). Besides Leishmania parasite may be detected in cardiac tissue (3-6, 8).

Given canine myocarditis in relation with *L. infantum* infection has already been determined (6, 9), with pathological changes composed of degeneration and necrosis of cardiomyocytes and interstitial infiltration (5, 8), it should not be unwise to draw conclusion that cardiac involvement in dogs with CVL of different stages should be investigated with electrocaridographic and cardiac troponin I investigations, which was the puposes of this study.

MATERIALS AND METHODS

Animal material

A total of 24 dogs, from both sexes and different ages with presenting none, one or more of the complaints of hypotrichosis, periocular alopecia, weight loss, onichogrypphosis, skin lesions (scalling, alopecia in association with exfoliative dermatitis) and/ oranorexia, lymphadenopathy, hepatosplenomegalia. The study protocol was approved by the institutional laboratory animals ethics committee of Adnan Menderes University, Local Ethics Committee HADYEK (with no: 64583101/2013/060). Informed written consent was obtained from all of the owners for participating of their animals in this study.

Indirect Floresan Antibody Test (IFAT)

This test was applied at Adnan Menderes University, Faculty of Medicine, Parasitology Department as previously described (10). Bright yellow green fluorescence were deemed positive, pale/none yellow-green flourescence were denoted as negative. Highest serum dilution fluoresced were deemed antibody titer regarding related sample. Immuno fluorescent antibody titer 1/64 and above for samples were denoted as positive against CVL (10).

Classification to the groups

Following IFAT analysis allowing us to diagnose precise diagnosis of CVL, dogs were further enrolled into 4 different groups (n=6 in each). The dogs were denoted oligosymptomatic [in group I, solely one or two clinical signs (12-14) present] (Figure 1), polisymptomatic (in group II, even if presented three or more of the clinical signs in association with CVL) (Figure 2), and asymptomatic (in group III, even if free from clinical symptoms) (Figure 3), as was also described (14-16). Group IV were composed of IFAT negative, non CVL, healthy cases.

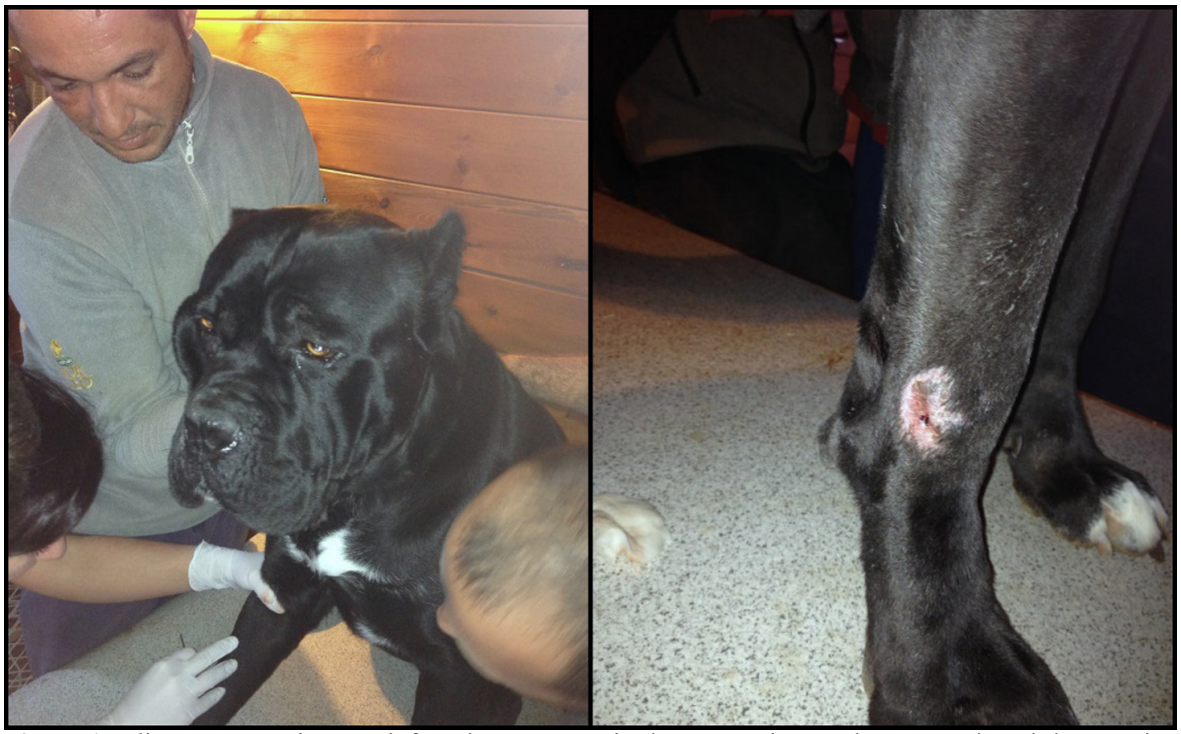

Figure 1. Oligosymptomatic CVL infected Presa Canario dog presenting ertyhematosus based demarcative and ulcerated lesion.

ECG examination with special reference to Pd

All dogs were subjected to ECG in standing position on BTL MT08 equipped with computerized 12 lead. The ECG signals were stored as direct electronic signals, every half minute by use of computer software BTL. Besides computerized system within the ECG record, permitted us to prevent the interference of muscles on the records and

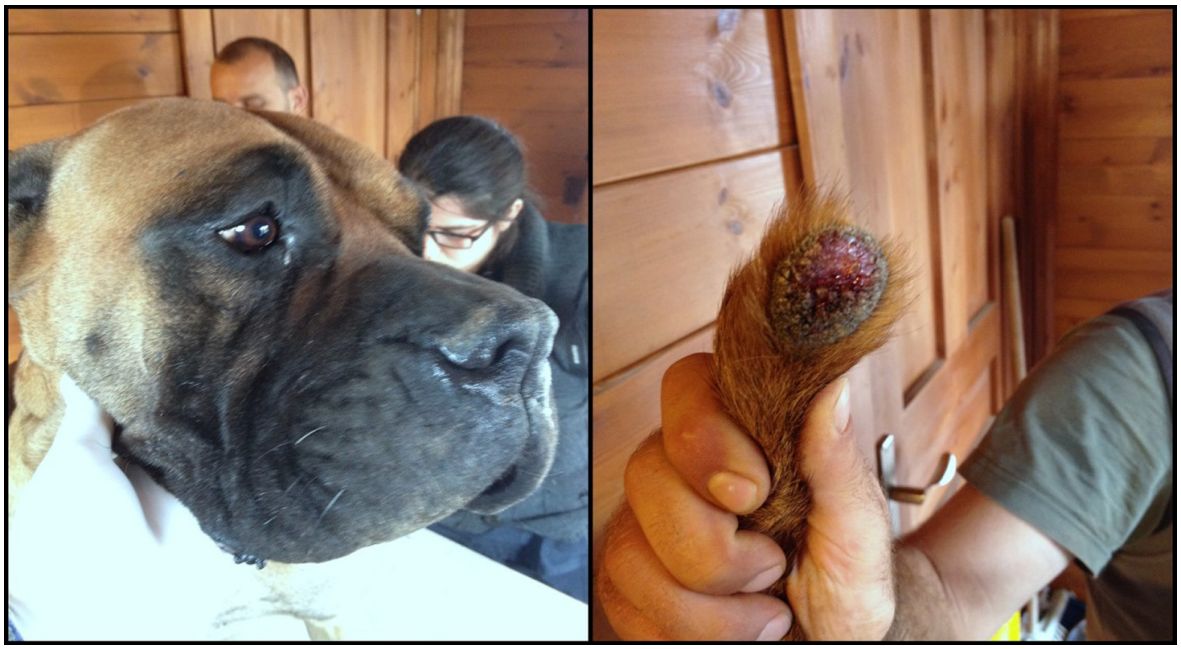

Figure 2. Polisymptomatic CVL infected Presa Canario dog. There was no traumatic history, with an ulcerated, haemorrhagic, crusted and alopecic lesion on tail.

besides eliminate probable artefacts, as was also described previously (17). The BTL system is capable of enlarging the record 200 times during usage of the computer display. Necessary electrodes such as red (right arm); yellow (left arm); black (right leg); and green (left leg) were introduced respectively. The precordial leads were subjected as: V1 on right of sternum (at the 5th intercostal space), V2 - beneath the left of the sternum, V4 –on to the left at the costochondrial junction (at the 6th intercostal space) (17, 18). The record was analyzed with at most care in an attempt to analyzed on 9 ECG leads (I, II, III, IV, aVR, aVL, aVF, V1, V2, V4) at five cardiac cycles (17) (Figure 4). Each established lead presented the duration of P-wave, calculated as the distance among the onset (positive/negative deflection from the isoelectric line) and the offset (return to the isoelectric line) with precision to 1 ms. This was followed bycalculation of Pmin (minimum) and Pmax(maximum)values of P-wave. The Pd was measured within the difference between Pmax and Pmin and to those of average from 5 measurements have been used (figure 5) (17-20).

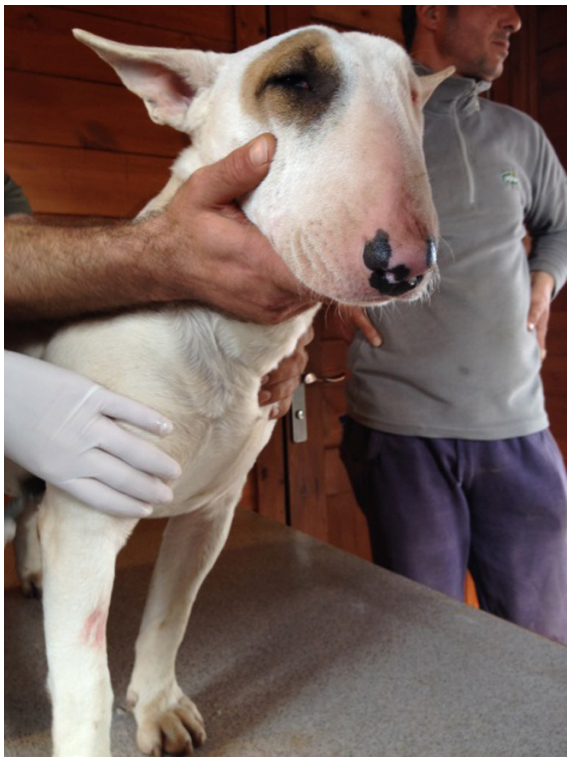

Figure 3. Asymptomatic Bullterrier dog with CVL.

Figure 4. Preparation for the ECG application under field conditions

Laboratory applications

Physical and hematologic examinations

In animals infected with CVL, history, age, gender, physical examination and laboratory findings were recorded (Table 1-5). In all cases, immediately prior to any application, in an attempt to perform IFAT and serum

biochemical analysis, blood samples were withdrawnfrom *vena cephalica antebrachii* into anticoagulant tubes (5 ml) for a single application and were evaluated.

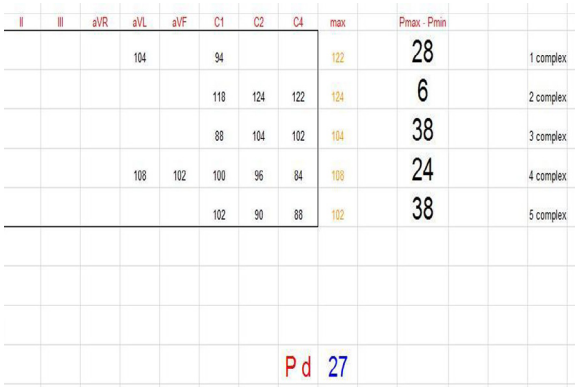

Figure 5. P wave dispersion (Pd) measurement. University of Adnan Menderes, Faculty of Veterinary Medicine Archive.

Serum cardiac troponin I concentrations

Serum cTnI concentrations were evalauted comperatively (in healthy group) and for detecting severity, presence and concentrations for probable mycoardial injury (in CVL infected dogs). Dog specific commercially available ELISA test kits (Kamiya Biomedical Company - Dog Cardiac Troponin-I, High Sensitive ELISA Cat. No: KT-640) were used in a private laboratory (Bilim Laboratory, İstanbul, TURKEY). Samples were stored at 80°C till analysis.

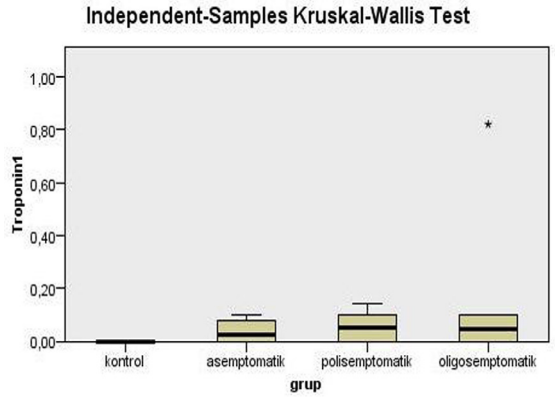

Figure 6. Intergroup distribution box plot graph of cardiac troponin I concentration (Kruskal-Wallis test p = 0.145)

Statistical analysis

Statistical analysis were performed by use of SPSS statistical software package (version 22; SPSS Inc. Chicago, IL). Both control and infected groups data were not normally distributed. Following logaritmic transformation, as data were abnormally distributed, non-parametric Kruskal-Wallis test andpaired way post-hoc were used for comparison.

RESULTS

cTnI analysis results

cTnI analysis were presented on tables 1-5. Among CVL infected dogs, 10/18 presented elevated levels. Interestingly 3/6 of asymptomatic and oligosymptomatic dogs, and 4 out of 6 polisymptomatic dogs presented higher cTnI concentrations. Among control groups 6/6 had cTnI concentrations in reference ranges [<0,03 ng/dL]. Min-max along with mean±st. dev. (ng/dL) values were shown in table 5. As was also shown in table 5, there was no statistical significance (p>0,05) between control and infected groups (Figure 6).

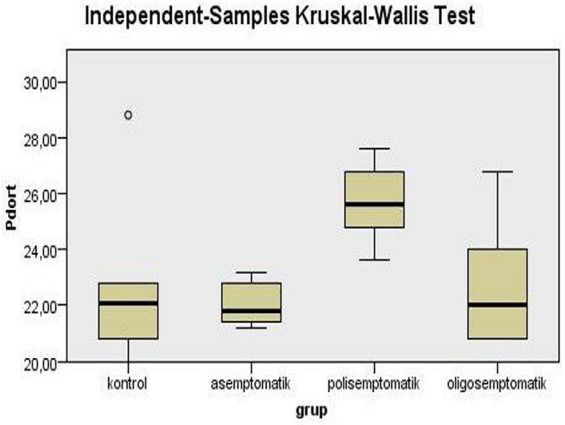

Figure 7. Pd result of intergroup distribution box on the chart (boxplot) (Kruskal-Wallis test p = 0.042)

Table 1. Demographic data regarding control group; breed, gender (sex),age distribution, IFAT titers, cTnI concentration and Pd analysis.

CONTROL GROUP															
No.	**Breed**	**Gender**	**Age (years)**	**IFAT**	**Troponin I ng/ml**	**P- wave dispersion**									
						I	**II**	**III**	**aVR**	**aVL**	**aVF**	**P max**	**P min**	**Pd**	**mean Pd**
1	Bull Terrier Shepherd crossbred	Male	2	Negative	<0.03	58	50	46	56	52	74	74	46	28	22
						60	70	50	58	68	50	70	50	20	
						50	38	48	40	62	58	62	38	24	
						48	54	56	56	58	66	66	48	18	
						66	54	50	70	62	56	70	50	20	
2	Bull Terrier	Female	3	Negative	<0.03	53	58	64	50	69	50	69	50	19	22,8
						37	48	74	42	58	53	74	37	37	
						32	53	42	40	63	42	63	32	31	
						48	53	64	48	48	48	64	48	16	
						53	42	53	53	53	53	53	42	11	
3	Golden Retriever	Male	4,5	Negative	<0,03	74	64	86	74	68	78	86	64	22	20
						68	74	66	68	78	58	78	58	20	
						48	64	64	64	68	66	68	48	20	
						64	76	64	62	80	64	80	62	18	
						56	68	70	66	76	72	76	56	20	
4	Presa Canario	Female	3.5	Negative	<0.03	74	54	48	56	68	60	74	48	26	20,8
						58	52	64	62	82	58	82	52	30	
						68	56	66	58	78	66	78	56	22	
						64	54	62	66	56	66	66	54	12	
						62	56	58	68	70	62	70	56	14	
5	Presa Canario	Female	4	Negative	<0.03	66	58	54	72	68	74	74	54	20	22,8
						68	60	58	80	58	58	80	58	22	
						58	72	50	64	56	66	72	50	22	
						64	60	58	78	64	48	78	48	30	
						68	56	48	64	62	68	68	48	20	
6	Presa Canario	Male	3	Negative	<0.03	48	53	53	48	58	53	58	48	10	22,2
						64	85	48	53	64	53	85	48	37	
						48	48	48	58	69	58	69	48	21	
						42	53	64	69	58	48	69	42	27	
						53	64	48	64	64	53	64	48	16	

Table 2. Demographic data regarding asymptomatic control group; breed, gender (sex),age distribution, IFAT titers, cTnI concentration and Pd analysis.

ASYMPTOMATIC GROUP															
No.	Breed	Gender	Age years	IFAT	Troponin I ng/ml	P- wave Dispersion									
						I	II	III	aVR	aVL	aVF	P max	P min	P dispersion	mean Pd
1	Bull Terrier	Female	1.5	1/256	0,08	53	48	58	64	58	64	64	48	16	21,4
						58	64	42	58	58	58	64	42	22	
						53	58	58	64	53	69	69	53	16	
						64	58	58	53	48	69	69	48	21	
						42	69	58	53	53	74	74	42	32	
2	İzci Köpeği Zağar	Female	4,5	1/256	0,1	98	102	80	96	92	74	102	74	28	22
						48	66	72	58	68	48	72	48	24	
						62	66	48	50	68	46	68	46	22	
						64	50	66	56	64	70	70	50	20	
						64	56	70	54	68	64	70	54	16	
3	Presa Canario	Female	4,5	1/64	<0,03	48	48	53	42	58	48	58	42	16	21,6
						58	48	53	42	48	42	58	42	16	
						42	53	48	48	48	64	64	42	22	
						48	42	69	53	53	53	69	42	27	
						42	42	53	58	53	69	69	42	27	
4	Alman Çoban	Female	2,5	1/128	<0,03	76	74	74	74	68	80	80	68	12	22,8
						58	76	78	68	64	74	78	58	20	
						76	84	74	72	54	74	84	54	30	
						74	78	48	84	58	64	84	48	36	
						82	82	68	84	68	74	84	68	16	
5	Melez	Female	5	1/64	<0,03	48	42	53	58	53	64	64	42	22	21,2
						53	42	48	53	58	53	58	42	16	
						42	48	64	58	58	58	64	42	22	
						40	42	53	53	69	42	69	42	27	
						45	48	53	58	64	48	64	45	19	
6	Presa Canario	Male	4	1/64	0,05	79	69	58	74	53	53	79	53	26	23,2
						85	64	64	64	58	53	58	53	32	
						69	64	48	58	58	58	69	58	11	
						79	74	64	64	53	53	79	53	26	
						74	64	58	79	58	58	79	58	21	

Table 3. Demographic data regarding oligosymptomatic group; breed, gender (sex),age distribution, IFAT titers, cTnI concentration and Pd analysis

OLIGOSYMPTOMATIC GROUP																
No.	**Breed**	**gender**	**Age (years)**	**IFAT**	**Troponin I ng/ml**	**P- wave dispersion**										
						I	**II**	**III**	**aVR**	**aVL**	**aVF**	**P max**	**P min**	**P dispersion**	**mean Pd**
1	Presa Canario	Female	7	1/512	<0,03	68	52	58	70	66	58	70	52	18	24
						84	58	66	70	76	52	84	52	32	
						70	56	46	50	52	44	70	44	26	
						58	48	44	42	54	50	58	42	16	
						76	60	50	48	62	54	76	48	28	
2	Kurzhaar (short haired German Pointer)	Female	3,5	1/256	<0,03	60	52	66	56	50	42	66	42	24	22
						56	60	86	56	66	62	86	56	30	
						46	50	56	60	58	54	60	46	14	
						50	48	54	50	72	48	72	48	24	
						46	40	58	48	58	50	58	40	18	
3	İzci Köpeği Zağar	Male	4	1/512	0,10	56	48	40	50	64	62	64	40	24	20,8
						42	68	50	52	46	56	68	42	26	
						40	48	42	48	50	38	50	38	12	
						46	52	46	36	50	48	50	36	14	
						56	66	44	40	38	64	66	38	28	
4	German Shepherd	Male	2	1/256	0,09	76	50	40	58	46	56	76	40	36	20,8
						50	42	46	50	42	44	50	42	8	
						48	56	50	46	58	52	58	46	12	
						66	50	40	64	38	54	66	38	28	
						68	56	54	56	48	62	68	48	20	
5	Presa Canario	Male	4	1/256	<0,03	58	50	70	50	62	40	70	40	30	26,8
						48	46	48	64	46	34	64	34	30	
						54	66	68	54	52	48	68	48	20	
						60	58	76	46	58	46	76	46	30	
						50	48	72	48	62	58	72	48	24	
6	Kurzhaar (short haired German Pointer)	Female	3	1/256	0,821	66	62	64	76	60	72	76	60	16	22
						86	82	68	74	72	60	86	60	26	
						66	62	76	66	58	64	76	58	18	
						56	60	58	68	54	76	76	54	22	
						58	74	84	72	56	72	84	56	28	

Table 4. Demographic data regarding polisymptomatic group; breed, gender (sex),age distribution, IFAT titers, cTnI concentration and Pd analysis

POLYSYMPTOMATIC GROUP															
No.	Breed	Gender	Age (years)	IFAT	Troponin I ng/ml	P- Wave dispersion									
						I	II	III	aVR	aVL	aVF	P max	P min	P dispersion	mean Pd
1	Presa Canario	Female	4	1/64	0,14	64	53	53	48	42	42	64	42	22	24,8
						69	48	42	53	48	48	69	42	27	
						64	58	48	53	42	48	64	42	22	
						58	42	48	74	53	48	74	42	32	
						48	48	53	64	69	53	69	48	21	
2	Presa Canario	Female	2	1/256	0,173	78	68	64	58	76	66	78	58	20	26,8
						82	56	50	62	86	64	86	50	36	
						58	54	68	70	62	68	70	54	16	
						54	72	90	62	70	54	90	54	36	
						64	58	58	58	84	58	84	58	26	
3	Bull Terrier	Female	2,5	1/512	<0,03	58	74	53	64	48	58	74	48	26	23,6
						48	48	80	53	53	48	80	48	32	
						74	74	74	69	69	63	74	63	11	
						48	80	74	48	48	53	80	48	32	
						58	42	69	69	69	58	69	42	27	
4	German Shepherd	Female	4	1/512	0,06	48	52	42	70	54	54	70	42	28	25,6
						46	42	64	62	56	46	64	42	22	
						50	54	44	46	76	50	76	44	32	
						48	46	66	44	66	48	66	44	22	
						58	66	56	42	60	50	66	42	24	
5	Melez	Male	7	1/512	0,04	64	53	69	64	85	64	85	53	32	27,6
						53	69	74	69	74	69	74	53	21	
						48	69	69	69	64	58	69	48	21	
						53	58	64	69	69	69	69	53	16	
						58	69	74	64	106	69	106	58	48	
6	Melez	Female	5	1/64	<0,03	48	69	58	74	53	53	79	53	26	23,2
						53	64	64	64	58	53	58	53	32	
						42	64	48	58	58	58	69	58	11	
						40	74	64	64	53	53	79	53	26	
						45	64	58	79	58	58	79	58	21	

Table 5. Mean (standard deviation) cTnI and Pd values in dogs with CVL.

Grup	cTnI (ng/mL) Referance <0,03			Pd (ms) Referance <36		
	Mean	S.d	Min-Max	Ortalama	S.d	Min-Max
Healthy Control	0	0	0	22,7667[a]	3,12517	20,00-28,80
Asymptomatic	0,0383	0,04491	0,00-0,10	22,0333[ac]	0,80416	21,20-23,20
Oligosymptomatic	0,1685	0,04491	0,00-0,82	22,7333[acd]	0,80416	20,80-26,80
Polisymptomatic	0,0567	0,05574	0,00-0,14	25,6777[b]	1,41798	23,60-27,60
P values	0,145			**0,042**		

* a, b, c, d; The differences between the averages with different letters in the same column are significantly (p <0.05).

p value; Control and statistical comparisons of the patient groups.

IFAT results

Regarding IFAT titers, intra group comparison revealed that there was a significant difference between control and asymptomatic (p<0,05) groups. Among all dogs involved and subjected to IFAT analysis 12 out of 18 (66,66%) presented 1/128 or higher seropsitivity, 6 (5,88%) at 1/64 positivity (border or suspected), whereas control group were entirely negative (Table 1-5).

Pd results

Pd values (mean±standard deviation) in control, asymptomatic, oligosymptomatic and polisymptomatic groups were deemed as 22,76±3,12, 22,03±0,80, 22,73±0,80 and 25,67±1,41 ms, respectively. Intragroup comparison revealed significant differences among polisymptomatic group within control (p=0,026), asymptomatic (p=0,012) and oligosymptomatic (p=0,027) groups (Figure 7, Table 1-5).

DISCUSSION

P-wave dispersion (Pd) is a biomarker as an ECG index used both in human cardiology and veterinary medicine (20-22). It has been recognized as the difference between the maximum and the minimum P-wave duration recorded from several ECG leads (19, 23). Alterations in the Pd might be presenting the prolonged intra-and inter-atrial conduction (23-25). Pd is a prognostic biomarker for detecting atrial fibrillation (AF) (22, 26). It was denoted that by determining Pd, there would be a probability to evaluate cases not presenting visible cardiac disorders although having an increased risk for development of AF. Regarding veterinary medicine, up to 2008 the Pd has been evaluated only on healthy dogs (20), whereas afterwards studies subjected the relationship among Pd and cardiac disorders (20, 27, 28).

Taking into account the supprotive role of ECG for diagnosing mycoarditis in CVL (6), a detailed literature search revealed lacking data regarding ECG examination in CVL. A recent survey indicated left atrial and ventricular enlargement and to those of myocaridal hypoxia in some of the animals

infected with CVL (25). Another study was focused on evaluation of the cardiotoxic effects of 75 mg/kg meglumine antimoniate sc for 60 days in 28 dogs with leishmaniasis. Electrocardiographic monitoring (before the onset and at the end of treatment) and serum cTnI concentrations were determined. No abnormality was detected in routine and 24 h electrocardiographic tracings before and after treatment. There was no statistical difference determined between serum cTnI concentrations, nor evidence of electrocardiographic features of cardiac toxicity in dogs with CVL treated with meglumine antimoniate (26). None of the studied pointed out Pd analysis. To this context a prior and important study established Pd values among 21 healthy dogs of various breeds and of both sexes (body weights ranging from 3- 70 kg) at the age of 1-12 years. All cases submitted to the ECG examinationin a standing position with BTL SD-8 electrocardiographic device. In that study P-wave duration was standardly calculated in six ECG leads (I, II, III, aVR, aVL, aVF) from five cardiac cycles. The mean Pmax, Pmin and Pd were detected as55.8±11.3 ms, 38.9±12.5 and 16.9±9.4, respectively. Consequently researchers concluded proper Pd upto 36 ms (22).

Same researcher group was involved in another study with the purpose of detecting proper value of Pd in healthy dogs (group I, n=53), chronic valvular diseased dogs (group II, n=23) and dogs with supraventricular conduction disturbances of (group III, n=12) from various breeds, of both sexes and from different body weight (1,5-80 kg), at the age of 0,5-17 years. All cases were subjected to ECG examination in a standing position with BTL SD-8 electrocardiographic device. P-wave duration was calculated in 9 ECG leads (I, II, III, aVR, aVL, aVF, V1, V2, V4) from 5 cardiac cycles. Among healthy dogs the proper P-wave dispersion was found as up to 24 ms. Pd was significantly ($p<0.01$) elevated in dogs with chronic valvular diseased and dogs with supraventricular conduction disturbances (23).

In a prior study in Turkey CVL infected dogs were subjected to 12-lead ECG examination. In that limited research authors involved solely oligosymptomatic dogs and found Pd as 34±7.76 ms (27). In the present study mean ± standard deviation of Pd values were 22.76±3.12, 22.03±0.80, 22.73±0.80 and 25.67±1.41 in the control, asymptomatic, oligosymptomatic and polysymptomatic groups, respectively. Intragroup comparison revealed that polysymptomatic group was significantly different than control ($p=0.026$), asymptomatic ($p = 0.012$) and oligosymptomatic ($p = 0.027$) groups. Those findings might be briefly suggested that even if increased Pd values were comperatively evaluated among different stages of the infection with evidence of progression of the disease caused increased vcalues. It may also be claimed that Pd values interpreted along with cTnI might be a predictor of myocardial injury or arrythmia such as atrial fibrillation. Pd might have predictive valuein dogs with chronic valvular diseases, supraventricular conduction disturbances, dilated cardiomyopathy, or enlarged atria due to mitral/tricuspid insufficiency (17). There-

fore it may not be unwise to draw conclusion that earlier detection dogs under arrythmia risk such as those in dogs with CVL, causing myocarditis (6, 9) and other cardiac problems (5, 8), may have helped prevention of the disease by early medical interventions.

In the present study as detected from 6 different derivations (I, II, III, aVR, aVL, aVF), especially within polisymptomatic dogs, mean Pd values were increased in comparison to healthy controls, whereas might be seen in reference ranges such as Pd 16.9±9.4 ms (20) or upper limit as 24 ms (23) previously detected in healthy dogs. On the other hand it should be taken into account that solely 6 dogs met inclusion criteria, warranting larger population studies.

cTn have long been recognized biomarkers with high sensitivity and specificity for myocardial degeneration in man and animals. In literature few studies are available detecting troponin concentrations among CVL infected dogs.In prior retrospective study, serum cTnI concentration was measured in dogs with leishmaniasis. Investigators detected reference ranges as > 0.06 µg/L increased for cTnI concentration and compared among dogs with and without anemia, azotemia, and proteinuria. In 40 dogs with CVL median cTnI concentration was higher (P = .011) than in 11 control dogs. Sixteen cases (40%) with CVLpresented elevated cTnI concentration.In contrast to nonproteinuric dogs, cTnI levels were higher (P = .017) in proteinuric dogs (28). The latter findings indicated that increased serum cTnI concentration was indicative of cardiac injury in CVL infection (28). In another study dogs with a precise diagnosis of CVL were subjected to meglumine an timoniat+allopurinoleorallopurinolein which serum cTnI concentration were not above reference ranges (>0.5 ng/mL) prior to treatment, and posttreatment days 14. and 28 (30).In the present study increased cTnI concentrations were detected among 10 out of 18 CVL infected dogs to those of asymptomatic (3/6), oligosymptomatic (3/6), polisymptomatic (6/6) groups. The mean values of 0.03±0.04, 0.16±0.04 and 0.05±0.05 ng/dL were determined in asymptomatic, oligosymptomatic, polisymptomatic groups, respectively. Inter group comparison did not reveal sstatistical significance (p>0,05) wehereas individual elevations might be briefly explained within some degree of myocaridal injury.

CONCLUSIONS

As myocarditis and arrhythmias may occur in both humans and dogs with Leishmaniasis, in an attempt to evaluate ECG changes, especially assessing combination of Pd and cTnI concentration, is essential. In this study, the evaluation of this two parameters together, especially the assessment made according to stages in infected dogs CVL, is important. In human medicine, serum cTnI concentrations is used as an important marker to detect myocardial ischemia and necrosis, and it may also be reported that this parameter have high sensitivity and specificity in animals with primary or secondary cardiac disorders. Although a statistically significant difference was not found between CVL positive and control dogs in the present

study, it was suggested that the individual increase may be associated with myocarditis due to disease. Besides, it may be claimed that the mean Pd values determined in especially polysymptomatic dogs was higher compared to the control group, whereas this may be accepted in the reference ranges based on mean Pd values reported in healthy dogs. However considering infected dogs population as 6 in each group, it may be safely claimed that further investigations regarding greater number of cases the may be warranted.

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