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Evaluation of Plasma D-Dimer Concentration in Dogs with Chronic Mitral Valve Insufficiency

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Abstract : D-dimer is a fibrin degradation product (FDP), a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis. D-dimer concentration is widely used for determining thrombosis and thromboembolism. Because one major cause of thromboembolism is congestive heart failure in human, we investigated the degree and risk of thromboembolism in dogs with different stage of congestive heart failure caused by chronic mitral valvular insufficiency (CMVI). The plasma level of d-dimer was evaluated in 20 healthy dogs and 30 dogs with different stage of congestive heart failure caused by CMVI. The d-dimer concentrations were measured by a commercialized assay kit. The plasma levels of d-dimer were not significantly different between healthy and CMVI dogs. Furthermore, there was no association of d-dimer concentrations to left atrium to aorta (LA/Ao) ratio, left ventricular dimension at diastole to aorta (LVIDd/Ao) ratio and severity of heart failure in our study population. Our study results implied that the degree of thromboembolism in canine heart failure might be minimal or the plasma d-dimer test might not be reliable for detecting thromboembolism in dogs.

Key words **:** d-dimer, thromboembolism, chronic mitral valvular insufficiency, heart failure, dog.

Introduction

D-dimer is a byproduct from the pathway of thrombin-mediated cleavage of fibrinogen and degradation of fibrin clot and widely used for detecting active thrombosis process including pulmonary thromboembolism and disseminated intravascular coagulation (DIC) in dogs (12). Human study found that congestive heart failure could be a major cause of hypercoagulable state predisposing to thrombosis and thromboembolic events (7). Arterial thromboembolism (ATE) is a common and fatal complication of hypertrophic cardiomyopathy in cats (6,8,9). However, thromboembolism in dogs with chronic mitral valvular insufficiency (CMVI) occurred uncommonly, although thrombosis may develop as a complication (13). One recent study evaluated changes in hemostatic markers for Cavalier King Charles Spaniels with CMVI and found the increased thromboembolic risk with the elevation of plasma fibrinogen concentration in dogs with advanced stage of heart failure (13). Unfortunately, more concrete study for assessing thromboembolic risk in dogs with CMVI is warranted. Therefore in this study, we evaluated the plasma concentration of d-dimer in CMVI dogs with different stage of heart failure and assessed correlation to certain echocardiographic indices.

Materials & Methods

Study population

The study population consisted of 20 healthy control (median age: 7.1 ± 2.8 years; range: 7-13 years) and 30 dogs (median age: 10.3 ± 3.4 years; range: 7-16 years) with CMVI. The dogs were presented to either the Veterinary teaching hospital of Kangwon National University from 2012 to 2014. All dogs were heartworm-antigen negative and were receiving heartworm prophylaxis. We excluded dogs with other systemic diseases (e.g., diabetes, adrenal and thyroid diseases, renal failure). The diagnosis of CMVI was based on a complete cardiac examination that included electrocardiography, thoracic radiography, and echocardiography. The severity of CMVI in the study population was classified according to the criteria proposed by the International Small Animal Cardiac Health Council (ISACHC) (4). The dogs were classified into 4 groups: Group C (control group), ISACHC Class I (asymptomatic heart failure), ISACHC Class II (compensated heart failure), and ISACHC Class III (decompensated heart failure).

Evaluation of plasma d-dimer concentration

Blood samples were obtained by jugular venipuncture using a 23-gauge butterfly needle, attached to a connecting tube with a multiple sample Luer adapter. Precautions were taken to avoid excessive tissue trauma and coagulation activation during blood collection. Blood was directly transferred into appropriate evacuated collection tubes containing 3.2% buffered sodium citrate in a ratio of 9 parts blood to 1 part anticoagulant. Citrated plasma was then separated within 45 minutes of collection by centrifugation at 3000 g for 10 minutes and frozen at −80ºC in 500 uL aliquots until analyzed. All assays were performed on citrated plasma, in duplicate and according to the manufacturer's recommendations. D-dimer plasma concentration was determined with the

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use of the chromogen substrate method by relying on the colometric reaction kinetics at a wavelength of 405 nm. These paremeters were determined with the use of an automated coagulometer (K-3003 Chrom, SLAMED, Germany).

Echocardiography

Echocardiographic examinations were conducted in accordance with recommended standards for dogs. M-mode, Doppler, and 2-dimensional echocardiography were performed in left and right lateral recumbency. M-mode echocardiography was used to measure left ventricular dimension at systole (LVIDs) and diastole (LVIDd), left atrium (LA) and proximal aortic (Ao) diameter. These measurements were used to determine the LA to proximal Ao diameter (LA/Ao) ratio and LVIDd to Ao diameter (LVIDd/Ao) ratio.

Statistical analysis

Statistical analysis was performed using SPSS version 19.0 for Windows. A 1-stage nested design for variation partition was used to estimate intra-assay variability of d-dimer in healthy dogs and dogs with CMVI. Continuous variables were described as the mean ± standard deviation (SD). The statistical methods used were a 1-way analysis of variance (ANOVA) and a Pearson's coefficient of correlation. Differences in d-dimer concentration among groups were evaluated using ANOVA. Pearson's coefficient of correlation was used to test the strength of association between d-dimer concentration and the echocardiographic indices (i.e., LA diameter, LA/Ao and LVIDd/Ao ratio) and the severity of heart failure. In all comparisons, a probability value of $P < 0.05$ was considered statistically significant, unless stated otherwise.

Results

The mean age, body weight, sex and breeds of each group was summarized in Table 1. D-dimer concentrations in dogs from ISACHC Class I to Class III did not differ significantly from those in the control group (Table 2; $P > 0.05$). The echocardiographic indices, those are LA/Ao ratio and LVIDd/Ao ratio, were closely related to the severity of heart failure (ISACHC score; $P < 0.01$). However, those echocardiographic indices were not associated with plasma level of d-dimer (Table 2).

Table 1. Demographic characteristics of the study population

	ISACHC class			
	Control	Class I	Class II	Class III
n (50)	20	10	10	10
Age	7.1 ± 2.8	8.3 ± 1.1	10.2 ± 1.3	11.4 ± 1.4
Sex	M(10), F(10)	M(6), F(4)	M(5), F(5)	M(5), F(5)
BW	5.2 ± 1.8	4.9 ± 3.2	5.1 ± 3.2	4.5 ± 2.1
Breeds				
Chihuahua		1	1	
Cross breed	5			1
Maltese	5	4	3	3
Poodle	1	1	1	1
Pekingese		2	1	1
Pomeranian			1	1
Shih-Tzu	8	2	3	3
Yorkshire Terrier	1			

All data are expressed with the mean value (± SD).
BW, body weight; M, male; F, female;

Discussion

Indications for plasma d-dimer measurement are deep venous thrombosis (DVT), pulmonary embolism (PE) or disseminated intravascular coagulation (DIC). It is also used for detecting arterial thromboembolism (ATE) in cats. Unfortunately, although various assays for d-dimer detection are commercially available, only few assays have been validated for clinical use in dogs and cats (3,10,12). A negative d-dimer test will virtually rule out thromboembolism if the test assay is fully validated. However, if the d-dimer concentration is high, further testing for validation for result is required to confirm the presence of thrombus. Since one major cause of thromboembolism is congestive heart failure in human and cats, the degree and risk of thromboembolism in dogs with different stage of congestive heart failure is necessary to be answered.

The results of our study indicate that the severity of heart failure resulting from CMVI is unrelated to the plasma levels of d-dimer in dogs. In contrast, many human and feline

Table 2. Echocardiographic indices and plasma d-dimer concentration in this study population

	LA/Ao ratio	LVIDd/Ao ratio	D-dimer (ug/mL)	Correlation[$]
Control	1.23 ± 0.31	1.43 ± 0.11	0.55 ± 0.15	< 0.10 / < 0.10
ISACHC I	1.43 ± 0.42*	1.63 ± 0.22**	0.48 ± 0.15[#]	< 0.10 / < 0.10
ISACHC II	1.78 ± 0.27*	2.03 ± 0.71*	0.32 ± 0.10[#]	< 0.10 / < 0.10
ISACHC III	2.23 ± 0.48*	2.63 ± 0.58*	0.36 ± 0.09[#]	< 0.10 / < 0.10

LA/Ao ratio, left atrium to aorta diameter ratio; LVIDd/Ao ratio, left ventricular dimension at diastole to aorta ratio; ISACHC, international small animal cardiac heath council
*$P < 0.01$, **$P < 0.05$
[#]$P > 0.05$
[$]Correlation (1) d-dimer to LA/Ao ratio (2) D-dimer to LVIDd/Ao ratio

studies found that elevated d-dimer levels were related to a greater risk of arterial thromboembolism and myocardial infarction, owing to its promotion of systemic hypercoagulability (1,2,5). One human study found the LA diameter in hypertrophic cardiomyopathy (HCM) was significantly correlated with concentrations of thrombin-antithrombin complex (TAT; 7). In addition, two feline studies found close correlation with coagulation markers to the LA diameter in cats with HCM (1,11). However, in this study, the LA diameter in dogs with CMVI was not closely associated with the d-dimer concentration. Furthermore the LV dilation was not also associated with the d-dimer concentration. One study also found plasma d-dimer concentration was not closely related to the severity of heart failure in dogs, although other coagulation marker (anti-thrombin III) was closely correlated to the LA diameter in a certain breed of dogs (13). Recent feline study (11) found that d-dimer concentration was high in 50% of cats with arterial thromboembolism (ATE). In contrast, two other feline studies (2,5) found only a few ATE cats had high plasma d-dimer concentration. Although the use of different reagents and assay methods were suggested to be responsible for this difference, the last studies could not detect the actual elevation of d-dimer concentration in cats with LA dilation (5,11), because the cats could rapidly clear d-dimer. Therefore, our study results implied that the degree of thromboembolism in canine heart failure might be minimal or the plasma d-dimer test might not be reliable for detecting thromboembolism in dogs.

There are several limitations of our study. Firstly, relatively low numbers of dogs in each disease group were enrolled so that decreases statistical power and increases risk of a type II error. Secondly, we could not exclude dogs with medication, although none of the drugs used are known to impact the plasma d-dimer assay. Thirdly, we only evaluated d-dimer concentration for evaluating systemic hypercoagulability in dogs. Future study should be included more markers indicating hypercoagulability such as fibrinogen, FVIII:C, antithrombin (AT), thrombin-antithrombin complex (TAT).

In conclusion, we evaluated plasma d-dimer concentration in dogs with different stage of congestive heart failure caused by CMVI. The plasma levels of d-dimer were not significantly different between healthy and CMVI dogs. Furthermore, there was no association of d-dimer concentrations to LA/Ao ratio, LVIDd/Ao ratio and severity of heart failure (ISACHC stage) in our study population. Our study results implied that the degree of thromboembolism in canine heart failure might be minimal or the plasma d-dimer test might not be reliable for detecting thromboembolism in dogs.

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만성 이첨판 폐쇄부전증에 걸린 개에서 혈장 D-dimer 농도 측정 연구

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요 약 : D-dimer란 섬유소분해산물로 응고혈액이 섬유소용해 후에 혈액 내에 보이는 작은 단백질 파편이다. D-dimer의 농도는 혈전증과 혈전색전증을 결정하기 위해서 널리 사용되고 있다. 인의에서 혈전색전증의 주요 원인 중 하나는 울혈성 심부전이기 때문에, 금번 연구에서 만성 이첨판 폐쇄부전증에 의한 울혈성 심부전의 다양한 심각도를 가진 개들에서 혈전색전증의 정도와 위험성을 조사하였다. 혈장 d-dimer의 농도는 건강한 개 20마리와 만성 이첨판 폐쇄부전증에 의해 울혈성 심부전에 이환된 다양한 중등도의 30마리 개에서 평가되었다. D-dimer의 농도는 상품화 된 키트로 측정하였다. 혈장 D-dimer의 농도는 건강한 개체 집단과 만성 이첨판 폐쇄부전증에 이환된 집단 사이에 유의적인 차이는 존재하지 않았다. 게다가, d-dimer의 농도는 심초음파 인덱스 중 대동맥대 좌심방비, 대동맥대 좌심실 이완말기 직경비와 연관성이 보이지 않았고, 이번 연구 집단의 심부전의 심각도와도 연관성이 존재하지 않았다. 따라서 금번 연구는 심부전을 가진 개에서 혈전색전증의 정도가 심하지 않거나 혈장 d-dimer의 농도 검사 자체가 개의 혈전색전증을 발견하는데 신뢰하지 못하다는 점을 암시하고 있다.

주요어 : d-dimer, 혈전, 만성 이첨판 폐쇄부전증, 심부전, 개