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RESEARCH ARTICLE

Evaluation of D-Dimer Concentrations in Clinically Ill Dogs with High Risk of Thromboembolic Disease

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ABSTRACT

Many systemic and metabolic diseases are associated with increased risk factors that promote the development of thrombus. But early diagnosis of thromboembolism (TE) may difficult in general practice due to a lack of noninvasive diagnostic tests. This study was conducted to compare the plasma concentration of D-dimer, platelet numbers and fibrinogen degradation products (FDPs) between healthy and clinically ill dogs to evaluate the usefulness of these assays in detections of hypercoagulability. Eighty-one clinically ill dogs with high risk of TE and 25 healthy dogs were included in this study. The plasma D-dimer concentrations were measured through the immunometric assay, and FDPs concentration was measured by semi-quantitative latex agglutination assay. Results of the present study indicated D-dimer concentrations were mainly elevated in immune-mediated hemolytic anemia (IMHA), liver disease, neoplasia and miscellaneous inflammatory disease group. In addition, markedly increased D-dimer concentrations (>2000ng/ml) were also mostly presented in IMHA (33.3%), liver disease (20%), and neoplasia (14.3%) group. Platelet numbers were significantly different only in neoplasia and endocrine disorder group. The plasma concentrations of D-dimer and FDPs of clinically ill dogs were mainly increased compared to healthy dogs. However, almost 30% of dogs with normal D-dimer value showed positive FDP assay results in both healthy and disease group. Concurrently performed plasma D-dimer and FDPs assays can be rapid screening tests for hypercoagulability in canine patients; however, cautious interpretation is required and should not be used as single diagnostic tool for TE.

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INTRODUCTION

Thromboembolism (TE) is considered as one of the most fatal complication in clinically severe illness in dogs and cats (Kitrell and Berkwitt, 2012). It is common that canine patients with TE showed no evident symptom and there is no test sensitive and specific enough to predict the development of TE, which makes its early diagnosis and timely intervention difficult to achieve (Stokol, 2003). D-dimer is produced during physiologic or pathologic thrombosis from the degradation of cross-linked fibrin but only can be detected after fibrinolysis. Thus, D-dimer is specific for active coagulation and fibrinolysis (Machida *et al.*, 2010). D-dimer has been used as only useful laboratory marker to detect early thromboembolic disease, including disseminated intravascular coagulation (DIC) and pulmonary artery thrombosis in human medicine (Halaby *et al.*, 2015). Recently, many studies have been reported the utility of D-dimer to detect thromboembolism (TE) in veterinary medicine (Dewhurst *et al*., 2008; Machida *et al*., 2010; Epstein *et al*., 2013). They demonstrated that the measurement of D-dimer is highly sensitive to detect TE and DIC in dogs. Fibrin degradation products (FDPs) are one of the markers of activated coagulation or fibrinolysis (Stokol *et al*., 1999). Blood clot dissolution through the fibrinolytic system generates FDPs, and elevation of plasma FDPs has been used an indicator of activated fibrinolysis along with D-dimer (Bédard *et al*., 2007). The purpose of this study is to evaluate plasma D-dimer concentrations in clinically ill dogs with high risk of TE and/or DIC. Comparision of D-dimer concentrations, FDP concentrations and platelet

numbers in clinically ill and healthy dogs and evaluated the usefulness of these tests in early diagnosis of hypercoagulability and TE.

MATERIALS AND METHODS

Study designs and Animals: The medical records of dogs admitted to the Veterinary Medical Teaching Hospital of Konkuk University from February, 2006 to May, 2007 were searched for cases in which a D-dimer test had been performed. Clinically ill dogs with high risk factor for TE and/or DIC were included (disease group). The disease group was subdivided into the following groups; neoplasia, heart failure, endocrine disorders, immune-mediate hemolytic anemia (IMHA), liver disease, and miscellaneous. Dogs which were diagnosed as pneumonia, bronchopneumonia, pyometra and pancreatitis were included in the miscellaneous group. The dogs admitted for routine check up without systemic disease during the same period were included as control group (clinically healthy dogs).

Plasma D-dimer assay: 2ml of whole blood sample was collected from the jugular vein with vacutainers containing 3.2% sodium citrate. The citrated plasma samples were centrifuged 3000g for 15 minutes within 15minutes and immediately frozen (-20℃) for overnight. Concentrations of D-dimer were measured through the immunometric assay in commercial veterinary diagnostic laboratory (Neodin Veterinary Diagnosis Laboratories, Seoul, Korea) by use of an automated chemistry analyzer (NycoCard® Reader II, Norway). D-dimer molecules are trapped on a membrane carrying D-dimer specific monoclonal antibodies against human D-dimers then bind the gold-antibody conjugate. In the presence of D-dimer levels above 0.1mg/L in plasma, the membrane appears reddish with color intensity proportional to the D-dimer concentration. The color intensity is evaluated using NycoCard® Reader II. The D-dimer result is expressed in micrograms per milliliter of fibrinogen equivalents.

Platelet numbers count: Platelet counts were measured all dogs in control and disease group. 1ml of blood samples were collected from the jugular vein with EDTA-coated tubes. Platelet numbers were measured within 30 minutes by an automated counter (HEMA Vet 850, FUJI, Japan).

Fibrinogen degradation products (FDPs) assay: The FDPs assays were evaluated only 10 dogs of control group and 48 dogs of disease group. Blood samples were collected and prepared following the same method used in D-dimer assay. FDPs concentrations in plasma were measured by use semi-quantitative latex-agglutination assay in same commercial veterinary diagnostic laboratory. Plasma samples were diluted 1:2 and 1:8 and observed for agglutination. The FDPs concentration is expressed as negative (FDP concentration, <5 µg/ml) or positive (FDPs concentration, 5 to 20 µg/ml or >20 µg/ml) follow previous report (Stokol *et al*., 1999).

Statistical analysis: Data was analyzed using commercial software, SPSS version 19.0 (SPSS, Inc., Chicago, IL, USA) and Excel 2010 (Microsoft, Redmond, WA, USA). The results are expressed as mean±SD. A Kruskal-Wallis test was used to test the hypothesis of differing D-dimer concentrations, platelet numbers and FDP concentrations among groups. Post hoc analyses of significant Kruskal-Wallis tests using Mann–Whitney U tests were conducted. A value of $P<0.05$ was considered statistically significant.

RESULTS

One hundred six dogs were evaluated in this study, 25 healthy dogs (control group) and 81 clinically ill dogs (disease group). Mean age of the dogs in control group was 7.2±4.1 years (range, 0.4 to 15 years), and 8.8±3.8 years (range, 1 to 20 years) in disease group. Fourteen dogs were male in control group and 36 dogs were male in disease group. In total, 14 breeds were presented, of which the most common breeds were Shih Tzu (n=17), Maltese (n=17), Mixed dog (n=13), and Yorkshire terrier (n=10) in disease group. In control group, Shih Tzu (n=7) was the most common breed, followed by Yorkshire terrier (n=4), Cocker spaniel (n=3), and Toy poodle (n=2) (Table 1).

The disease group was subdivided into 6 subgroups according primary diagnosis: neoplasia (34.6%), heart failure (27.2%), endocrine disorder (16%), IMHA (11.1%), liver disease (6.2%), and miscellaneous disease (4.9%) (Table 2). The plasma D-dimer concentration and platelet numbers for the control group and disease group were shown in Table 2. Forty-five dogs (n = 45, 55.6%) of disease group had higher D-dimer concentrations; however, only one dog of control group had higher D-dimer concentrations (600 ng/ml) than reference range (<500 ng/ml). One dog in IMHA disease had the highest plasma D-dimer concentrations (8000 ng/ml) of all patients in disease group. Markedly increased D-dimer concentrations (>2000 ng/ml) were mostly presented in IMHA (33.3%), liver disease (20%), and neoplasia (14.3%) group. On the other hand, over 50 percent of dogs in the heart failure and endocrine disorders showed normal plasma D-dimer concentrations (50% and 61.5%, respectively). The IMHA group dogs had the highest mean plasma D-dimer concentrations, followed by the liver disease, neoplasia, miscellaneous, heart failure, and endocrine disorders groups. The D-dimer concentrations ($P=0.004$) were significantly different among groups. Of the disease group, neoplasia ($P=0.002$), IMHA ($P=0.001$), liver disease ($P=0.019$) and miscellaneous ($P=0.001$) group had plasma D-dimer concentrations markedly different from those of the control group.

Table 1: Characteristics of study population

	Control group (n=25)	Disease group (n=81)
Gender (male/female; n)	14/11	36/45
Age (years)	7.2±4.1(0.4-15)	8.8±3.8 (1-20)
Breeds		
Shih Tzu	7	17
Maltese	1	17
Mixed dog	1	13
Yorkshire terrier	4	10
Cocker spaniel	3	5
Schnauzer	1	5
Pomeranian	1	4
Toy poodle	2	3
Other breeds	Alaskan Malmute (1), Bichon frise (1), West highland white terrier (1), Unknown breed (2)	Bichon Frise (1), Jindo (1), Pekingese (2), Rottweiler (1), Unknown breed (2)

Table 2: Plasma D-dimer concentrations and platelet numbers in healthy dogs and dogs with various diseases

Variable	Control group (n=25)	Disease group (n=81)						P value
		Neoplasia (n=28)	Heart failure (n=22)	Endocrine disorders (n=13)	IMHA (n=9)	Liver disease (n=5)	Miscellaneous (n=4)	
		Lymphoma (7) Mammary gland tumor (5) Hemangiosarcoma (2) Hepatic tumor (2) Nasal carcinoma (2) Adrenal tumor (2) Fibrosarcoma (2) Ovarian tumor (1) Osteosarcoma (1) Transitional cell carcinoma (1) Squamous cell carcinoma (1) Lung tumor (1) Meningioma (1)	Mitral valve insufficiency (18) Tricuspid valve insufficiency (1) Patent ductus arteriosus (1) Hypertrophic cardiomyopathy (1) Dilated cardiomyopathy (1)	HAC (13)	IMHA (9)	Chronic hepatitis (3) Cholangiohepatitis (1) Cholelithiasis (1)	Pneumonia (1) Bronchopneumonia (1) Pyometra (1) Pancreatitis (1)	
D-dimer (ng/ml)	n	n	n	n	n	n	n	
<500	24	12	11	8	3	2	-	
500-1000	1	7	3	2	2	1	4	
1000-2000	-	5	6	2	1	1	-	
>2000	-	4	2	1	3	1	-	
Median (Range)	244.0±158.3 (100-600)	1060.7*±1256.2 (100-3500)	772.7±873.0 (100-3400)	592.3±672.6 (100-2200)	1988.9**±2587.2 (200-8000)	1640.0*±2111.4 (200-5300)	800.0**±141.4 (600-900)	0.004
Platelet numbers (x 10^3 platelets /μL)								
Median (Range)	402.3±155.5 (210-799)	509.5*±141.6 (259-799)	439.6±157.9 (175-752)	519.4*±182.0 (282-877)	498.1±242.0 (199-782)	286.0±61.8 (224-362)	389.3±173.5 (211-542)	0.038

HAC, hyperadrenocorticism; IMHA, immune-mediated hemolytic anemia; N=number; *P<0.05 vs control group; **P<0.001 vs control group.

Table 3: The relation between plasma D-dimer and FDPs concentration in healthy dogs and dogs with various diseases

Group		D-dimer < 500ng/ml		D-dimer>500ng/ml	
		Negative FDPs (<5ug/ml)	Positive FDPs (>10ug/ml)	Negative FDPs (<5ug/ml)	Positive FDPs (>10ug/ml)
Control group (n=10)		7	3	-	-
Disease group (n=48)	Neoplasia (n=17)	4	4	-	9
	Heart failure (n=13)	6	-	2	5
	Endocrine disorders (n=8)	3	2	-	3
	IMHA (n=5)	1	-	-	4
	Liver disease (n=2)	1	1	-	-
	Miscellaneous (n=3)	-	-	1	2

Platelet counts were measured in all patients in control and disease group, and the results were shown in Table 2. Thrombocytosis (>500-600 x 10^3 platelets /μL) was detected in 12% of control group dogs and 44.4% of disease group dogs. Of the disease group, 50% of neoplasia and miscellaneous disease group, 46.7% of endocrine disorder, 44.5% of IMHA, and 40.9% of heart failure dogs showed thrombocytosis. On the other hand, thrombocytopenia (<200 x 10^3 platelets/μL) was detected 2.5% of disease group dogs. Eleven percent of IMHA group and 4.5% of heart failure group dogs showed thrombocytopenia respectively. Overall, 47% of the disease group dogs had abnormal platelet numbers. Among them, only neoplasia and endocrine disorders groups had significantly different platelet numbers than those of the control group (P=0.013 and P=0.039, respectively).

Among the study population, 58 dogs (10 dogs of control group and 48 dogs of disease group) had evaluated for the plasma FDPs concentration. The comparison between D-dimer and FDPs concentrations in 10 of 25 normal group and 48 of 81 disease group was described in Table 3. Three dogs of the control group (30%) had positive FDPs assay results (10 μg/ml), with normal D-dimer concentrations. Of the disease group, 23 of 26 dogs (88.5%) which had plasma D-dimer concentrations more than 500 ng/mL, showed positive result in FDPs assay. On the other hand, 7 of 22 dogs (31.8%) had positive FDPs assay results despite normal plasma D-dimer concentration. Over all, the FDPs concentrations among groups were not significantly different (P=0.378).

DISCUSSION

The D-dimer assay has been used as the first-line test in patient suspected of having thromboembolic disease including DIC and deep vein or pulmonary artery thrombosis in human (Halaby *et al*., 2015). Similarly, the increase of plasma D-dimer concentrations was reported after surgery, sepsis, IMHA, cancer, congestive heart failure, renal failure, and liver disease in veterinary medicine (Scott-Moncrieff *et al*., 2001; Zoia *et al*., 2012; Epstein *et al*., 2013).

Latex bead agglutination and immunoturbidimetry have been used as detection methods for D-dimer in dogs (Stokol *et al*., 1999; Boutet *et al*., 2009). In the present study, immunoturbidimetric assays were used to detect plasma D-dimer, which use antibody-coated beads that

react with D-dimer in plasma samples. These antibodies detect D-dimer and do not cross-react with degradation products produced from lysis of fibrinogen (Kroneman *et al*., 1990). The specificity and sensitivity of each methods were variable depend on the characteristics of study population and cut-off concentration of each study. However, measurement of D-dimer concentration using both methods seems to be accurate and reliable (Dewhurst *et al*., 2008; Boutet *et al*., 2009).

This study focused on the distribution of plasma D-dimer concentrations in clinically ill dogs with high risk of TE and the comparison with clinically healthy patients. In this study, 81 clinically ill dogs were selected by high risk of thrombosis based on the previous studies (Dewhurst *et al*., 2008; Kitrell and Berkwitt, 2012), and subdivided according to the primary diagnosis. The neoplasia group accounted for 34.6% of the disease group, followed by heart failure group. Results of the present study indicated D-dimer concentrations were mainly elevated in IMHA, liver disease, neoplasia and miscellaneous inflammatory disease group and the concentrations were significantly different from those of the control group. In addition, markedly increased D-dimer concentrations (>2000 ng/ml) were mostly presented in IMHA (33.3%), liver disease (20%), and neoplasia (14.3%) group. Furthermore, one dog with IMHA had the highest plasma D-dimer concentrations (8000 ng/ml) of all dogs in disease group. Similarly, a previous report demonstrated that 60% of canine TE cases had underlying neoplasia or protein-losing nephropathy, and plasma D-dimer concentrations were significantly different in dogs with TE and liver disease (Nelson and Andreasen, 2003). Other study demonstrated that Plasma D-dimer concentrations were increased in 80% of IMHA patients; moreover, 50% of the patients had plasma D-dimer value greater than 1000 ng/ml (Scott-Moncrieff *et al*., 2001). In cancer patients, the capability of tumor cells and their procoagulant products to interact with platelets, clotting and fibrinolytic proteins contributes to the development of TE (Lee, 2006). Previous studies demonstrated that high D-dimer value could have positive predict values of TE (Scott-Moncrieff *et al*., 2001; Nelson and Andreasen, 2003). However, many other situations, such as infection, inflammation, surgery, trauma, and bleeding can cause higher concentrations of D-dimer (Nelson and Andreasen, 2003; Dewhurst *et al*., 2008).

In the present study, one dog of normal group had higher D-dimer concentrations (600 ng/ml) than reference range (< 500 ng/ml). Otherwise, 37.5% of disease group had D-dimer concentrations within reference range. Similarly, previous study demonstrated that a few healthy dogs can have slightly higher D-dimer concentrations than reference range, whereas some dogs with DIC may have D-dimer concentrations within normal range (Stokol *et al*., 1999). Although high D-dimer value alone was not enough for positive prediction of TE, our data demonstrated the situations occurring of high D-dimer values in the clinically ill patients.

Abnormal platelet numbers could be linked to thrombotic or bleeding complications in various disease conditions and one report demonstrated abnormal platelet numbers in 65% of the TE dogs (Nelson and Andreasen, 2003). In this study, abnormalities in platelet numbers (mostly thrombocytosis) were detected 47% of disease group and 12% of control group dogs. Like other studies (Nelson and Andreasen, 2003; Neel *et al*., 2012), thrombocytosis was occurred most frequently in neoplastic disease group. However, the direct association of thrombocytosis and TE is still not well defined (Rinder *et al*., 1998).

In this study, almost 30% of normal D-dimer concentrated dogs showed positive FDPs assay results in both healthy and disease group. Although the result of FDPs assay was not always concordant to the elevation of plasma D-dimer concentration, the rise of FDPs concentration was detected substantially in disease group. The sensitivity and specificity of D-dimer assays and FDPs assay in detecting thrombosis in dogs were assessed in several previous studies and they suggested that plasma D-dimer may be more sensitive than FDPs (Griffin *et al*., 2003). Similarly, another report demonstrated that plasma D-dimer concentrations were increased in 13 dogs with TE, without a concurrent increase in FDPs (Nelson and Andreasen, 2003). Thus, those tests require careful interpretation for the diagnosis of TE. Further prospective studies for the confirmatory association of TE with higher D-dimer value should be conducted with complete assay of procoagulant, anticoagulant and fibrinolytic pathway proteins in future to evaluate its validity in various conditions.

Conclusions: In conclusion, although the direct occurrence of TE was not evaluated in this study, our data demonstrated that D-dimer concentrations were mainly elevated in clinically ill dogs with high risk of TE, such as IMHA, liver disease, and neoplasia. The measurement of D-dimer concentration would be simple and rapid screening test for clinically suspected TE patients and this would be more informative than the simple decision of the presence or absence of TE.

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Authors' contributions: HRY and PHM designed the study and directed its implementation. HRY executed the experiment and KMH conducted data analysis. The manuscript was prepared by KMH under the supervision of PHM. All authors interpreted the data, critically revised the manuscript and approved the final manuscript.

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