Short Communication

Detection of transforming growth factor beta 1 in normal dogs and dogs with degenerative mitral valve disease

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Abstract

Degenerative mitral valve disease (DMVD) is a common finding of acquired heart diseases in small and medium-sized adult dogs. Transforming growth factor beta 1 (TGF-β1) was found to increase in valves of DMVD dogs. However, plasma TGF-β1 concentrations in dogs with DMVD have not been evaluated before. The objective of the present study was to compare the plasma concentration of TGF-β1 between normal dogs and dogs with DMVD by using an ELISA technique. This measurement might help to distinguish dogs with DMVD from healthy dogs. The Mann-Whitney U test was used to compare plasma TGF-β1 concentrations between the two groups. A p-value less than 0.05 was considered statistically significant. Results showed no statistical difference (p=0.574) between the median plasma TGF-β1 concentrations of 22 normal dogs (1.14, range 0.94-1.33 ng/ml) and 27 DMVD dogs (1.21, range 0.92-1.32 ng/ml). In conclusion, the concentration of TGF-β1 is very low in the circulation; it may not be a suitable marker for the diagnosis of DMVD due to its sensitivity.

Keywords: canine, degenerative mitral valve disease, mitral valve, plasma, transforming growth factor beta 1

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Introduction

Degenerative mitral valve disease (DMVD) is a common finding of acquired heart diseases in dogs (Häggström et al., 2004). Small and medium-sized dogs such as Cavalier King Charles spaniels (CKCS), Miniature poodles, Dachshunds, Pomeranians, and Chihuahuas are prone to DMVD (Disatian, 2010). An accumulation of mucopolysaccharide within the valves leads to valve thickening, resulting in valve regurgitation or prolapse (Häggström et al., 2004). Severe mitral valve regurgitation can cause volume overload and left-sided congestive heart failure.

Transforming growth factor beta 1 (TGF-β1), a cytokine, has several roles in heart valve homeostasis including extracellular matrix turnover and valve interstitial cell differentiation (Disatian and Orton, 2009; Disatian et al., 2008). There are three isoforms of TGF-β including TGF-β1, β2, and β3. A previous study reported a marked increase in an expression of TGF-β1 protein in degenerative mitral valves (Aupperle et al., 2008). It has been proved that TGF-β1 is involved in increasing mucopolysaccharide synthesis, regulating interstitial cell proliferation, and apoptosis in cultured valvular interstitial cells of human heart valves (Jian et al., 2003; Khan and Shepperd, 2006). Plasma and serum of TGF-β1 concentrations in dogs and humans have been measured in several studies and diseases (Sharma et al., 1997; Wang et al., 1997; Syrris et al., 1998; Tashiro et al., 2002; Zois et al., 2012; Moesgaard et al., 2014). However, information on TGF-β1 in the circulation of DMVD dogs is scarce. In addition, it is unclear whether TGF-β1 concentrations in the circulation is associated with the disease severity or in the valve itself.

This study aimed to compare the plasma concentration of TGF-β1 in normal and DMVD dogs by a canine ELISA test kit and to correlate TGF-β1 concentrations with the severity of DMVD.

Materials and Methods

Animals: Plasma samples were collected from dogs that were 6 years of age or older and had less than 15 kg body weight presented to the Small Animal Veterinary Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University, Thailand with the owners' permission. The protocol used for sample collection in this study was approved by the Animal Use and Care Committee, Faculty of Veterinary Science, Chulalongkorn University, Thailand (The animal use protocol No. 1431027). Physical examination, thoracic radiography, echocardiography, and blood collections for complete blood count and blood chemistry profiles were performed on all dogs. Dogs with pregnancy, lactation, and other systemic diseases were excluded from the present study. Forty-nine dogs were then divided into two groups: the control (n=22) and DMVD (n=27) groups. Inclusion criteria for the control group were dogs with normal physical examination, normal complete blood count and blood chemistry profile values, and unremarkable cardiac abnormalities on radiography and echocardiography. The DMVD group comprised dogs presented with thickening of mitral valve leaflets and valvular regurgitation assessed by echocardiography. Thoracic radiography was performed on all dogs to evaluate heart shape, abnormality of pulmonary parenchyma, and thoracic cavity. Vertebral heart score was calculated to determine heart size.

Echocardiography was performed to diagnose DMVD and determine cardiac remodeling using an ultrasound machine with 6-10 MHz multi-frequency phased array and 5-6 MHz microconvex transducers operated by an experienced veterinarian. The dogs were non-sedated and restrained in right lateral recumbent position. Left ventricular remodeling was assessed by M-mode echocardiography. Cornell's allometric normalized values were used to normalize the M-mode echocardiographic values (Cornell et al., 2004). DMVD dogs were categorized according to the American College of Veterinary Internal Medicine (ACVIM) classification (Atkins et al., 2009) including Stage A: pre-disposing breed dogs with no murmurs, clinical signs, or cardiac structural changes; Stage B: asymptomatic DMVD dogs with no sign of congestive heart failure, i.e. pulmonary edema evaluated by radiography with or without cardiac remodeling; Stage C: symptomatic DMVD dogs with sign of congestive heart failure on radiography and cardiac remodeling, i.e. left ventricular dimension ≥17 mm and LA/Ao ≥1.6 by echocardiography.

Blood sample collection: Two and a half ml of blood samples were collected from the cephalic or saphenous vein of the dogs by venipuncture. Half ml of EDTA blood samples was collected for complete blood count. Plasma samples for measuring TGF-β1 concentrations were separated from other 1 ml EDTA blood samples by centrifuging at 1000 g for 15 minutes at 2-8°C within 30 minutes after collection, and stored immediately at -20°C until assay. Finally, heparinized blood samples were prepared for blood chemistry measurement.

Measurement of plasma TGF-β1 concentrations: Plasma samples were prepared for measuring TGF-β1 concentrations by the canine TGF-β1 ELISA test kit (BlueGene Biotech, Shanghi, China), and run a duplicate. Briefly, the plasma samples were incubated with TGF-β1 conjugated to horseradish peroxidase (HRP) for 1 hour at 37°C. Then, the plates were washed manually 5 times. The plates were incubated with a substrate for HRP. The reaction was stopped with a stop solution. Optical density was measured at 450 nm using a microplate reader. TGF-β1 concentrations were calculated by comparing their absorbance with a standard curve.

Statistical analysis: Computer-based software (IBM SPSS Statistics 22, Armonk, NY, USA) was used for statistical analysis. Normally distributed data were tested by the Shapiro-Wilk test before statistical analyses. Data of sex and breed of dogs in the normal and DMVD groups were reported descriptively. Age and weight differences between the two groups were analyzed by the independent t-test. Effect of sex on an evidence of DMVD was analyzed by the Fisher's exact test. The Mann-Whitney U test was used to compare echocardiographic values and plasma TGF-β1 concentrations between the normal and DMVD dogs. Differences in plasma TGF-β1 concentrations between

stages (normal, stage B, and stage C) were evaluated by the Kruskal-Wallis test. Relationship between plasma TGF-β1 concentrations and age and echocardiographic values were analyzed by the Spearman's rank correlation.

Results and Discussion

The data of normal dogs and dogs with DMVD are presented in Table 1. The mean age of DMVD dogs in this study was 11.85 years. This finding is similar to a previous study reporting that dogs more than 9 years old were at a high risk of DMVD (Whitney, 1974). The DMVD dogs were older than the normal dogs in the present study (Table 1). However, age did not correlate with plasma TGF-β1 concentrations (r=-0.056, p=0.701). Poodle was the major population of DMVD dogs in this study. This result is different from a previous study showing that Dachshund and CKCS were the top breeds affected by DMVD (Olsen et al., 1999; Swenson et al., 2009). It is possible because Dachshund and CKCS breeds are not popular breeds in Thailand. In the DMVD group, the number of males was higher than females. In addition, the statistical result confirmed the effect of sex on an evidence of DMVD (p=0.029). Similar result was found in a prior study reporting that males had a higher risk of developing DMVD than females (Ware, 2003). The weights of normal and DMVD dogs were not different (p=0.349) (Table 1). The DMVD dogs had larger left atrium and left ventricle than the normal dogs (Table 2).

Table 1 Data of dogs in normal and degenerative mitral valve disease groups

	Normal (n=22)	**DMVD (n=27)**
Breeds	7 Poodles, 5 Mixed breeds, 4 Shih-Tzus, 2 Yorkshire-Terriers, 2 Pomeranians, 1 Miniature Pinscher, and 1 Chihuahua	10 Poodles, 5 Mixed breeds, 4 Shih-Tzus, 2 Pomeranians, 2 Chihuahuas, 1 Miniature Schnauzer, 1 CKCS, 1 Miniature Pinscher, and 1 Pekingese
Sex	15 females and 7 males	10 females and 17 males
Age	8.86±2.05 years	11.85±2.12 years
Weight	5.35±2.05 Kg	5.88±2.12 Kg
VHS	9.95±0.55	11.26±0.89
		15 dogs were newly diagnosed with DMVD. 12 dogs were on cardiovascular medicine including furosemide, pimobendan, ACEi, and spironolactone.

ACEi= angiotensin converting enzyme inhibitor; CKCS= Cavalier King Charles spaniels; DMVD= degenerative mitral valve disease; VHS= vertebral heart scale

Table 2 Echocardiographic values of normal and degenerative mitral valve disease dogs

Parameter	**Normal (n=22)**	**DMVD (n=27)**	**p-value**	**Correlation between echocardiographic values and TGF-β1**
LVd (^0.294mm/kg)	12.67 (11.15-13.92)	14.39 (11.72-17.29)	0.021*	r=0.078, p=0.594
LVs (^0.315mm/kg)	7.81 (6.29-8.91)	8.53 (6.49-9.85)	0.300	r=0.039, p=0.790
IVSd (^0.241mm/kg)	4.49 (4.14-5.19)	3.96 (3.46-4.38)	0.004*	r=0.884, p=-0.021
IVSs (^0.240mm/kg)	5.93 (5.28-6.86)	7.01 (6.23-7.83)	0.006*	r=-0.008, p=0.959
LWd (^0.232mm/kg)	4.45 (3.74-4.71)	4.44 (3.88-5.36)	0.355	r=0.036, p=0808
LWs (^0.222mm/kg)	6.49 (5.62-7.07)	7.27 (5.84-7.88)	0.082	r=-0.180, p=0.217
LA (^0.345mm/kg)	8.67 (8.30-9.39)	10.58 (9.75-12.29)	<0.00001*	r=0.029, p=0.841
Ao (^0.341mm/kg)	6.79 (6.20-7.82)	7.09 (6.45-7.91)	0.587	r=-0.113, p=0.441
LA/Ao	1.29 (1.16-1.44)	1.48 (1.36-1.73)	0.002*	r=0.222, p=0.126

* indicates significantly different at p-value <0.05.
DMVD= degenerative mitral valve disease; LVd= left ventricular end diastolic diameter; LVs= left ventricular end systolic diameter; LWd= thickness of left ventricular free wall during diastole; LWs= thickness of left ventricular free wall during systole; IVSd= interventricular septal thickness during diastole; IVSs= interventricular septal thickness during systole; LA= left atrium dimension; Ao= aortic dimension; LA/Ao= the ratio of left atrium to aorta dimension; r= correlation coefficient

This study demonstrated that a small amount of TGF-β1 was measured in the circulation. Also, there was no difference in plasma TGF-β1 concentrations in the normal (1.14; 0.94-1.33 ng/ml) and DMVD (1.21; 0.93-1.3 ng/ml) dogs (p=0.574). A previous study showed that besides the valvular interstitial cells, mononuclear cells within the circulation might be another source of TGF-β1 in canine DMVD (Mavropoulou et al., 2016). However, the mononuclear cells may store but not release TGF-β1 into the circulation because only a small amount of TGF-β1 was found in the plasma. The difference in plasma TGF-β1 concentrations could not be found between each stage of DMVD classification; stage B (0.96; 0.93-1.31 ng/ml) and stage C (1.26; 0.96-1.35 ng/ml) (p=0.352). Moreover, echocardiographic values did not correlate

with plasma TGF-β1 concentrations (Table 2). These findings suggest that TGF-β1 in the circulation is not related to the progression of DMVD.

In conclusion, a small amount of TGF-β1 can be found in the circulation. The concentration of plasma TGF-β1 is not different between normal and DMVD dogs. Therefore, TGF-β1 may not be a good marker for diagnosing DMVD in dogs.

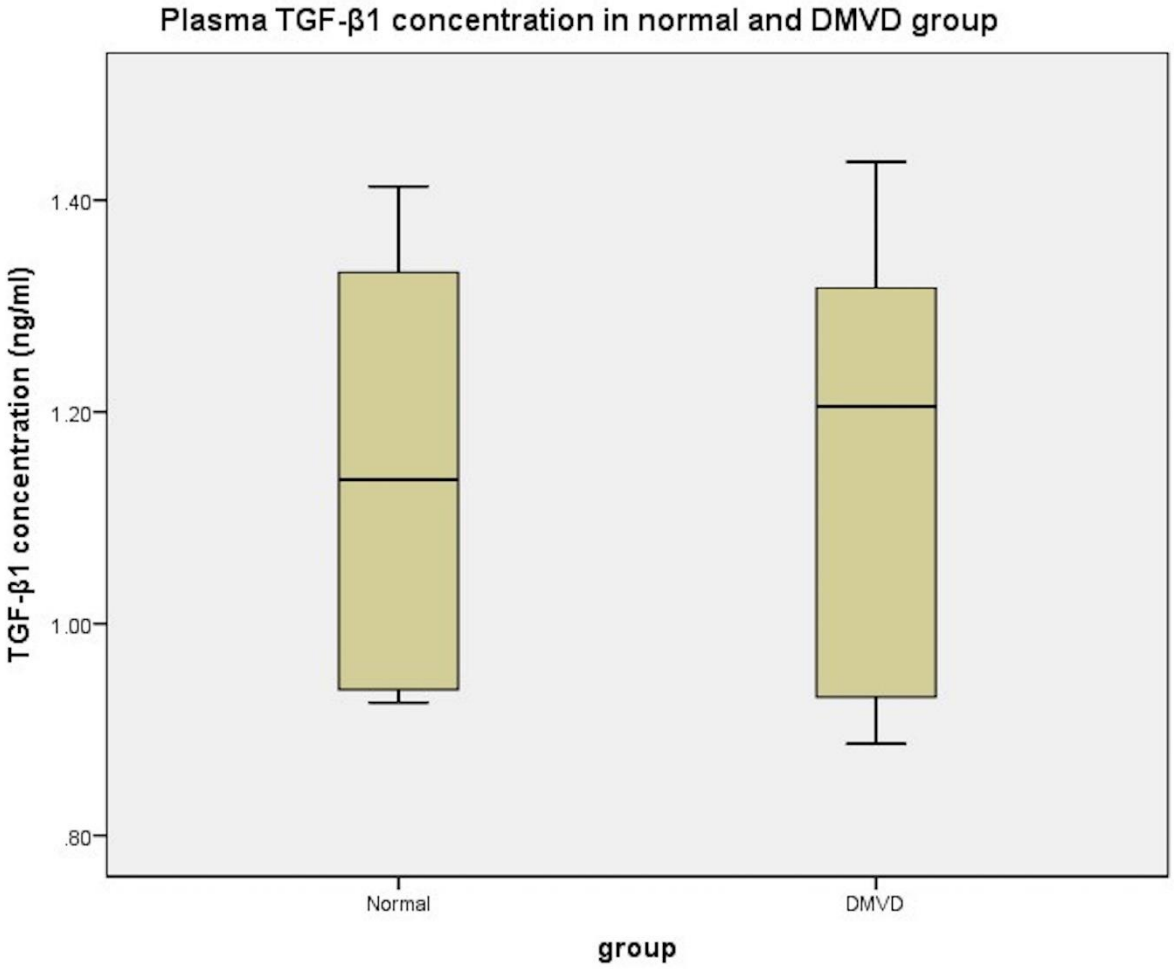

Figure 1 The boxplot of plasma TGF-β1 concentrations in normal and DMVD dogs. Lines within the boxes are median values. Lines at the bottom are minimum values, and lines at the top are maximum values. The 25th and 75th percentile values represent the limits of the box.

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References

Atkins CE, Bonagura J, Ettinger S, Fox P, Gordon S, Häggström J, Hamlin R, Keene B, Luis-Fuentes V and Stepien R 2009. Guidelines for the diagnosis and treatment of canine chronic valvular heart disease. J Vet Intern Med. 23(6):1142-1150.

Aupperle H, März I, Thielebein J and Schoon HA 2008. Expression of transforming growth factor-beta-1,-beta-2 and –beta3 in normal and diseased canine mitral valves. J Comp Pathol. 139(2-3):97-107.

Cornell CC, Kittleson MD, Della Torre P, Häggström J, Lombard CW, Pedersen HD, Vollmar A and Wey A 2004. Allometric scaling of M-mode cardiac measurements in normal adult dogs. J Vet Intern Med. 18(3):311-321.

Disatian S 2010. Myxomatous degenerative mitral valve disease: An update. Thai J Vet Med. 40(1):151-157.

Disatian S and Orton EC 2009. Autocrine serotonin and transforming growth factor beta 1 signaling mediates spontaneous myxomatous mitral valve disease. J Heart Valve Dis. 18(1):44-51.

Disatian S, Ehrhart EJ 3rd, Zimmerman S and Orton EC 2008. Interstitial cells from dogs with naturally occurring myxomatous mitral valve disease undergo phenotype transformation. J Heart Valve Dis. 17(4):402-411.

Häggström J, Pedersen HD and Kvart C 2004. New insights into degenerative mitral valve disease in dogs. Vet Clin North Am Small Ani Pract. 34(5):1209-1226.

Jian B, Narula N, Li QY, Mohler ER and Levy RJ 2003. Progression of aortic valve stenosis: TGF-β1 is present in calcified aortic valve cusps and promotes aortic valve interstitial cell calcification via apoptosis. Ann Thorac Surg. 75(2):457-465.

Khan R and Sheppard R 2006. Fibrosis in heart disease: understanding the role of transforming growth factor-β1 in cardiomyopathy, valvular disease and arrhythmia. Immunology. 118(1):10-24.

Mavropoulou A, Guazzetti S, Borghetti P, De Angelis E and Quintavalla C 2016. Cytokine expression in peripheral blood mononuclear cells of dogs with mitral valve disease. Vet J. 211:45-51.

Moesgaard S, Aupperle H, Rajamäki M, Falk T, Rasmussen C, Zois NE and Olsen LH 2014. Matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs) and transforming growth factor-β (TGF-β) in advanced canine myxomatous mitral valve disease. Res Vet Sci. 97(3):560-567.

Olsen LH, Fredholm M and Pedersen HD 1999. Epidemiology and inheritance of mitral valve prolapse in Dachshunds. J Vet Intern Med. 13(5):448-456.

Sharma K, Ziyadeh FN, Alzahabi B, McGowan TA, Kapoor S, Kurnik BR, Kurnik PB and Weisberg LS 1997. Increased renal production of transforming growth factor-β1 in patients with type II diabetes. Diabetes. 46:854-859.

Swenson L, Häggström J, Kvart C and Juneja R 1996. Relationship between parental cardiac status in Cavalier King Charles spaniels and prevalence and severity of chronic valvular disease in offspring. J Am Vet Med Assoc. 208(12):2009-2012.

Syrris P, Carter ND, Metcalfe JC, Grainger DJ, Kaski JC, Crossman C, Francis SE, Julian G, Jeffery S and Heathcote K 1998. Transforming growth factor-β1 gene polymorphisms and coronary artery disease. Clin Sci. 95:659-667.

Tashiro H, Shimokawa H, Sadamatu K and Yamamoto K 2002. Prognostic significance of plasma concentrations of transforming growth factor-β in patients with coronary artery disease. Coro Artery Dis. 13:139-143.

Ware W 2003. Acquired valvular and endocardial diseases. In: Small animal internal medicine St. Louis: Mosby 39-150.

Wang X, Liu SX and Wilcken D 1997. Circulating transforming growth factor β1 and coronary artery disease. Cardiovasc Rec. 34(2):404-410.

Whitney J 1974. Observations on the effect of age on the severity of heart valve lesions in the dog. J Small Anim Pract. 15(8):511-522.

Zois NE, Moesgaard SG, Kjelgaard-Hansen M, Rasmussen CE, Falk T, Fossing C, Häggström J, Pedersen HD and Olsen LH 2012. Circulating cytokine concentrations in dogs with different degrees of myxomatous mitral valve disease. Vet J. 192(1):106-111.

บทคัดย่อ

การตรวจทรานส์ฟอร์มมิ่งโกรทแฟกเตอร์ เบต้า 1 ในสุนัขปกติและสุนัขที่เป็นโรคลิ้นหัวใจไมทรัลเสื่อม

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โรคลิ้นหัวใจไมทรัลเสื่อมเป็นโรคหัวใจภายหลังกำเนิดที่พบได้บ่อยในสุนัขโตเต็มวัยพันธุ์ขนาดเล็กถึงกลาง พบการเพิ่มขึ้นของทรานสฟอร์มมิ่งโกรทแฟกเตอร์เบต้า 1 ในลิ้นหัวใจของสุนัขที่เป็นโรคลิ้นหัวใจไมทรัลเสื่อม อย่างไรก็ตามยังไม่มีการประเมินพลาสมาทรานสฟอร์มมิ่งโกรทแฟกเตอร์เบต้า 1 มาก่อน วัตถุประสงค์ของการศึกษานี้เพื่อเปรียบเทียบความเข้มข้นของพลาสมาทรานสฟอร์มมิ่งโกรทแฟกเตอร์เบต้า 1 ในสุนัขปกติและสุนัขที่เป็นโรคลิ้นหัวใจไมทรัลเสื่อมด้วยวิธีอีไลซ่า การวัดนี้อาจช่วยในการแยกสุนัขที่เป็นโรคลิ้นหัวใจไมทรัลเสื่อมจากสุนัขสุขภาพดีได้ วิธีทดสอบแมนวิทนี่ถูกใช้เพื่อการเปรียบเทียบพลาสมาทรานสฟอร์มมิ่งโกรทแฟกเตอร์ เบต้า 1 ระหว่างสองกลุ่ม p-value น้อยกว่า 0.05 แสดงถึงนัยสำคัญทางสถิติ ผลการศึกษาแสดงความไม่แตกต่างทางสถิติ (p=0.574) ระหว่างค่ากลางของพลาสมาทรานสฟอร์มมิ่งโกรทแฟกเตอร์ เบต้า 1 ในสุนัขปกติ 22 ตัว (1.14, 0.94-1.33 นาโนกรัม/มิลลิลิตร) และสุนัขที่เป็นโรคลิ้นหัวใจไมทรัลเสื่อม 27 ตัว (1.21, 0.92-1.32 นาโนกรัม/มิลลิลิตร) โดยสรุปความเข้มข้นของพลาสมาทราสฟอร์มมิ่งโกรทแฟกเตอร์ เบต้า 1 น้อยมากในกระแสเลือด จึงไม่เหมาะสมที่จะเป็นตัวชี้วัดทางชีวภาพในการวินิจฉัยโรคลิ้นหัวใจไมทรัลเสื่อม

คำสำคัญ: สุนัข โรคลิ้นหัวใจไมทรัลเสื่อม ลิ้นหัวใจไมทรัล พลาสมาทรานสฟอร์มมิ่งโกรทแฟกเตอร์ เบต้า 1

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