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Evaluation of Thromboelastography Analysis for Treatment of Heartworm Disease in Dogs over Time: a Pilot Study

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Abstract : Thromboelastography (TEG) analysis consists of α, G, K, MA, and R types of values and tests the effectiveness of blood clotting, which can be assessed for platelet function, clotting strength, and fibrinolysis. Canine heartworm diseases caused by *Dirofilaria immitis*, a vascular nematode, can lead to hyperfibrinolysis and hypercoagulation. In this study, G and MA values showed a statistically significant decrease over time after treatment of heartworm disease. Additionally, the α-value showed a high correlation with G, K, MA, and R values. The G value showed a high correlation with K and MA values, while The K value showed a high correlation with MA and R values. This study clearly found a gradual decrease in G and MA values in dogs with heartworm disease over time, both before and after treatment. This suggests that the clot formation time is longer and that the intensity of clot formation is lowered and may improve the risk of thromboembolism in dogs with heartworm disease.

Key words **:** dog, heartworm, hypercoagulability, thromboelastography, thromboembolism.

Introduction

Blood coagulation tests associated with blood clotting, such as prothrombin time (PT) and partial thromboplastin time (aPTT) have limitations such as not being able to assess platelet function, clotting strength, and fibrinolysis (1). Thromboelastography (TEG), however, can test the effectiveness of blood clotting without these limitations. TEG analysis is determined by different types of values. They are R value or reaction time, K value, angle (α), and maximum amplitude (MA) associated with the formation of blood clots. R value indicates the time when the first evidence of blood clotting is detected. The K value represents the rate of formation of clot i.e., the time until the coagulation reaches 20 mm after R is completed. The α-value provides information similar to K, and is the tangent of the curve formed when K is reached. The intensity of clotting is measured by the value of MA. The G value, which is the data for indicating the clotting strength, is the log-derivation of MA and it increases when the risk of blood clotting or the risk of venous thromboembolism increases in human medicine (2). Although there are not many studies on TEG in veterinary medicine (3-8), its usefulness has been proposed in clinical evaluation or monitoring of patients related to hypercoagulability or fibrinolysis (9).

Dirofilaria immitis, called the heartworm, is a roundworm parasite that spread from host to host via mosquitoes that suck the blood of the host. The final host is a dog, but it can also infect other animals such as cats, wolves, coyotes, foxes, ferrets, bears, seals, and sea lions, with some cases also reported in humans (10). Heartworm-infected dogs suffer severe complications including glomerulonephritis, eosinophilic pneumonitis, right heart failure (11,12), caval syndrome with very poor prognosis, and pulmonary thromboembolism leading to a fatal prognosis in the patient. Additionally, canine heartworm diseases caused by *D. immitis*, a vascular nematode, can cause hyperfibrinolysis and hypercoagulation (13,14).

Generally, dogs infected with heartworm infection, except those with caval syndrome, receive three injections of melarsomine dihydrochloride based on the treatment guidelines by the American Heartworm Society (AHS) (15). The aim of our pilot study was to determine the time course of risk factors associated with hypercoagulation using TEG analysis over time, before and after treatment of dogs infected with heartworm, by following the standard treatment guidelines protocol by AHS.

Materials and Methods

Animals

The study was conducted on client-owned dog patients of heartworm (I-III/IV Grade [Gr]) with no other disease and included 6 dogs (5 intact females and 1 castrated male, weighing 4.7-8.1 kg). All the dog patients were cross-breeds and ages were not clarified. Those with heartworm disease were under medication for heart diseases with drugs such as pred-

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nisolone, doxycycline, and melarsomine dihydrochloride, except anticoagulant agent such as clopidogrel and aspirin.

Study designs

Dogs participating in this study were selected through various criteria. For study probabilities, those with hypercoagulable diseases other than heartworm disease, such as immune-mediated hemolytic anemia (IMHA), neoplasia, hyperadrenocorticism, protein-losing nephropathies (PLN) and enteropathies (PLE), early disseminated intravascular coagulation (DIC), sepsis and heart disease, or chronic kidney diseases were not included. Physical examination, antigen test for heartworm, radiography, and echocardiography were performed for all the dogs with a diagnosis of heartworm disease. However, dog patients with caval syndrome as IV/IV Gr heartworm disease were excluded from this study because injecting melarsomine dihydrochloride as an initial therapy was not recommended by AHS (15). Anticoagulants such as clopidogrel and aspirin were excluded from the treatment of patients, in order to avoid interference with hypercoagulability.

Schedule of events

Based on the guidelines by AHS for heartworm treatment, the patients received a total of three injections of melarsomine dihydrochloride (IMMITICIDE®, Boehringer Ingelheim, France) (2.5 mg/kg, deep intramuscular) at the lumbar epaxial muscles between 3rd and 5th lumbar vertebrae.

In this study, we collected and analyzed the blood samples four times, before and after treatment for heartworm disease (T 0-3). ‘T 0’ was the analysis result obtained before the 1st injection of melarsomine dihydrochloride for heartworm treatment; ‘T 1’ was the result of the blood sample collected one day after the 1st melarsomine dihydrochloride injection, while the 2nd injection of melarsomine dihydrochloride was given one month after the 1st injection, and the 3rd was given one day after the 2nd injection; ‘T 2’ was the result obtained one day after the 2nd injection; and ‘T 3’ was the result after the 3rd injection. D-dimer and TEG analyses tests were performed once before (T 0) and thrice after (T 1-3) the injection of melarsomine dihydrochloride during the heartworm treatment.

Thromboelastography and D-dimer analysis

Blood was collected from the jugular vein using a 3 ml syringe with 23-G 1 and 1/2" needles, after a 12-hour fasting period for all the dogs. The whole blood was diluted with anticoagulants using 3.2% sodium citrate at a ratio of 9:1. The anticoagulated blood specimens were immediately transferred to the laboratory in a refrigerated state and analyses were performed and completed immediately upon arrival of the sample at the reference laboratory.

TEG concentration was measured using a TEG® 5000 Hemostasis Analyzer System (Thromboelastograph, Haemonetics Corporation, Braintree, MA, USA; Haemaru Small Animal Clinical Research Institute, Seongnam, Korea). The reference ranges for TEG analysis for dogs, where the blood was collected using a 23-G syringe, as provided by the laboratory were 25.5-73.3 degree for α, 3.4-9.9 Kdyn/cm^2 for G, 0.5-6.9 min for K, 45.3-67.2 mm for MA, and 0.5-13.2 min for R.

D-dimer analysis was measured using the immunometric assay (NycoCard® Reader II, Alere, Norway) in a commercial veterinary diagnostic laboratory (Neodin Veterinary Diagnosis Laboratories, Seoul, Korea). According to the reference laboratories, reference intervals of D-dimer for healthy dogs were established as 0-0.3 μg/mL.

Statistical analysis

Statistical analyses were performed using commercially available statistical software (Microsoft Office Excel 2016 for Mac). Continuous variables are presented as mean ± standard deviation (SD). Differences in the various indices that changed before and after treatment for heartworm disease were assessed using one-way analysis of variance (ANOVA). Pearson’s coefficient of bivariate correlation analysis was used to test the strength of association between each item of TEG. Median values and SD ranges are reported. In all comparisons, a probability value of $P < 0.05$ was considered statistically significant, unless stated otherwise.

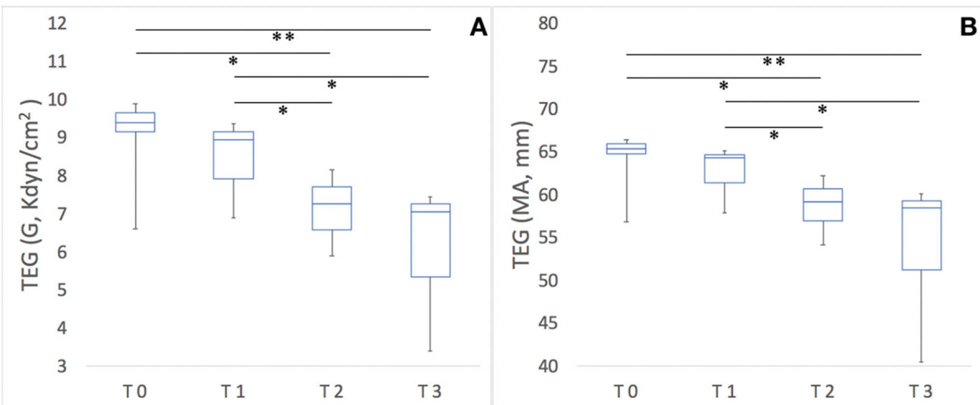

Fig 1. The values of thromboelastography (TEG) in this study. A. TEG (G) statistically significant decrease over time after treatment of heartworm disease in dogs. B. TEG (MA) statistically significant decrease over time after treatment of heartworm disease in dogs. ‘T 0’ indicates the result before 1st injection of melarsomine dihydrochloride for heartworm treatment; ‘T 1’ indicates the result one day after the 1st melarsomine dihydrochloride injection, ‘T 2’ is the result one day after the 2nd injection; and ‘T 3’ is the result one day after the 3rd injection. *, $P < 0.05$; **, $P < 0.01$.

Results

The breeds of dogs included in this study were all cross-breeds.

Compared to the references presented by the laboratory that analyzed the blood sample, concentrations of D-dimer and values of all five items in the TEG did not deviate from the normal range during the entire study period.

G and MA values demonstrated a statistically significant decrease over time after the treatment of heartworm disease ($p = 0.003$, $p = 0.009$, respectively). G and MA values were 9.4 ± 1.22, 65.4 ± 36.2 at ‘T 0’ (before treatment), 9.0 ± 1.03, 64.3 ± 3.02 at ‘T 1’, 7.3 ± 0.87, 59.2 ± 3.01 at ‘T 2’, and 7.1 ± 1.68, 58.5 ± 7.92 at ‘T 3’ (after treatment), respectively.

G and MA were statistically significantly lower in dogs with heartworm disease over time at each step from ‘T 0’ to ‘T 3’ (Fig 1A and B). Especially, the gap between mean value of G and MA were estimated at 2.3 Kdyn/cm^2 and 6.9 mm, between ‘T 0’ and ‘T 3’, respectively. Differences in the various indices that changed before and after treatment for heartworm disease in this study population are summarized in Table 1.

In the statistical test of correlation, D-dimer did not show a high correlation ($r > 0.7$) with any item of TEG. In the analysis of the correlation between the items of the TEG, α highly correlated with G ($r = 0.79$, Fig 2A), K ($r = -0.96$, Fig 2B), MA ($r = 0.77$, Fig 2C), and R ($r = -0.91$, Fig 2D). G showed high correlation with K ($r = -0.82$, Fig 2E) and MA ($r = 0.98$, Fig 2F). K showed high correlation with MA ($r = -0.84$, Fig 2G) and R ($r = 0.94$, Fig 2H).

Discussion

Our study clearly found a gradual decrease in G and MA values in dogs with heartworm disease in the time before and after treatment.

The condition in which heartworms are found in the vena cava and right atrium of dogs is called “caval syndrome” (16). This results in severe pulmonary artery injury and is classified as IV/IV Gr. However, if dogs with caval syndrome are treated with melarsomine dihydrochloride, the risk of post-treatment pulmonary thromboembolism increases dramatically resulting in a high risk of morbidity and mortality after treatment (17). Since hypercoagulability increases in dogs suffering from IMHA, neoplasia, hyperadrenocorticism, PLN, PLE, early DIC, and sepsis (3-8), excluding the interfering effects of this study on the problem was crucial. Therefore, the treatment group of this study was limited to dogs with heartworm disease between I-III/IV Gr. Furthermore, the patients without any other disease than heartworm disease was selected.

The pharmacokinetics of melarsomine dihydrochloride, an

Table 1. Comparison of various indices in dogs with heartworm disease over different time points, before and after treatment

Analysis (value, unit)	T 0	T 1	T 2	T 3	*p* value
D-dimer (μg/mL)	0.16 ± 0.41	0.05 ± 0.11	0.00 ± 0.14	0.16 ± 0.08	0.293
TEG (α, degree)	52.3 ± 9.87	55.4 ± 20.57	41.9 ± 9.74	37.7 ± 16.01	0.406
TEG (G, Kdyn/cm^2)	9.4 ± 1.22	9.0 ± 1.03	7.3 ± 0.87	7.1 ± 1.68	0.003
TEG (K, min)	3.0 ± 1.31	2.6 ± 3.35	4.3 ± 1.35	5.3 ± 3.96	0.264
TEG (MA, mm)	65.4 ± 3.62	64.3 ± 3.02	59.2 ± 3.01	58.5 ± 7.92	0.009
TEG (R, min)	8.9 ± 1.96	7.9 ± 5.19	8.9 ± 2.05	11.4 ± 4.61	0.449

All data expressed with the mean value (± standard deviation)
TEG: Thromboelastography; T 0: results before 1st injection of melarsomine dihydrochloride for heartworm treatment; T 1: results one day after the 1st melarsomine dihydrochloride injection; T 2: results one day after the 2nd injection; T 3: results one day after the 3rd injection.

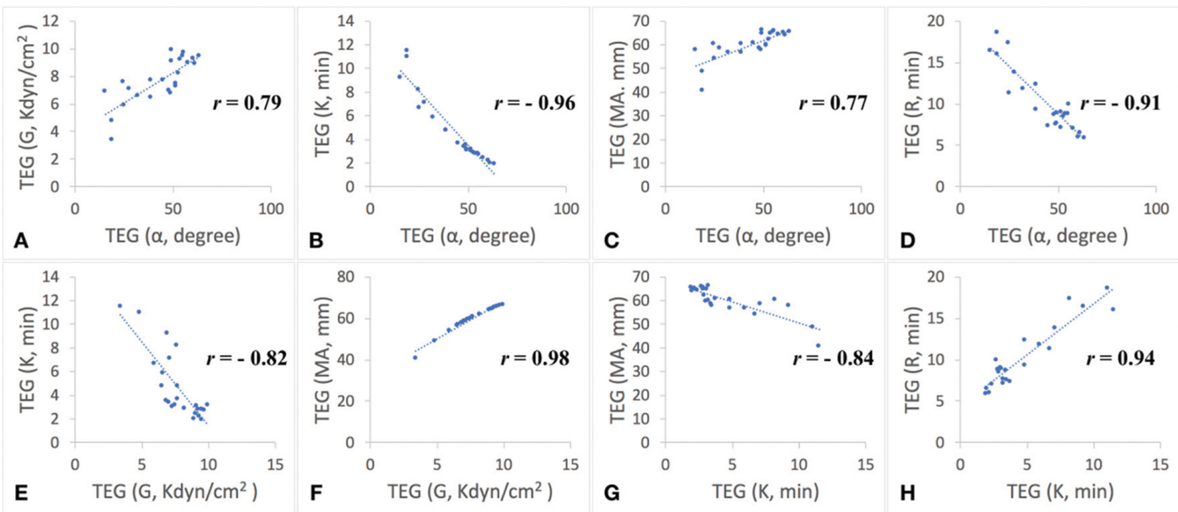

Fig 2. The correlation between the items of the thromboelastography (TEG) A-D: α is highly correlated with G, K, MA, R values. E and F: G showed high correlation with K, MA. G and H: K showed high correlation with MA and R.

intramuscular injection for the treatment of dogs with heartworm disease, has an apparent volume of distribution of approximately 0.7 L/kg, an estimated terminal half-life of 3 hours and takes around 11 minutes to reach peak plasma concentration. Using melarsomine dihydrochloride to kill heartworms in dog blood is essential to restore dog's health, but at the same time the death of very long adults can pose a risk to the patient. Therefore, on the day of melarsomine dihydrochloride injection, it could be even more dangerous. When the heartworm dies, its broken body fragments may block the blood vessels of the lungs and cause potentially fatal pulmonary embolism. In order to avoid these hazards, the TEG analysis in our study was conducted with blood drawn after a day of melarsomine dihydrochloride injection.

R and K values increased during treatment. The increase in these values indicates that the clot formation time was delayed, which means that clot formation is delayed through treatment but there was no statistical significance before and after treatment. The values of α, G, and MA decreased during treatment, indicating that the intensity of clot formation had reduced. This means that the intensity of clot formation is reduced during treatment, but only G and MA are statistically significant. R and K values are related to the clot formation time and showed a high correlation with one another, and the curve formation slope decreases with increasing clot formation time indicating that α value was inversely correlated to K. G representing the intensity of the clot showed a high correlation with MA, and α expressed a high correlation of inverse proportion to the time of clot formation.

This study had some limitations. First, it was a pilot study in which the involved population was too small. This weakness can be rectified with further extensive collective studies on heartworm diseases for hypercoagulability. Second, there was a lack of sampling schedule for TEG. In our study after treatment for heartworm disease, we only collected blood thrice to express TEG and other data. However, the statistical results indicate that the hypercoagulability of heartworm disease is reduced. Third, as a study of blood coagulation, the results of the traditional tests, such as PT and aPTT were not performed together. However, a recent study of TEG for hypercoagulable disease showed that PT was not significantly correlated to R, and aPPT was not found to be significant between control group and case group (18).

In conclusion, the evaluation of hypercoagulability after treatment of dogs with heartworm disease suggests that the clot forming time is longer and the intensity of clot formation is lowered. This may improve the risk of thromboembolism in heartworm disease in dogs.

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