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Evaluation of prothrombin and activated partial thromboplastin time in dogs with disseminated intravascular coagulation

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Abstract
The coagulation profile testing plays a major role in diagnosing Disseminated Intravascular Coagulation (DIC) in dogs. The aim of this study is to assess the changes in Prothrombin Time (PT) and activated partial thromboplastin time (aPTT) in dogs with DIC affected with various diseases such as Haemoprotozoan diseases, Pyometra, Leptospirosis, Neoplasia and others. The PT and aPTT recorded was 8.2±1.2 sec and 25±1.5 sec in healthy (N=10), 12.98±1.66 sec and 132.11±26.79 sec in Haemoprotozoan diseases (N=15), 8.2±0.5 sec and 62±12.34 sec in Pyometra (N=3), 13.6±5.2 sec and 73.7±8 in Leptospirosis (N=2), 75.28±41.47 sec and 258.32±120.57 sec in Neoplasia (N=5), 17.35±2.98 sec and 67.56±26.05 sec in others (N=3) respectively. This study showed that 46% of the dogs with DIC had prolonged PT and 92% had prolonged aPTT.

Keywords: Activated Partial Thromboplastin Time (aPTT), Prothrombin Time (PT), Disseminated Intravascular Coagulation (DIC), Thrombocytopenia

Introduction
The interplay of endothelial cells, platelets, circulating clotting factors, fibrinolytic agents, and inhibitors of haemostasis makes up the extremely intricate, balanced and coordinated haemostatic system. Disseminated intravascular coagulation (DIC) is also known as consumptive coagulopathy and it occurs when the haemostatic system is activated as a result of an external stimulus, such as inflammation brought on by a disease (Stokol 2012) [2]. This potentially fatal illness commences as a hypercoagulable state before changing into a hypocoagulable state when bleeding occurs after consuming an excessive amount of platelets and clotting factors. Since, there is no single specific laboratory test for the diagnosis of DIC (Bruchim *et al.*, 2008) [4], a series of laboratory tests can be conducted when DIC is suspected which includes platelet count, prothrombin time (PT), activated partial thromboplastin time (aPTT), Antithrombin (AT), fibrinogen, FDPs (Fibrin/ fibrinogen degradation product), and D-dimer levels. Abnormalities of both PT and aPTT may be seen in dogs with consumptive coagulopathy, but in the early stages of DIC, it is more common to see a mild to moderate prolongation of the aPTT and a normal PT. This may be because the main coagulation machinery for generating fibrin is the intrinsic pathway, so once coagulation is initiated, the majority of clot propogation is supported by this way (Brainard, 2014) [5]. The aim of this study is to evaluate the PT and aPTT levels in dogs with disseminated intravascular coagulation and the significance of these results in DIC diagnosis.

Materials and Methods
For this study, the thrombocytopenic dogs which were suspected for DIC, presented to the Critical Care Unit, Madras Veterinary College Teaching Hospital were chosen, while the healthy dogs served as the control group. Sub grouping was done based on the etiology of diseased dogs as Haemoprotozoan disease (N=15), Neoplasia (N=5), Leptospirosis (N=2), pyometra (N=3), hyperthermia (N=2) and others (N=3) which includes acute hepatitis, snake envenomation and Haemorrhagic gastro enteritis.

Two millilitre of whole blood was collected aseptically from each dog by venipuncture of cephalic or saphenous vein in vacutainers coated with 10% EDTA as anticoagulant to estimate platelet count using automated haematology analyser® (Mindray-BC-2800 VET, Exigo EVS). As the aPTT is more sensitive to *in vitro* activation of clotting factors than the PT and may be prolonged with difficult venipuncture or if insufficient blood is collected in citrate. To avoid this error, double syringe collection method as suggested by Tvedten, (2012) [8] was carried out. For coagulation analysis 0.9 ml of blood was collected in a syringe with 0.1ml of 3.2% Trisodium citrate solution (0.109M) and centrifuged immediately for 15 min at 3000 RPM. The undiluted plasma samples were analysed using a semiautomatic coagulometer, MISPA CLOG from M/s. Agappe Diagnostics, India. It is an opto-mechanical coagulation analyser, which applies turbodensitometric measuring principles for estimation. PT and aPTT was estimated as per the guidelines of the manufacturer of the kits. The fibrinogen and D-dimer values were estimated using immunoturbidimetric assay used for human plasma according to Caldin *et al.* (2000) [6] and Bauer *et al.* (2009) [1].

Results

Among these twenty-eight patients there were ten females and eighteen males between the age group from 3 months to 10 years. Haemoprotozoan disease was the commonest underlying condition for DIC. Prothrombin (PT) and activated partial thromboplastin (aPTT) time in healthy dogs were 8.2±1.2 sec and 25±1.5 sec, respectively and the PT and aPTT values recorded in the dogs with DIC were given in the table 1. The dogs which were suspected for DIC were subjected to platelet count, PT and aPTT, D-dimer and Fibrinogen concentration. Among these twenty-eight patients, thirteen patients showed elevated PT values and twenty-six patients showed elevated aPTT value. Diagnosis is generally based on presence of an underlying condition that could trigger DIC, together with three or more of the following anomalies: thrombocytopenia, prolongation of the PT, aPTT or TT, elevated D-dimers, hypofibrinogenemia, reduced antithrombin activity, and/ or evidence of red blood cell fragmentation (schistocytes) on blood smear examination. These dogs were confirmed with DIC due to presence of an underlying etiology that predisposes DIC along with atleast three anamolies in the laboratory tests which includes thrombocytopenia, elevated PT or aPTT, the elevation of D-dimer (> 0.5 µg/ ml) and low level of Fibrinogen concentration (< 100mg /dl).

Statistical Analysis

One-way ANOVA has been used to test the significance

Table 1: Observed values of PT and aPTT in dogs with various underlying etiology

Group	Platelet count (/µl)	PT (sec)	aPTT (sec)
Control (10)	3,26,000±82,600[a]	8.2±1.2[a]	25±1.5[a]
Hemoprotozoan Diseases (15)	55,786±10,598[b]	12.98±1.66[a]	132.11±26.79[b]
Pyometra (N=3)	89,000±7,000[b]	8.2±0.5[a]	62±12.34[a]
Leptospirosis (N=2)	1,03,500±66500[b]	13.6±5.2[a]	73.7±8[b]
Neoplasia (N=5)	40,000±5167[b]	75.28±41.47[b]	258.32±120.57[b]
Miscellaneous(N=3)	1,32,500±31435[b]	17.35±2.98[a]	67.56±16.05[b]
F value	15.274*	2.854*	2.807*

* Sig at the level of 0.05

Means bearing the same superscript do not differ significantly

The mean platelet count was reduced significantly in the diseased group, when compared with the control group. No significant difference in the mean PT values of the diseased group, when compared with the Control group, except the Neoplasm group, which had high mean PT values. However, the diseased group had significantly high mean aPTT values when compared with control group, and also the mean aPTT values differ significantly within the diseased group. But there is no significant difference in mean aPTT values between control and Pyometra group.

Discussion

PT and aPTT were estimated in dogs which were suspected for DIC, with an underlying etiology. DIC was confirmed in those dogs with the parameters such as thrombocytopenia, prolonged PT and aPTT values, fibrinogen level and elevated D-dimer levels. This study shows that 46% of the dogs with DIC had prolonged PT and 92% had prolonged aPTT. Similarly, Dolente *et al.* (2002) reported that the aPTT appears to be more sensitive for detecting DIC in animals than the PT or TCT in the routine coagulation screening tests. The least time PT recorded was 6 and aPTT was 30 and both cases had elevated D-dimer values without any bleeding signs. The highest recorded time of PT and aPTT was 200 sec and 600 sec respectively in a Lymphoma case which had bleeding signs succumbed to death the next day, which indicates overt type of DIC. One leptospirosis case had mild thrombocytopenia, normal PT value, but prolonged aPTT value and elevated D-dimer level, recovered after treatment of the underlying cause, since the cornerstone of DIC management is providing treatment for the underlying disorders where DIC spontaneously resolves in many cases. Similarly, in a dog with Trypanosomiasis there was normal PT and mild prolongation of aPTT level without any bleeding signs had organ failure symptoms which indicates non-overt type of DIC which is in consistent with Brainard (2014) [5] who stated that abnormalities of both PT and aPTT may be seen in dogs with consumptive coagulopathy, but in the early stages of DIC (prior to excessive clotting factor consumption), it is more common to see a mild to moderate prolongation of the aPTT and a normal PT.

Conclusion

Estimation of PT and aPTT in dogs with disseminated intravascular coagulation revealed PT has been prolonged in 46% of the cases and aPTT has been prolonged in 92% of cases which indicates aPTT is more sensitive than PT.

Conflict of Interest

Not available

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Not available

Reference

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