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Research Article

EVALUATION OF OXIDATIVE STRESS INDICES AND DISSEMINATED INTRAVASCULAR COAGULATION IN *BABESIA GIBSONI* INFECTED DOGS WITH RESPECT TO DIFFERENT THERAPEUTIC MODALITIES

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ABSTRACT

The present study was conducted to evaluate status of oxidative stress and disseminated intravascular coagulation in *Babesia gibsoni* infected dogs under different therapeutic regimens. Dogs under study were screened for *B. gibsoni* infection based on characteristic clinical signs and confirmatory peripheral blood smear staining examination. Infected dogs were divided randomly into 3 groups for different therapeutic interventions. Infected dogs of Group I received Diminazine Aceturate (5mg/Kg B.Wt. IM) which was repeated on day 7. Dogs of Gr. II were treated with the combination of Doxycycline @ 10mg/Kg B.Wt. PO q12h for 21 days, Clindamycin @ 25mg/Kg B.Wt. PO q12h for 10 days. Dogs of Gr. III subjected to combination of Doxycycline @ 10mg/Kg B.Wt. PO q12h for 21 days, Clindamycin @ 25mg/Kg B.Wt. PO q12h for 10 days, Metronidazole @ 5-7mg/Kg B.Wt. PO q12h for 10 days. Both the Gr. (II and III) of dog receiving combination therapy revealed elevated levels of catalase and superoxide dismutase (SOD) after completion of therapy. However, dogs of Gr. III recorded significantly ($p<0.05$) decreased level of prothrombin time (PT) and von Willebrand factor (vWF) denoting good prognosis with respect to onset and severity of disseminated intravascular coagulopathy (DIC) and subsequent multiple organ failure by triple drug combination therapy.

Keywords: *Babesia gibsoni*, Combination Therapy, DIC, Oxidative stress

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Canine babesiosis, a tick borne hemoprotozoan disease caused by *B. gibsoni* is widely distributed throughout the world (Ettinger *et al.*, 2017). Acute *B. gibsoni* infection is characterized by remittent fever, lethargy, thrombocytopenia, anemia while chronic infection may be completely asymptomatic or may be characterized by intermittent fever, lethargy and weight loss (Gonde *et al.*, 2016; Ettinger *et al.*, 2017). Clinical signs in severe form may be due to excessive systemic inflammatory response syndrome (SIRS) which results in multiple organ dysfunction syndromes (MODS) (Matijatko *et al.*, 2010). The disproportion between scavenging and radical generating mechanism leads to oxidative stress (Omar *et al.*, 2015). When reactive oxygen species (ROS) exceeds the ability of antioxidant system, oxidative stress occurs which damages the tissues and cells (Celi, 2011). Anti-oxidant elements such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) play a vital role in counteracting ROS damage (Omar *et al.*, 2015). *B. gibsoni* infection may cause disturbance in the blood coagulation mechanism which results in disseminated intravascular coagulopathy (DIC) and further leads to multiple organ failure (Rafael *et al.*, 2007). *B. gibsoni* parasite damages the endothelium of blood vessels which release von Willebrand factor (vWF) that leads to a consumptive thrombocytopenia (Levy *et al.*, 2005).

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Use of various therapeutic modalities like Clindamycin, Diminazine aceturate and Imidocarb dipropionate combination (Lin *et al.*, 2012) or Atovaquone and Azithromycin combination (Kirk *et al.*, 2017) suppresses the parasite replication. To explore newer successful treatment modality not only targeting *B. gibsoni* but also improvements of general systemic condition of infected dogs is the need of present time. Considering the facts, the present study envisaged to analyze oxidative stress indices and DIC status in *B. gibsoni* infected dogs treated with combination therapy (Table 1).

MATERIALS AND METHODS

Screening and diagnosis of dogs for *Babesia gibsoni*: Dogs presented with characteristic clinical signs at Referral Veterinary Polyclinic, IVRI were screened for hemoprotozoan disease. Blood samples were collected from jugular vein in EDTA containing tubes, heparin vials and sodium citrate vials for microscopic examination, oxidative stress and DIC parameters estimation, respectively. *B. gibsoni* was confirmed by small, singular, signet shaped organism in Giemsa stained peripheral blood smear examination.

Measurement of oxidative stress indices: Blood samples were collected in heparin vials for estimation of oxidative stress indices which included lipid peroxides (LPO), superoxide dismutase (SOD) and catalase. Level of lipid

Table 1

Different therapeutic regimens of *B. gibsoni* infection in dogs

Group (n=5)	Therapeutic regimen
I	Diminazine aceturate @ 5mg/kg B.Wt. IM repeated after 7 days
II	Doxycycline @ 10mg/kg B.Wt. PO q12h for 21 days, Clindamycin @ 25mg/kg B.Wt. q12h for 10 days and Supportive therapy SOS (Antipyretic, Liver Stimulant, Haematinics)
II	Doxycycline @ 10mg/kg B.Wt. po q12h for 21 days, Clindamycin @25mg/kg B.Wt. q12h for 10 days, Metronidazole @ 5-7mg/kg B.Wt. q12h for 10 days and Supportive therapy SOS (Antipyretics, Liver Stimulant, Haematinics)

peroxides in erythrocyte haemolysate was determined by estimating malondialdehyde (MDA) level (Placer *et al*., 1966). Superoxide dismutase (SOD) in haemolysate was measured using nitro blur tetrazolium as substrate (Mainami and Yoshikawa, 1979). Catalase activity in erythrocyte haemolysate was determined photometrically (Scott and Harrington, 1990).

Measurement of Disseminated intravascular coagulation (DIC): Status of DIC was determined based on platelet count and prothrombin time (PT) and vWF level. Platelet count was measured by using autoanalyser (KT6200, China). Blood sample was collected in sodium citrate vial for harvesting plasma for estimation of PT and vWF level. PT was estimated by using human commercial kits. Level of vWF in the plasma was determined by ELISA kit (Ca vWF ELISA kit, Blue gene) as per the kit procedure.

Statistical analysis: Data were analysed by using two way ANOVA using SPSS version 20.0.

RESULTS AND DISCUSSION

Dogs in all the three groups were monitored for response till day 21 of therapy. Blood samples were collected at 7th day to see changes in blood and microscopic picture. In Gr. I and II, few dogs were positive (+1) for babesiosis by microscopic examination at 7th day. But dogs in Gr. III were completely cleared from parasitic infection microscopically.

LPO level of erythrocyte hemolysate was determined by estimating malondialdehyde (MDA) level. LPO level on post therapy was significantly ($P<0.05$) lower in Gr. II dogs than Gr. I and III. With respect to catalase activity, non-significant elevated level of catalase was observed in Gr. II and III post therapy. SOD level was measured using nitro blur tetrazolium as substrate and non-significant increased level of SOD was noticed post therapy in Gr. II and III as compared to Gr. I (Table 2).

Non-significantly elevated level of platelet count was noticed post therapy in all the three groups. With respect to prothrombin time (PT), significantly (<0.05) decreased level of PT was noticed in Gr. III as compared to Gr. I and II post therapy. Non-significantly decreased vWF level was noticed post therapy in Gr. III. Results are shown in Table 2 and 3.

Parasitic load is positively correlated with oxidative

Table 2

Oxidative stress indices in dogs with *Babesia gibsoni* infection (mean± SE)

Group	LPO (µmol/l))		CATALASE (unit/ml)		SOD (unit/ml)	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
I	3.98 ± 0.1^{Ab}	4.59 ± 0.3^{Aa}	5230.05 ± 876.53^{Aa}	4260 ± 78.68^{Aa}	0.19 ± 0.01^{Aa}	0.16 ± 0.01^{Aa}
II	3.87 ± 0.1^{Ab}	3.25 ± 0.08^{Ab}	5545.39 ± 1387.83^{Aa}	5731.68 ± 1421.19^{Aa}	0.19 ± 0.01^{Aa}	0.21 ± 0.01^{Aa}
III	5.52 ± 0.3^{Aa}	4.67 ± 0.4^{Aa}	2022.62 ± 415.45^{Ab}	3896.60 ± 34.5^{Aa}	0.12 ± 0.02^{Aa}	0.17 ± 0.01^{Aa}

(A, B superscripts indicate significant difference within a group and a, b superscripts indicate significant difference between groups)

Table 3

Pre and post treatment changes in platelet count, prothrombin time and vWF level in dogs with *Babesia gibsoni* infection (mean± SE).

Group	Platelet count(Lakhs)		Prothrombin time (Sec)		vWF (ng/ml)	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
I	1.57 ± 0.23^{Aa}	2.24 ± 0.35^{Aa}	15.00 ± 1.10^{Aa}	12.48 ± 0.71^{Aa}	25.10 ± 5.66^{Aa}	27.42 ± 8.60^{Aa}
II	1.13 ± 0.37^{Aa}	2.18 ± 0.52^{Aa}	14.00 ± 0.71^{Aa}	$12.48\pm.22^{Aa}$	37.73 ± 6.52^{Aa}	45.70 ± 11.86^{Aa}
III	0.45 ± 0.27^{Ab}	1.46 ± 0.44^{Aa}	17.80 ± 2.35^{Aa}	11.80 ± 0.37^{Ba}	28.77 ± 7.33^{Aa}	7.24 ± 0.55^{Ab}

(A, B superscripts indicate significant difference within a group and a, b superscripts indicate significant difference between groups)

stress (Murase *et al.*, 1996). In recent years, pathogenesis of anemia with babesiosis is correlated with lipid peroxidation and oxidative process (Nazifi *et al.*, 2011). RBC membrane is rich in polyunsaturated fatty acids which is highly prone to oxidative damage in babesiosis resulting into direct damage to red cells (Omar *et al.*, 2015). Presence of hemo-protozoan infection inside the RBCs disturbs the key anti-oxidant system of red cells, which leads to release of oxidant mediators from red cells that eventually causes cell injury and hemolysis (Martusevich and Karuzin, 2015). The increase in oxidant markers causes the exhaustion of antioxidant markers (GPx and SOD), which are important indicators of oxidative stress. Increased level of LPO and decreased level of catalase and SOD post therapy in Gr. I denoted constant oxidative stress because of incomplete clearance of parasite from the blood at 7th day of study. It was in accordance with the study of Baneth (2018) who reported that oxidative stress is directly associated with persistent parasitemia. But in Gr. II dogs treated with combination therapy, elevated level of catalase and SOD and decreased level of LPO were observed post therapy. Oxidative stress indices were not much conclusive in this study before and after treatments. Likewise, blood and serum changes were not significantly conclusive before and after treatment in *B. gibsoni* infected dogs treated with different combination therapy (Narayani *et al.*, 2021).

Multiple Organ Dysfunction Syndrome can occur after infection with most pathogenic *Babesia* species, particularly *B. canis* sub sp. *rossi* and *B. gibsoni*. Median vWF and propeptide levels were significantly higher in patients with parasitic diseases (Hollestelle *et al.*, 2006). Status of DIC can be determined based on platelet count and prothrombin time (PT) and vWF level. In the present study, platelet count was below the normal range in all three groups and prothrombin time was prolonged in all the groups before treatment. Significantly decreased ($p<0.05$) PT along with decreased vWF level post therapy in Gr. III denoted minimal vascular endothelial cell damage and less chance for development of DIC. This finding indicated minimal endothelial cell injury induced by parasite and/or free radicals in the Gr. III dogs receiving combination therapy.

In conclusion, *B. gibsoni* infected dogs receiving combination therapy with Doxycycline, Clindamycin, and Metronidazole recorded significantly decreased level of prothrombin time and von willebrand factor denoting good prognosis with respect to the onset and severity of disseminated intravascular coagulopathy.

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