

Evaluation of clinical, biochemical and haematological markers in natural infection of canine monocytic ehrlichiosis

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Abstract Canine monocytic ehrlichiosis caused by *Ehrlichia canis* has gained wider significance owing to its potential to inflict significant deleterious effect on the health of companion animals. In the present study, 46 confirmed ehrlichiosis positive dogs were evaluated for the alterations in clinical, haematological and biochemical attributes. Depression, anorexia, pyrexia, anaemia, weakness, jaundice, melana, vomiting and diarrhoea were the main clinical symptoms observed. Haematological alterations included pancytopenia especially thrombocytopenia. Significant changes were noticed in WBC, RBC, Hgb, McHc, Platelets, ALT values while rest all the studied haematological and biochemical parameters showed non-significant alterations within normal range in comparison to normal healthy controls. The findings substantiate that ehrlichiosis cause significant clinical, haematological and biochemical alterations of the varied intensity in dogs, even with lower grades of parasitaemia.

Keywords Biochemico-haematological alterations · Dogs · Ehrlichiosis · Natural infection

Introduction

Ehrlichia canis the causative agent for canine monocytic ehrlichiosis (CME) is known to possess special affinity for the monocytes and macrophages. The condition is worldwide with a higher incidence rates in tropical and subtropical regions (Milanjeet et al. 2014). So far as Indian scenario is concerned, canine ehrlichiosis has been reported from different parts of the country (Harikrishnan et al. 2009; Milanjeet et al. 2014). Clinically the disease is encountered in three forms viz., acute, subclinical and chronic (Heerden 1982). The diagnosis of canine ehrlichiosis is very challenging to the clinician due to wide range of non-specific clinical symptoms along with resemblances to a number of diseases that mimics ehrlichiosis in clinical presentation. The aim of this study was systematically describe the clinical, haematological, biochemical findings of dogs naturally infected with *E. canis*, so as to develop a clear clinical pictorial model the disease presentation.

Materials and methods

Blood samples were collected from dogs already confirmed for *E. canis* infection by routine Giemsa staining technique. Briefly, 2 ml of blood was collected from each animal by cephalic vein puncture in vacutainer tubes with and without EDTA. Collected blood samples were equally used to separate out serum and whole blood for analyzing different blood parameters (Jain 1993). Complete blood count was carried out using fully automated Haematology analyser (Diatron). The serum samples were subjected to the estimation of various parameters by using Semi auto chemistry analyser (Electronics India) using commercially available

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kits (Span cogent diagnostic). The data on clinical manifestations upon clinical examination was done as per score card method described by Varshney et al. (2008) using the clinical histories filled out during the consultation, clinical examination and parasitological results. Alongside, 10 healthy dogs from a kennel were used as control for comparison of attributes.

Statistical analysis

The haemato-biochemical parameters were analyzed by independent samples *T* test using SPSS statistical software.

Results and discussion

Clinical manifestations

The dogs affected with canine ehrlichiosis showed a wide variability in clinical presentations. The main clinical signs noticed were pyrexia followed by weakness, anorexia, anaemia, epistaxis, melena, ocular signs, jaundice and yellow coloured urine (Table 1).

Haemato-biochemical alterations

The details of the blood parameters examined viz., red blood corpuscle (RBCs) count, white blood corpuscle (WBCs) count, haemoglobin (Hb), haematocrite value

Table 1 Clinical signs observed in the dogs naturally infected by CME (n = 46)

Symptoms	Animals	Percentage (%)
Pyrexia	44	95.65
Weakness	30	65.21
Ticks on body	10	21.73
Recumbancy	22	47.82
Dehydration	12	26.08
Anorexia	26	56.52
Anemia	22	47.82
Vomiting/nausea	18	39.13
Epitaxis	24	52.17
Constipation	6	13.04
Diarrhoea	20	43.47
Abdominal distension	2	4.34
Melena	10	21.73
Jaundice	10	21.73
Congested mucus membrane	8	17.39
Dull/depression	22	47.82
Urological signs (yellow coloured urine)	18	39.13
Ocular signs (eye discharge)	20	43.47

Table 2 Haematological alterations in clinical cases of CME

Attributes	Control	<i>Ehrlichia</i> infected dogs	P value
W.B.C ($\times 10^9/L$)	10.84 \pm 0.46 ^a	7.42 \pm 0.40 ^b	0.000
R.B.C ($\times 10^{12}/L$)	8.71 \pm 0.27 ^a	5.70 \pm 0.52 ^b	0.002
Hgb (g/dL)	12.96 \pm 0.20 ^a	10.64 \pm .61 ^b	0.022
HcT (%)	51.46 \pm 1.88	47.74 \pm 1.02	0.080
MCV (fl)	71.08 \pm 1.40	66.94 \pm 1.73	0.146
RDWc (%)	20.70 \pm 0.67	19.50 \pm 1.38	0.569
McHc (g/dL)	31.10 \pm 2.76 ^a	22.91 \pm 2.07 ^b	0.049
MCH (pg)	14.94 \pm 0.60	12.95 \pm 2.54	0.079
Platelets ($\times 10^3/\mu L$)	246.60 \pm 10.53 ^a	154.10 ^b \pm 5.08	0.000
Lymphocytes (%)	24.62 \pm 1.89	20.90 \pm 1.06	0.197
Monocytes (%)	10.87 \pm 0.89	9.74 \pm 1.00	0.430
Granulocytes (%)	69.36 \pm 1.95	65.26 \pm 1.01	0.058

Mean bearing different superscript differs significantly (P < 0.05)

(HCT), mean corpuscular volume (MCV), red blood cells distribution width concentration (RDWc), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet blood test (PLT) along with lymphocyte, monocyte and granulocyte count are compiled in Table 2 alongside the variability in levels ALT, AST, ALP, albumin, total protein, calcium, phosphorus, creatinine (Table 3). Significant changes were noticed in WBC, RBC, Hgb, McHc, Platelets, ALT values while rest all the parameters showed non-significant alterations within normal range in comparison to normal healthy controls.

More or less similar types of clinical manifestations have earlier been reported by several workers. The mild variations in these clinical signs could be very much attributed to a number of factors including variability in pathogenicity of various strains of *Ehrlichia*, breeds of dogs, concurrence with other diseases, immune status of dogs, etc. The most important clinical findings that may raise suspicion for ehrlichiosis included pyrexia, depression, pale mucosa, bleeding tendencies in form of epistaxis and melena. Amongst these, epistaxis may be considered as the single most finding attributing suspicion of ehrlichiosis (Troy et al. 1980). The melena and epistaxis and other bleeding tendencies could be attributed to thrombocytopenia and the deposition of immune complexes on the vascular wall (Rungsipipat et al. 2009). Amongst the main haemato-biochemical variables, significant pancytopenia, decreases in plasma proteins along with haemoglobin levels are thought to be cardinal for ehrlichiosis (de Castro et al. 2004). The alterations in the protein metabolism in liver due to tissue damage alongside bone marrow

Table 3 Biochemical alterations in clinical cases of CME

Attributes	Control	Ehrlichia affected	P values
ALT	27.68 ± 1.17 ^{ab}	45.00 ± 3.32 ^{ba}	0.003
AST	19.00 ± 1.55	23.47 ± 1.58	0.099
ALP	54.86 ± 3.54	66.53 ± 6.47	0.248
CREATININE	0.90 ± 0.09	0.73 ± 0.10	0.281
CALCIUM	7.29 ± 0.39	7.27 ± 0.34	0.971
PROTEIN	6.86 ± 0.28	6.54 ± 0.33	0.545
PHOSPHORUS	4.01 ± 0.33	3.56 ± 0.16	0.196
ALBUMIN	2.68 ± 0.24	2.15 ± 0.26	0.587

Mean bearing different superscript differs significantly (P < 0.05)

suppression may be supposed to be the contributing factor for these changes (de Castro et al. 2004). Significant decrease in the plasma protein levels and colloidal osmotic pressure subsequent to hepatic necrosis eventually accounts for oedema in CME (Rungsipipat et al. 2009). Hematological abnormalities like non regenerative anemia and thrombocytopenia alongside hyperglobulinemia, hypoalbuminemia and low albumin-globulin ratio are the other biochemical abnormalities frequently encountered in CME (Neer and Harrus 2006). Platelet consumption, increased splenic sequestration and decreased platelet lifespan are the possible attributes for thrombocytopenia (Harrus et al. 1999). Depleted synthesis of coagulation proteins in the liver due to necrosis may also cause increased platelet aggregation further aggravating the thrombocytopenia (Rungsipipat et al. 2009). Thrombocytopenia, mainly due to large-scale destruction of the cells in the spleen that begins a few days after the infection (Smith et al. 1975) alongside bone marrow hypoplasia, are also amongst the primary causes of pancytopenia including thrombocytopenia (Waner 2008).

Nonregenerative anemia, thrombocytopenia and mild leucopenia are the generally accepted three cardinal hematological attributes subsequent to acute to subacute infection of CME. The non regenerative anaemia is incriminated to bone marrow hypoplasia leading to impaired production of cellular components of blood. Many of the clinical symptoms in CME like fever are attributed to over production of interleukin-1 by the antigen-presenting cells and B cells or even because of exogenous pyrogenic products of the causative agent (de Castro et al. 2004). Again immunopathological mechanisms involving significant increase in levels of serum antiplatelet IgG resulting in the removal of antibody adsorbed thrombocytes by the mononuclear phagocyte system in the liver and spleen also contribute significantly to thrombocytopenia (Waner et al. 2001). Transient suppression of bone marrow activity along with its hypoplasticity due to accumulation

of marrow fat and subsequent decrease of erythroid cell series is the prime factor responsible for anemia and thrombocytopenia (Rungsipipat et al. 2009). Thrombocytopenia is often seen as a diagnostic identity for CME. Though a number of supporting factors are stated in literature pertaining to thrombocytopenia yet the basics lies in the increase of antiplatelet antibodies and subsequent decrease in circulating half-life of the platelets causing acute destruction of platelets accounting for the foundations of pathogenesis of thrombocytopenia. These phenomena also demonstrates the other clinical signs in CME like skin and subcutaneous hemorrhages pale visceral organs, ascites, and jaundice.

Along with these changes, generalized lymphadenopathy and splenomegaly also occurs due to proliferation of lymphocytes of medullary and paracortical region and is further aggravated by the aggregation of reactive histiocytes (Rungsipipat et al. 2009). This is histopathologically demonstrated by the presence of increased extramedullary hemopoiesis and hemosiderosis (de Castro et al. 2004; Rungsipipat et al. 2009). A linear relationship exists between the increase in titers of immunolabeled IgM and IgG coated cells and presence of anti-*E. canis* IgG antibodies in the lymph nodes (Cadman et al. 1994). All these contributing factors signify the central role of spleen and lymph nodes in the immunopathogenesis of CME. Alongside, liver is widely regarded as the target organ affected with CME as is exemplified by seen in the increase in activity of specific liver enzymes (AST, ALT, AP, bilirubin) along with histological alterations pertaining to severe diffuse centrilobular degeneration and chronic active hepatitis along with generalized hypoproteinemia, hypoalbuminemia (Rungsipipat et al. 2009). Functional loss of several of the liver functions is depicted on the host in the form of ascites, bleeding disorders, prerenal azotemia, hepatic encephalopathy, and is the major cause of death (Rungsipipat et al. 2009).

In conclusion, it can be concluded that anaemia, leucopaenia and thrombocytopenia must be included in the differential diagnosis when observed during routine laboratory evaluations for diagnosis of canine ehrlichiosis. Besides these, the clinical score card is also very important for routine and timely diagnosis of CME.

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