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Clinico-pathological and haemato-biochemical changes associated with immune mediated haemolytic anaemia in dogs

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Abstract

The present study was carried out to study the clinico-pathological changes of haemato-biochemical parameters in Immune mediated haemolytic anaemia associated with haemoparasitic infections in dogs in Aizawl, Mizoram. Immune mediated haemolytic anaemia as we know is a life-threatening condition in dog, in which the dog's own immune system attacks its own erythrocytes and cause anaemia. So, the clinico-pathological changes observe in IMHA associated haemoparasitic infections in dogs exhibited significant decrease in RBC, Hb, PCV, MCV and thrombocytes values and exhibited significant increase in WBC, Prothrombin time, clotting time, ALT, Total Bilirubin, Creatinine, Urea and globulin.

Keywords: Immune mediated haemolytic anaemia, haemoparasite, dogs

Introduction

IMHA is one of the most common autoimmune diseases diagnosed in dogs (Klotsman *et al.*, 2019) [7]. The production of antibodies specific for normal molecules on the surface of RBCs results in the development of IMHA in dogs. These antibodies lead to activation of the complement cascade, resulting in intravascular lysis of RBCs or them opsonise RBCs to enable phagocytosis by cells of the monocyte phagocyte system (MPS) in the liver and spleen. IMHA is one of the most common manifestations of canine immune-mediated disease. It is an immune system disease in which the body attacks and destroys its own RBCs. In IMHA, RBCs are still being manufactured in the bone marrow but once released into the circulation, they have a shorter-than-normal life span (Swann and Skelly, 2016) [14].

Methods and Materials

In the present study, a total of 100 dogs of either sex which was brought for treatment to the Teaching Veterinary Clinical Complex, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram and Veterinary dispensaries of Mizoram state was screened to find out the occurrence of Immune mediated haemolytic anaemia associated with haemoparasitic infections in dogs in and around Aizawl. These dogs were selected based on the symptoms like icterus, pale mucus membrane, lethargy, anorexia, fever and other symptoms associated with haemolytic anaemia. The confirmation was done based on blood smear examination, Antibody test kit, Coomb's test, haemato-biochemical screening of whole blood, serum/plasma and urinalysis for the evidence of haemolytic anaemia. The statistical data obtained during the study was analyzed as per standard statistical procedure (Snedecor and Cochran, 1995) [12].

Results

In the present study, dogs with IMHA associated with haemoparasitic infections in dogs, the values of Red Blood Cell (RBC), Haemoglobin (Hb), Packed cell volume (PCV), Mean corpuscular volume (MCV), thrombocyte ($p \leq 0.01$) showed high significant decreased and White Blood Cell, Prothrombin time, clotting time ($p \leq 0.01$) showed high significant increased as compared to the Control group (Table 1). There was no significant difference in the values of Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Lymphocytes, monocytes and granulocytes.

Table 1: Haematological and coagulation profile analysis in IMHA associated with haemoparasitic infections in dogs. Mean±SE

Parameters	Control group (n=6)	IMHA associated with haemoparasitic infected dogs (n=12)	P value
RBC (10 ⁶ /μL)	6.12±0.22	3.07±0.12	0.00**
Hb (g/dL)	12.67±0.54	5.34±0.19	0.00**
PCV (%)	39.60±1.08	17.24±0.36	0.00**
MCV (fL)	66.21±1.64	52.25±0.48	0.00**
MCHC (g/dL)	33.20±0.45	32.60±0.81	0.63 ^{NS}
MCH (pg)	21.17±1.04	17.27±1.08	0.37 ^{NS}
WBC (10 ³ /μL)	10.17±0.59	36.44±0.81	0.00**
THR (10 ³ /μL)	226.83±12.11	135.50±9.00	0.00**
LYM (%)	22.83±1.74	27.58±2.21	0.43 ^{NS}
MON (%)	6.98±0.55	6.84±0.6	0.89 ^{NS}
GRA (%)	70.25±2.13	67.12±2.08	0.36 ^{NS}
Clotting Time (Sec)	82.16±3.97	121.95±5.01	0.00**
PT (Sec)	22.50±1.59	35.43±0.58	0.00**

The Biochemical analysis revealed significant decrease in albumin ($p \leq 0.01$) and significant increase in Globulin, Total bilirubin (TB), Creatinine, Urea and Alanine aminotransferase

($p \leq 0.01$) when compared the Control group. There was no significant difference in total protein (Table 2).

Table 2: Biochemical analysis in IMHA associated with haemoparasitic infections in dogs. Mean±SE

Parameters	Control group (n=6)	IMHA associated with haemoparasitic infected dogs (n=12)	P value
Total protein (g/dL)	6.40±0.20	6.25±0.11	0.48 ^{NS}
Albumin (g/dL)	2.80±0.14	2.14±0.05	0.00**
Globulin (g/dL)	3.61±0.24	4.10±0.10	0.00**
ALT (IU/L)	49.16±4.14	167.16±9.56	0.00**
Total bilirubin (mg/dL)	0.21±0.02	1.58±0.19	0.00**
Creatinine (mg/dL)	0.76±0.11	1.26±0.05	0.00**
Urea (mg/dL)	19.95±1.19	46.24±0.83	0.00**

*Significant ($p \leq 0.05$); **Highly significant ($p \leq 0.01$); NS: Non-significant

Discussion

In the present study, dogs with IMHA associated with haemoparasitic infection exhibited significant decreased in RBC, Hb, PCV, MCV values signifying a state of severe anaemia in comparison to Control dogs (Healthy group). This could be because, IMHA is predominantly a hypersensitivity type II immune reaction in which the immune system produces auto-antibodies that bind to the host's own erythrocytes, triggering both intravascular and extravascular haemolysis as reported by Swann and Skelly (2013) [13].

Dogs with secondary IMHA also exhibited significant increase in WBC and decreased thrombocytes. The alterations in the haemogram and leucogram values in the present study were in agreement with the reports of Shah *et al.* (2009) [11], McAlees (2010) [8] and Piek *et al.* (2011) [10].

Ashwini *et al.* (2017) [2] stated that leukocytosis in IMHA might be due to tissue necrosis as a consequence of anaemic hypoxia and thromboembolic condition or might be due to inflammation, stress response, or sepsis. Archer and Mackin 2013 also described leukocytosis might be due to severe tissue injury.

Thrombocytopenia and increase in PT and clotting time was observed in the present study. Since the present study was mainly focused on secondary IMHA associated with haemoparasites, this might be one of the reasons for thrombocytopenia or due to consumptive coagulopathy as cause of thrombocytopenia as stated by Jutkowitz *et al.*, (2013) [6]. Immune mediated destruction of thrombocyte or haemorrhagic loss might also be the reason for decreased platelet count (Carr *et al.*, 2002) [4]. Weinkle *et al.* (2005) described that severe thrombocytopenia in IMHA was mainly due to DIC, failure of production, concurrent immune

mediated thrombocytopenia and splenic sequestration.

In the present study, the serum biochemical analysis revealed an increased in ALT, Total Bilirubin, Creatinine, Urea, globulin and reduction in albumin when compared to the Control group, which is in agreement with the findings of Ashwini *et al.* (2017) [2]. ALT in IMHA dogs increases due to hypoxic damage of liver as reported by Archer and Mackin (2013) [1].

Nassiri *et al.* (2005) [9] reported findings of hematological and biochemical test for IMHA in dogs, which include mean Haematocrit (21.4±1.4%), anisocytosis in 8 dogs (61%), spherocytosis in 7 dogs (54%), polychromasia in 5 dogs (38.5%), thrombocytopenia in 7 dogs (54%), hyperbilirubinemia in 8 dogs (80%) out of the 10 dogs which were evaluated. And 13 dogs that was evaluated for ALT and ALP showed increased in 6 (46.2%) and 8 (61.5%), respectively.

Balch and Mackin (2007) [3] conveyed that either haemolytic or hepatobiliary could be the reason for an elevated bilirubin level and it was common in patients with IMHA. Swann and Skelly (2013) [13] described that rise in BUN levels in dogs might be as a result of prerenal, renal or gastrointestinal cause resulting from hypoxia or thromboembolism. And rise in globulin could be due to high immune response to IMHA as stated by Ashwini *et al.* (2017) [2]. Ishihara *et al.* (2010) [5] reported that decrease in albumin concentration might be due to reduced synthesis by liver or loss through haemorrhage, as most IMHA dogs have thrombocytopenia and are hypercoagulable.

Conclusions

The present study concluded that the clinico-pathological

changes of haemato-biochemical parameters observe in IMHA associated haemoparasitic infections in dogs exhibited significant decrease in RBC, Hb, PCV, MCV, thrombocytes values and exhibited significant increase in WBC, Prothrombin time, clotting time, ALT, total bilirubin, creatinine, urea and globulin.

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Animal Ethics

The study was conducted with the approval of Institutional Animal Ethics Committee (IAEC), College of Veterinary Sciences & Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram-796015 *vide* reference number CVSC/CAU/IAEC/20-21/P-14 dated 24th September 2021.

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