

## **ALTERED BIOCHEMICAL PARAMETERS IN DOGS AFFECTED WITH IMMUNE MEDIATED HAEMOLYTIC ANAEMIA– RESEARCH ARTICLE**

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**Abstract:** Immune-mediated hemolytic anemia (IMHA) is the common immunohaematological diseases associated with production of anti-erythrocyte antibodies and complements in dogs. It causes severe anaemia, morbidity and mortality in dogs. The present study records the biochemical changes in dogs affected with IMHA. Anaemic dogs presented to Teaching Veterinary Clinical Complex Mannuthy and Kokkale, Thrissur were diagnosed with IMHA by performing saline agglutination test and direct Coombs' test. Twenty five dogs positive for IMHA were studied for biochemical changes on day of presentation. These dogs were characterized by hyperbilirubinemia and increased levels of alkaline phosphatase. The results indicate that dogs affected with IMHA have significant affection of liver which may be due to acute intravascular haemolysis and hypoxic damage.

**Keywords:** Immune mediated haemolytic anaemia; Biochemical changes; Anaemia; Dog.

### **INTRODUCTION**

Immune-mediated haemolytic anaemia (IMHA) is a common clinical disorder in dogs in which, antibodies/complement are produced against the erythrocytes. The antibodies IgG, IgM and complement C<sub>3</sub> are produced against the antigenic determinants expressed on the surface of the RBC, thus resulting in extravascular or intravascular haemolysis (Jackson and Kruth, 1985). Canine IMHA can be of two types, idiopathic / Primary ie with no specific etiology or secondary IMHA can occur as a result of various triggering factors such as infection, parasite, neoplasia, drug reaction, other autoimmune diseases and vaccination (Duval and Giger, 1996; Piek, 2011; Kidd *et al.*, 2014). The present study was carried out in order to evaluate the alterations in biochemical parameters in dogs affected with IMHA.

### **MATERIALS AND METHODS**

Dogs presented to the Teaching Veterinary Clinical Complex Mannuthy and University Veterinary hospital Kokkalai with pale / icterus mucous membrane were screened by saline agglutination test for autoagglutination. Blood samples giving positive result for saline

agglutination test were confirmed with direct Coombs' test for immune mediated haemolytic anaemia (Caviezel *et al.*, 2014). Twenty five dogs positive for saline agglutination test and direct Coombs' test were selected for the study. A drop of anti-coagulated whole blood was placed on clean grease free slide and equal quantity of normal saline was added to it and mixed. The slide was evaluated for the presence of macroscopic and microscopic agglutination (Balch and Mackin, 2007 and Caviezel *et al.*, 2014). While microtiter method of direct antiglobulin /Coombs' test was employed as described by Overman *et al.*, 2007. Blood smear, buffy coat smear, wet film examination, faecal examination, microscopic agglutination test, ultrasonography, radiography were carried to determine the possible causes of IMHA.

Biochemical parameters such as total protein, albumin, globulin, albumin-globulin ratio, blood urea nitrogen, creatinine, alkaline phosphatase, alanine amino transferase, total bilirubin and direct bilirubin were estimated with biochemical analyzer using standard diagnostic kits manufactured by Erba and semiautomatic blood analyser Erba Mannheim Chem-5 plus V2, Transasia Biomedicals Ltd, Mumbai. The mean values were compared with the normal reference range on the day of admission.

## RESULTS

Among twenty five dogs diagnosed for IMHA, majority were secondary IMHA (76 per cent) caused by various haemoparasites such as *Babesiacanis*, *Ehrlichia canis*, *Babesiagibsoni* *Mycoplasma haemocanis* and sheathed microfilaria. Faecal examination and MAT was found to be negative. Twenty four per cent of dogs no specific etiology could be determined.

The mean serum total protein, albumin, globulin and A/G ratio in the serum of IMHA dogs were  $7.82 \pm 0.43$  g/dl,  $3.02 \pm 0.27$  g/dl,  $4.83 \pm 0.46$  g/dl,  $2.05 \pm 1.45$  respectively, these values were within the reference range. The mean values of total bilirubin, direct bilirubin and indirect bilirubin were  $9.37 \pm 3.43$ ,  $1.06 \pm 0.21$  and  $7.27 \pm 0.63$  mg/dl respectively which is above the reference range indicating a state of hyperbilirubinemia. The ALT and ALP levels on the 0th day were  $56.39 \pm 20.73$  U/L and  $262.19 \pm 80.11$  U/L respectively. The ALP value was higher than the normal reference value. While the mean BUN and creatinine values were within the normal range i.e.  $26.6 \pm 3.71$  and  $1.08 \pm 0.12$  mg/dl respectively.

## DISCUSSION

In the present study the protein parameters on an average were within the normal range. But one dog had hypoproteinemia, 5 dogs had hypoalbuminemia and 9 dogs had

hyperglobulinemia. Decrease in albumin concentration, might be due to acute phase reaction or decreased production by impaired liver as stated by Carr *et al.* (2002). Similar finding of hypoalbuminemia and hypoproteinemia was recorded by Ishihara *et al.* (2010) which was attributed to decreased synthesis by liver or loss through haemorrhage, as most IMHA dogs have thrombocytopenia and are hypercoagulable. The high levels of globulin in some cases might be due to high immune response seen in IMHA.

A state of hyperbilirubinemia was seen in the IMHA dogs on the day of presentation. This might be due to acute intravascular haemolysis, severe anaemia leading to hypoxic damage of liver and thus improper bilirubin conjugation and excretion (Klag *et al.*, 1993). Increase in serum bilirubin levels were also observed by Burgess *et al.* (2000), Carr *et al.* (2002). Both unconjugated and conjugated levels were high which indicated a severe affection of liver. In severe haemolysis there is an overload of unconjugated bilirubin on liver, in turn increased levels in circulation causes haemoglobinemia and increased excretion in urine. Thus signs such as icterus and haemoglobinuria are observed in IMHA (Giger, 2005). Also hyperbilirubinemia is considered as a negative prognostic indicator of IMHA (Klag *et al.*, 1993 and Swann and Skelly, 2013)

The ALP was above the reference range on the day of presentation but hepatic enzyme ALT was within the normal range in the present study. But elevation of both these levels were recorded by Burgess *et al.* (2000) indicating a liver dysfunction due to hypoxia resulting from severe anaemia. Unlike ALP which is present on the bile canalicular surface, ALT is present in cytoplasm of hepatocyte. Being a leakage enzyme damage of hepatocytes by infectious disease, inflammation, or necrosis causes release of it into the serum (Kaneko *et al.*, 2008), which are seen in IMHA. The serum creatinine and BUN on the day of presentation was within the normal range in the current study. An increase in BUN levels were observed by Swann and Skelly (2013) which might be due to pre-renal, renal or gastrointestinal origin resulting due to hypoxia or thromboembolism.

### CONCLUSION

Alteration in the biochemical parameters in IMHA dogs are discussed which evinced increased bilirubin and ALP levels in the serum. Results suggest that estimation of biochemical parameters holds importance in understanding the severity of the condition.

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**Table 1. Changes in biochemical parameters noticed on the day of presentation in dogs affected with IMHA**

Variables	Normal range	Values on the day of presentation
Total protein (g/dl)	5.4 - 7.1	7.82 ± 0.43
Albumin (g/dl)	2.6 - 3.3	3.02 ± 0.27
Globulin (g/dl)	2.7 - 4.4	4.83 ± 0.46
A:G	0.8 – 2.0	2.05 ± 1.45
Total bilirubin (mg/dl)	0.10 - 0.5	9.37 ± 3.43
Direct bilirubin (mg/dl)	0.06 - 0.12	1.06 ± 0.21
Indirect bilirubin (mg/dl)	0.01 - 0.49	7.27 ± 0.63
ALT (U/L)	21- 102	56.39 ± 20.73
ALP (U/L)	23 - 66	262.19 ± 80.11
BUN (mg/dl)	10 - 28	26.6 ± 3.71
Creatinine (mg/dl)	0.5 – 1.5	1.08 ± 0.12

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