Low haemoglobin density as an anaemic indicator in canine babesiosis

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ABSTRACT

Low Hemoglobin Density (LHD) is related to functional iron availability and hemoglobin content. To assess the use of LHD as an anaemic indicator in canine babesiosis, 108 dogs with symptoms of canine babesiosis were selected and screened for disease by polymerase chain reaction. Blood samples were collected for estimating haematological variables, serum iron, TIBC, UIBC and LHD. Based on the haemoglobin, PCV and erythrocyte count the animals were grouped into anaemic and non-anaemic populations. LHD% was higher in the anaemic population (P<0.001) and it was negatively correlated to haemoglobin, packed cell volume, erythrocytes, MCHC and serum iron. LHD% was highly depend on serum iron (P<0.001). Receiver operating characteristics (ROC) curve analysis revealed LHD was 100.00% specific and 80.43% sensitive in identifying the functional iron deficient anaemia with cut-off value of 1.83%. This study reveals that LHD is a cost-effective parameter for identifying the functional iron deficient anaemia in canine babeisosis.

Key Words: Anaemia, Canine babesiosis, Iron deficiency, LHD.

INTRODUCTION

Anaemia is an imperative clinical sign of many systemic, infectious and parasitic diseases. Evaluating the cause and type of anaemia will provide adequate information about patho-physiology of diseases (Aird, 2000; Morrison, 2005). Complete blood count, haemoglobin level, packed cell volume, and erythrocyte indices were used for diagnosing anaemia (Vieth and Lane, 2017). Apart from these normal haematological parameters estimation of micro-minerals, like iron can help in diagnosing different types of anaemia such as iron deficiency anaemia, anaemia due to chronic diseases, anaemia of inflammatory disease and iron overload anaemia (Lieu *et al*., 2001; McCown and Specht, 2011). It also aids in characterising the anaemia into regenerative or non- regenerative type (Mitchell and Kruth, 2010). Serum iron, serum total iron binding capacity (TIBC), serum ferritin and per-cent transferrin saturation (%TSAT) were normally used parameters to measure the iron status of animals (Wians *et al.,* 2001). Among these parameters serum ferritin concentration has limited use in veterinary practice because of its species-specificity and limited assay availability (Schaefer and Stokol, 2015).

Currently available iron tests were influenced by acute phase response and are also expensive (Bovy *et al*., 2007; Brugnara, 2003; Coyne, 2006; Mast *et al*.,

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2002). To evade these problems, Urrechaga (2010) proposed a parameter, Low hemoglobin Density (LHD %) derived from the mathematical sigmoid transformation of mean cell hemoglobin concentration (MCHC). MCHC is a traditional estimator of iron availability preceding 90–120 days. As like MCHC, LDH is linked to iron availability and haemoglobin content of erythrocyte. The clinical reliability of LHD as an iron availability marker for erythropoiesis have been documented early (Urrechaga *et al.,* 2010; Urrechaga *et al.,* 2012).

Canine babesiosis is a hemoprotozoan parasitic disease caused by apicomplexan parasites of the genera Babesia, which is characterized by haemolyticanaemia, jaundice, lethargy, pyrexia and haemoglobinurea (Solano-Gallego and Baneth, 2011). *Babesia gibsoni* (small form) and *Babesia canis* (large form) are the two important species reported in natural infections in dogs in India. The clinical presentation is diverse and ranges from transient anorexia to a complex syndrome in which multiple organ systems are affected. Anaemia and thrombocytopenia are the most common haematological abnormalities observed in canine babesiosis. Initially, anaemia will be mild, normocytic and normochromic, as the disease progresses it becomes macrocytic, hypochromic and regenerative (Lobetti, 2003).Even though anaemia is an important clinical sign of babeisosis, some of the infected dogs did not show the clinical signs of anaemia and also noalterations in their haematological parameters like erythrocyte count, haemoglobin level and erythrocyte indices (Zamokas *et al.,* 2014). Considering these facts we aimed to identify the changes in LHD among babesia infected dogs (anaemic and non-anaemic groups) and also to know about the ability of LHD in diagnosinganaemia in these dogs.

MATERIALS AND METHODS

A total of 108 dogs (47 males and 61 females), aged 2 months to 13 years, brought to Madras Veterinary College Teaching Hospital and Blue Cross of India, Chennai with clinical signs such as pyrexia coupled with haemoglobinuria, tick infestation, lymphadenopathy and lethargy were included in the study group. PCR was done to confirm the presence of *Babesia*infection. Blood samples were collected from all animals on the first day of presentation. Based on the traditional anaemic markers levels (Haemoglobin, PCV, erythrocytes), the affected animals were grouped into anaemic (n = 58, Male - 25, Female – 33) and non-anaemic group (n = 50, Male - 22, Female – 28). The haematological parameters like Haemoglobin (Hb) by acid hematin method, packed cell volume (PCV) by microhematocrit method, erythrocyte count (TEC) by hemocytometer method were recorded and erythrocyte indices calculation were done as per the method given by Coles (1986). Serum iron, transferrin iron binding capacity (TIBC) and unsaturated iron binding capacity (UIBC) were estimated from all the samples by Ferrozine method using colorimetric kit supplied by Coral clinical systems, Goa, India- 403202. LHD% was calculated from the MCHC values using the formula described by Urrechaga (2010).

$$LHD\% = 100 * \sqrt{1 - (1/(1 + e^{1.8(30-MCHC)}))}$$

Unpaired Student'st-test was used to identify the statistical difference of estimated parameters between these groups. Relationship between LHD and other parameters was estimated by Pearson correlation. Linear regression model was used to identify the dependency between LDH and serum iron. Receiver operating characteristics (ROC) curve analysis was used to identify the anaemic diagnostic cut-off value, sensitivity, specificity, area under curve (AUC) and Youden's index of LHD. All analyses were performed using SPSS IBM Version 23 software.

RESULTS AND DISCUSSION

Serum iron was significantly reduced and LHD, TIBC and UIBC were significantly ($P<0.001$) elevated in the infected dogs with anaemia (Fig. 1). In the current study, reduction in the serum iron concentration of anaemic population indicates the ineffective erythropoiesis leading to anaemia which was evidenced as decreased haemoglobin and erythrocytes count. Because during iron deficiency, the impairment in the haemoglobin synthesis causes reduction in circulating erythrocytes level and they will be hypochromic in nature (Brugnara, 2003). LHD is a marker for hypochromasia, which reflects the period of iron deficiency during erythropoiesis (Urrechaga, 2016). Elevation of LHD % in the anaemic group also reveals the on-going ineffective erythropoiesis which accelerates the prevailing anaemic condition. Increase in the transferrin iron binding capacity of the anaemic group indicates the effective binding capacity of transferrin molecule to increase the utilization of limited functional iron available in the circulation. Considering these variations in the anaemic and non-anaemic group of babeiosis dogs, LHD can also indicate the anaemic status as like other standard anaemic parameters like haemoglobin, erythrocyte and packed cell volume.

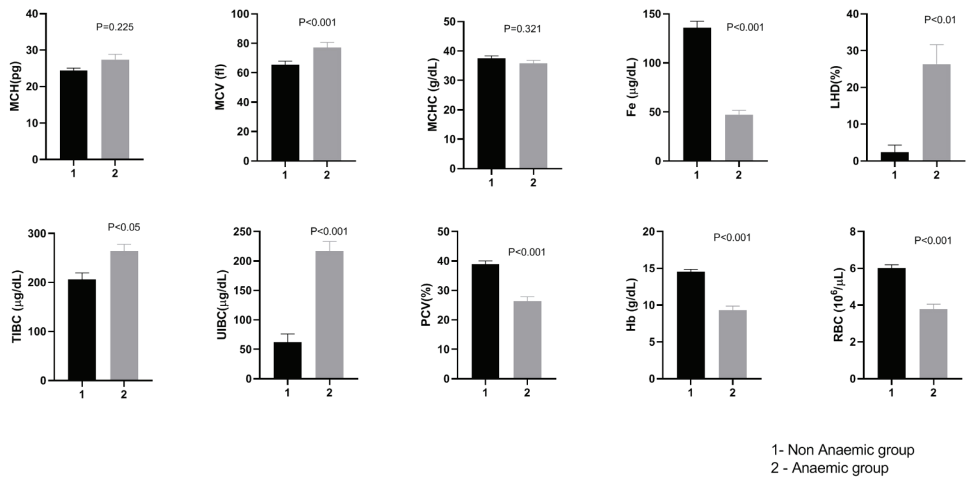

Figure 1: Results of unpaired t test for different parameters analysed between anaemic and non-anaemic groups of dogs with babesiosis.

Pearson correlation between LHD and other standard anaemic parameters revealed the significant (P<0.01) negative relationship of LHD to haemoglobin, packed cell volume, erythrocytes, MCHC and serum iron (Fig. 2). This indicates the increase in LHD will cause decrease in haemoglobin, packed cell volume, erythrocytes, MCHC and serum iron. Reduction in functional iron reserve for erythropoiesis may be a reason for this negative relationship between LHD and other anaemic parameters. Hence, evaluation of the LHD will be an added advantage in diagnosing different types of anaemia. In canine babesiosis, the serum iron level is not a static one and its concentration differs according the severity of anaemia (Lobetti, 2003). Thus, LHD evaluation along with other anaemic parameters will provide information about the functional iron reserve of the affected animal.

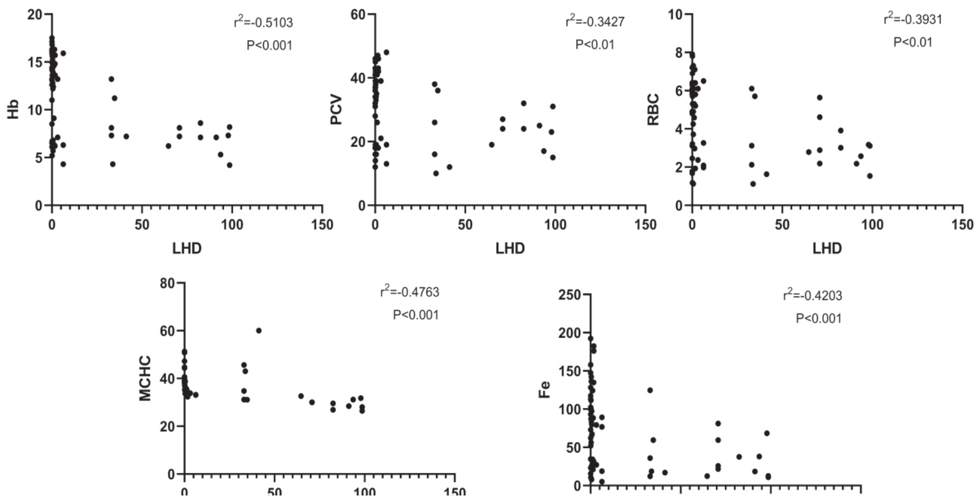

Figure 2: Results of Pearson correlation of LDH to other parameters analysed in the study.

Linear regression analysis revealed significantly (P<0.001) high dependency between LHD and serum iron (Fig. 3). This provides information for predicting the functional serum iron level by simple mathematical sigmoid transformation of MCHC. Currently available iron tests are influenced by acute phase response, expensive, species specific and limited in availability; which makes them ineffective in veterinary practice (Schaefer and Stokol, 2015). Use of this LHD % as an indicator of iron status in different clinical conditions can evade the above said problems.

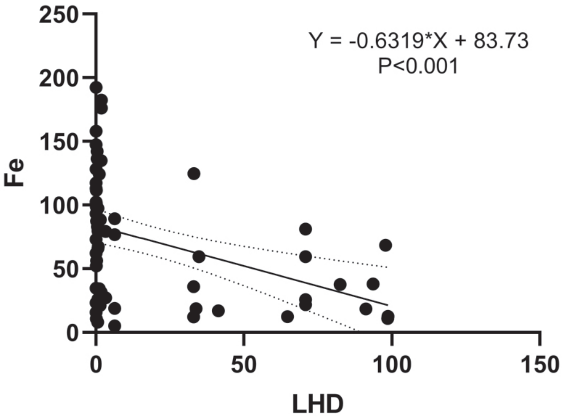

Figure 3: Linear regression model indicating the relationship between LHD and serum iron concentration.

Receiver operating characteristics (ROC) curve analysis identified the anaemic diagnostic cut-off value as >1.83% at the confidence interval of 84.3 and 98.2% (P<0.001) with 100.00% specificity and 80.43% sensitivity. Area under curve (AUC) of LHD was about 0.935 and the Youden's index was 0.804 (Fig. 4). This was similar to diagnostic cut-off value of LHD% for iron status indication in humans (Urrechaga *et al.*, 2010). In the current study, LHD was more specific in identifying the healthy population without anaemia and also it indicates that the increase in the LHD% more than the cut-off range will predispose the animal to functional iron deficient anaemia. Previous reports in humans also suggests that the LHD can be used as an indicator for the functional iron status and it can be used in the early investigation of anaemia(Urrechaga *et al.,* 2010; Urrechaga *et al*., 2012; Urrechaga, 2016).

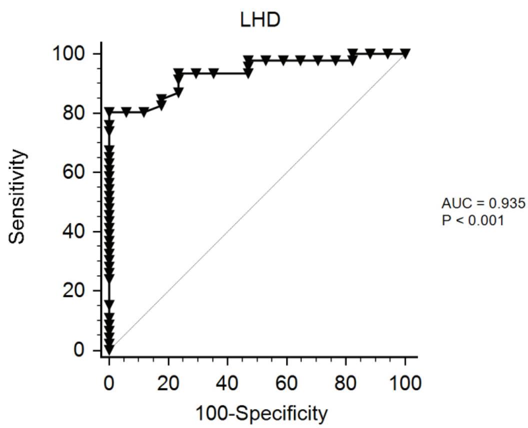

Figure 4: Receiver operating characteristics (ROC) curve analysis with area under curve for identifying the diagnostic sensitivity and specificity of LHD.

In the current study it was identified that use of LHD in conjunction with standard blood cell counts and iron parameters enable the more rapid and accurate diagnosis of anaemia in the babesiosis affected animals. Cost- effectiveness of LHD than other iron status indicator tests normally employed in veterinary practice is an added advantage to this parameter. First-hand information obtained from this parameter will be extremely useful to the veterinarians in diagnosis and monitoring the treatment response when the LHD% is above 1.83 %. Since this is a first study to evaluate the use of LHD as anaemic indicator in veterinary clinical medicine, still more researches are warranted to support this finding in different clinical conditions.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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