

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.708.508>**Haematobiochemical and Mineral Level Changes in Microfilaraemic Dogs**

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ABSTRACT**Keywords**

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In the present study, a total of 33 clinical cases of microfilaraemic dogs various regions of Gujarat state were assessed for alterations in haemato-biochemical and mineral level changes. Microfilariae were detected using modified Knott technique and microfilariae of *Dipetalonema (Acanthocheilonema) reconditum* was more common (23 cases; 69.69%) than *Dirofilaria immitis* (10 cases; 30.30%). Apparently healthy asymptomatic ten dogs were kept as control. The haematological findings for different circulating microfilariae in the present study indicated highly significant ($p \leq 0.01$) decrease in Hb concentration and increase in lymphocyte percentage in *Dirofilaria immitis* group. Highly significant ($p \leq 0.01$) decrease in platelet count and monocyte percentage and, increase in eosinophil count was observed in both the groups as compared to control. Highly significant ($p \leq 0.01$) increase in total protein (TP) and globulin concentration in both the groups was reported as compared to control. Mineral estimation indicated a significant ($p \leq 0.01$) decrease in zinc (Zn) and increase in calcium (Ca) concentration in dogs suffering from *Dipetalonema reconditum*.

Introduction

Filariasis is one of the most important life threatening parasitic disease affecting canines having worldwide distribution. In India, presence of *Dirofilaria immitis*, *Acanthocheilonema reconditum*, *Acanthocheilonema dracunculoides*, *Brugia malayi*, *Brugia ceylonensis* and *Brugia pahangi* have been reported in canines (Ananda *et al.*,

2006). Amongst them *D. immitis* commonly referred to as heart worm of dogs is most pathogenic, having zoonotic potential too (Miyoshi *et al.*, 2006). Adult worms are usually located in the right ventricle and pulmonary arteries (Quinn *et al.*, 1998) leading to right ventricular hypertrophy (McCall *et al.*, 2008; Seiler *et al.*, 2010). Clinical manifestations of canine heartworm disease include cough, dyspnoea, weight loss,

poor exercise tolerance, weakness, haemoptysis, cyanosis and congestive heart failure (Kramer, 2009; Cardoso *et al.*, 2012). Depending upon the worm load, *in situ* transfer of nutrients from the tissues of the animal body to the parasites contributes to deficiency of organic and inorganic nutrients (Sharma and Pachauri, 1982; Sharma *et al.*, 1985). The present study deals with the haemato-biochemical alterations and mineral level changes in dogs suffering from filariasis. Haemato-biochemical abnormalities are valuable in evaluating the progression of the disease in affected dogs.

Materials and Methods

A total of 418 clinical cases of dogs from the area of North Gujarat (TVCC, Deesa), South Gujarat (Nandini Veterinary Hospital, Surat) and Central Gujarat (private clinics, Ahmedabad) during July 2016 to May 2017 formed the basis of the research material.

Modified Knott Technique (MKT)

All the 418 clinical cases of dogs suspected for parasitemia were subjected to modified Knott's technique (MKT) as per standard procedures to detect circulating microfilariae (Castillo and Guerrero, 2006).

Haemato-biochemical analysis

Blood samples were collected from 33 dogs found positive for circulating microfilariae through MKT. Haematological analysis was performed from K₃ EDTA whole blood using blood auto analyzer (Exigo EOS, Sweden). The hematological parameters included total erythrocyte count (TEC), total leukocyte count (TLC), Hemoglobin (Hb), hematocrit (HCT), platelets, eosinophils, monocyte, granulocyte and lymphocyte. From the serum sample, total protein (TP), albumin, globulin, aspartate aminotransferase (AST), alanine

aminotransferase (ALT), total cholesterol, calcium (Ca), magnesium (Mg) and zinc (Zn) were estimated according to manufacturer instructions (Agappe Diagnostics LTD, Ernakulam, Kerala, India) using auto analyzer (Randox Monaco, UK).

Estimation of Zinc

Estimation of Zn was done using (Atomic Absorption Spectroscopy (AAS). Serum samples were digested as per procedure (Devi *et al.*, 2017). For that an aliquot of 1 ml of serum was mixed with 10 ml of triple acid mixture (Perchloric acid: Sulphuric acid: Nitric acid= 1:2:4) and the samples were kept overnight at room temperature before digestion on low heat (70-80°C) using digestion bench. Digestion was carried out until the volume of samples was reduced to about 1 ml or till the digested samples became watery clear and emits white fumes. The process was repeated for several times to get desired level of digestion and the volume of digested materials was reduced to approximately 0.5 ml with slow heating. Final last volume was adjusted to 10 ml with triple distilled deionized water.

Statistical analysis

The obtained data in this study was computed for mean values of different parameters studies and statistically analyzed by one way ANOVA Test using SPSS software (version 23.0).

Results and Discussion

The haematological changes recorded in the present study are given in Table 1. The present study indicated highly significant ($p \leq 0.01$) decrease in Hb concentration and, increase in lymphocyte percentage in *D. immitis* group and similar non significant changes were recorded in *D. reconditum* group. The

recorded reduction in the level of hemoglobin might be due to mechanical disruption of red blood cells due to presence of large numbers of adult heartworms in the right atrium and venae cavae, obstructing blood flow, causing intravascular haemolysis which further leads to decrease in the TEC, PCV, decreased erythrocytes fragility and platelets count as well. A previous hematological study in heart worm infected dogs also revealed low Hb concentration, increased erythrocyte

sedimentation rate (ESR) and lymphocyte percentage (Sharma and Pachauri, 1982). Lymphocytosis is an indicative of chronic infestation with the filarial worm.

Highly significant ($p \leq 0.01$) decrease in platelet count and monocyte percentage and, increase in eosinophil count was observed in both the groups as compared to control. The rise in the eosinophil count is indicative of body defensive response to parasitaemia.

Table.1 Haematological Values of Microfilaraemic and Healthy Dogs (Mean±SE)

Parameter	<i>Dirofilaria immitis</i> (n=10)	<i>Dipetalonema reconditum</i> (n=23)	Control (n=10)
RBC ($10^6/\mu\text{l}$)	6.54±0.39	6.53±0.33	6.79±.39
WBC ($10^3/\mu\text{l}$)	11.76±2.07	13.36±1.14	11.91±0.53
Haemoglobin (gm/dl)	10.46±0.82**	14.67±0.83	15.52±0.82
HCT (%)	43.45±3.21	44.03±2.35	48.47±1.90
Platelets ($10^3/\mu\text{l}$)	254.70±52.63**	219.60±24.14**	450.20±29.39
Eosinophils (%)	11.38±1.09**	9.14±0.79**	5.81±0.65
Monocyte (%)	3.21±0.19**	3.06±0.24**	5.96±0.84
Granulocyte (%)	69.85±3.96	75.71±2.64	70.76±3.44
Lymphocyte (%)	22.20±3.21**	16.76±2.31	10.01±0.71

* $P \leq 0.05$ Significant ** $P \leq 0.01$ Highly significant

Table.2 Serum Biochemical Values of Microfilaraemic and Healthy Dogs (Mean±SE)

Parameter	<i>Dirofilaria immitis</i> (n=10)	<i>Dipetalonema reconditum</i> (n=23)	Control (n=10)
ALT (U/L)	52.94±10.48	56.04±4.08	66.42±10.16
AST (U/L)	44.54±11.59	60.37±6.66	50.09±8.89
Total Protein (g/dl)	14.10±0.57**	13.39±0.66**	6.52±0.26
Albumin (g/dl)	2.62±0.11	2.67±0.10	2.46±0.15
Globulin (g/dl)	11.48±0.64**	10.72±0.59**	4.16±0.29
Total Cholesterol (mg/dl)	167.59±24.93	206.63±10.11	205.63±11.06
Ca (mg/dl)	8.47±0.40	9.57±0.31**	8.00±0.60
Mg (mg/dl)	1.82±0.09	2.15±0.10	2.40±0.29
Zn ($\mu\text{g}/\text{dl}$)	7.06±5.55	3.85±1.67*	10.97±3.09

* $P \leq 0.05$ Significant ** $P \leq 0.01$ Highly significant

In canine dirofilariasis values of ESR, reticulocyte, thrombocyte, total and differential leucocytic counts were significantly increased (Hashem and Badawy, 2008). Low grade non-regenerative anaemia (10%-60% cases), neutrophilia (20%-80% cases), eosinophilia (85% of cases) and basophilia (60% of cases) are among the important haematological abnormalities reported in the dogs suffering from dirofilariasis (Calvert *et al.*, 1999; Schaer, 2003). However, monocytosis is the inconsistent findings in dogs infected with *D. immitis* (Nelson and Couto, 2003). Mechanical destruction of RBCs causes anemia, hemoglobulinemia, and hemoglobinuria (Sodicof, 1995).

The biochemical parameter for two different circulating microfilariae in the present study (Table 2) indicated highly significant ($p \leq 0.01$) increase in the mean value of TP and globulin in both the groups as compared to control. Hypoproteinemia might be attributed to increase in the globulin concentration as a defensive response of animal body to parasitaemia and release of hemoglobin from destructed erythrocytes (Moustafa *et al.*, 1991; Schaer, 2003). Increased enzymatic activity along with hypergammaglobinaemia and hypoalbuminaemia was recorded in dogs suffering from dirofilariasis (Quinn *et al.*, 1998). Increased serum protein level as evidenced in the present investigation also has been reported earlier (Moustafa *et al.*, 1991; Hashem and Badawy, 2008). The observed hypoproteinemia could be attributed to either increased gamma globulin concentration (Schaer, 2003) in response to parasitic antigen or release of hemoglobin from the fragile RBC's and the later was further confirmed by an increase in MCV and decrease in Hb level. Non significant increase in total cholesterol concentration was recorded in both the groups in the present study. Heartworms accumulate in the right atrium or tricuspid valve area, and

or posterior vena cava increases venous pressure in the liver, causing damage to the liver parenchyma (Jagannath and Ananda, 2001). Liver dysfunction causes a decreased esterification of serum cholesterol, thus increases cholesterol content of the red blood cell membranes resulting in red blood cell fragility.

Serum mineral estimation recorded significant ($p \leq 0.01$) decrease in Zn and increase in Ca concentration in *D. reconditum* group whereas similar non significant changes were recorded in *D. immitis* group. It might be due to presence of worms in animal body causing reduction in food intake by the dog which further leads to imbalance of different minerals in the animal body. A significant decrease in the serum level of copper, iron and zinc was observed in the animal infected with the microfilaria (Sharma and Pachauri, 1982). Calcium a macro mineral and is supplied from different storage pools of the body into blood circulation, hence its level is difficult to be found deficient in blood.

It may be concluded that hemato-biochemical alterations and mineral level changes in dogs infected with filariasis are useful in assessing the disturbances in hemodynamics of the affected dogs.

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