

Study the effects of naturally acquired canine *Dirofilariasis* on some hematological and biochemical parameters

Amal Hassen Atyha and Donia A.A. Alani

Institute of Medical Technology, Baghdad-MTU, Iraq.

E-mail: amalhussen@yahoo.com

Received: 1/12/2015

Accepted: 7/6/2016

Summary

The present study was aimed to check the amount of variation in some hematologic and biochemical parameters accompanied with natural acquired canine *dirofilariasis*. Blood samples were collected from sixty five stray dogs (5-10) years old belonging to local breed dog in the villages of Al-Hindya area/ Karbala Governorate. The affected animal showed differences in hematological and biochemical values as compared with reference ranges. In conclusion, the disease showed no significant clinical signs although many pathological changes in some blood constituent with serum biochemical parameters were observed, before euthanasia for dogs.

Keywords: Canine *dirofilariasis*, Hematological, Biochemical parameters.

Introduction

Cardiopulmonary *dirofilariasis* is an important disease not because it is the task of veterinary diseases, but it has been classified as an emerging zoonosis disease in many countries of the world (1 and 2). Several Veterinary practitioners believed that heartworm infection could become endemic in their non-endemic area within the next 10 year (3). The reservoirs of heartworm are mostly canids. However heartworms may infect more than 30 species of animals. Heartworm disease is a vector-borne transmitted disease, mosquitoes can transmit heartworm disease. Approximately 70 species of mosquitoes are capable to transmitting the *D. immitis* (4). Their distribution have undergone upon the world climate change (5). The severity of disease in the dog is mainly a reflection of the number of adult heartworm present, the duration of the infection and the level of activity of the dog. Heartworm disease can cause serious damage to the heart and lungs (6). The most common early pathological changes are due to inflammatory processes that occur in the right ventricular of the heart and the arteries of the lower portion of the lungs, also the lesion is secondary commonly occur in association dysfunction of the liver and kidneys (7). The parasitic infestation lead to the occurrence of anemia, where there is a lack of blood hemoglobin either due to insufficient number of red blood cells, or lack of their hemoglobin content or both. Leukocytosis has been noted in dogs with

dirofilariasis (8). Also it was found that the most common serum biochemical abnormalities in microfilaremic dog an increased of: alkaline phosphatase, alanine and asparatate aminotransferases with creatinine and urea concentrations were elevated in infected dogs (9 and 10). Previous studies revealed variant results among seroepidemiology, morphology and the nephropathy in natural infected dog *dirofilariasis*, as well as histological changes of affected organs (11). The present study had been done to focus the light on the changes in some blood parameters and biochemical values associated with canine *dirofilariasis* in dogs.

Materials and Methods

I would like to note that the current study depended on blood samples that were collected in our previous study, PhD thesis (12). It studied sixty-five blood samples collected from stray dogs of Iraqi local breed at 5 to 10 years old, which were euthanasia during the period from April 2008 to March 2010, in the villages of Al-Hindya area/ Karbala Governorate. After, it studied the natural infection in dogs and then isolated heart worm and their numbers ranged between 4-31 worms per dog. Series studies have been done to clarify some aspects of the heartworm. The current work deals with pathological changes in some hematological and biochemical criteria after natural infection with *dirofilariasis*. The tests were carried out in the

laboratory units of Al-Hussein hospital. Whole blood tested was drawn from the dogs then stabilized so as not to clot, and were kept on ice in cool containers to avoid denaturation of proteins, and were taken to the laboratory, stained of the blood smear with Leishman stain and microscopically examined. A blood smear is often used to describe many disorders affecting number and type of blood cells such as anemia (13). After, the collected blood was divided into two parts in two tubes one of which was heparinized tube, used for estimation of some hematological parameters. The other sample was centrifuged to separate serum and then stored in the refrigerator before use. The collected whole blood samples were examined for total and differential WBCs count and RBCs counts by using Neubauer-haemocytometer; the microhematocrite method was used for packing cell volume PCV determination. The hemoglobin concentration Hb was measured by Sahli's method (14). Sera were separated from the second tube and stored at -20 °C until serum biochemical analysis, which involved alanine amino-transferase (ALT), aspartate aminotransferase (AST), creatinine and urea according to the method as described by (15 and 16). Statistical package of social science version 10.2 used to carry out statistical analyses for interpret the results if significant or none.

Results and Discussion

In this study no obvious clinical signs of the disease were observed in natural infected dogs. Although some dogs suffered from emaciation and general weakness. Sixty four percent of the carrier's dogs *Dirofilaria immitis* were without symptoms and the pathological changes might precede the onset of clinical symptoms of the disease (17-19). However, signs were mostly associated with the presence of adult worms, the duration of infection, and the host immune status. Also the severity of the signs is often related to the dog's activity level. Active dogs (such as hunters and performers) will typically show more dramatic signs of infection than will less active dogs. Even though they may have many worms, sedentary dogs may show few or no signs (20). The mean values of the investigated blood are presented in (Table, 1) showed low

of RBCs count, Hb concentration and PCV with significant difference $P < 0.1$. Twenty six (40 %) of infected dogs showed anemia, the origin of this anemia is not completely elucidated. Proposed mechanisms include hemolysis RBCs as a result of destructive motility of microfilaria as reported by (21) that showed a severe intravascular hemolytic anemia with a significant reduction of RBCs count and Hb concentration in dogs with dirofilariasis hemoglobinuria. The significant macrocytic anemia in dogs infested with three different microfilariae: *Dirofilaria immitis*, *D. reconditum*, and the third (mf3) were not identified (22). Mild to moderate anemia and decrease in sedimentation velocity were established in dogs (9).

Table, 1: Hematological and biochemical parameters of dogs infected with dirofilariasis.

Parameter/ unit	Mean ± SD	Observation range	Reference*
RBC 10^6 cells/µl	4.76 ±4.8	4.1-6.5	5.5-8.5
Hemoglobin g/dl	10.24 ±1.26	8-12.6	12.0-18.0
Packed cell volume%	32.96 ±7.71	27-39	37.0-55.0
White blood cell $\times 10^3$ cell/µl	15.73 ±14.76	5-20	6.0-17.0
Eosinophil %	3.82 ±5.02	0-9	2-10
Urea mg/dl	31.55 ±56.59	13.8-62.1	7-27
Creatinine mg/dl	1.43 ±0.27	0.5-2.8	0.4-1.8
Alanine amino transferase (GPT) IU/L	70.19 ±363.72	30-108	5-60
Aspartate amino transferase (GOT) IU/L	69.38 ±660.88	14.7-118	5-55

* Tilley and Smith 2000 (23).

These figures RBCs count (4.1-6.5), were lower than those reported by the previous studies (5.4 – 7.8), in America (8) and from reference values (23). The present data varied within the average of RBCs count 3.4 ± 1.12 in Egypt and 6.14 in Iran reported by (24 and 25) respectively. In accordance to these findings (9) studying four dogs were indicated to have been infected with *Dirofilaria immitis* by using modified Knott's method, from Turkey found mild to moderate anemia and decrease in sedimentation velocity were established in

dogs. If compare the figures of Hb concentration (10.24 ± 1.26) and PCV (32.96 ± 7.71) in present study, with another researchers (24 and 25) respectively. It observed lower than the average of hemoglobin 9.5 ± 1.12 and packed cell volume 28.0 ± 1.12 , and 18 ± 1.58 and 47 ± 2.07 respectively. The lowest RBC values were observed in natural infected dogs, suggesting the intravascular decomposition and increased fragility of the red blood cells in the blood circulatory system due to presence of large number of adult worms lodges in the blood vessels during the period of the infection. Immunity has no important role in pathogenesis of anemia in dogs with this disease (23). Elevated value of leukocytes occur in certain infected cases where the upper limit of observational range higher than normal value range (6-17) reported by (8 and 23), (Table, 1), with no statistically significant difference, also more than mean 6.10 ± 1.91 (25), but less than 16.6 ± 1.12 (24), also differ from 22.7, 9.4, 19.2 and 24.3 for four hospitalized dogs (9). The normal eosinophil count in infected animals in this study may be related to the number of infective larval stage injected by infective mosquitoes during bites in dogs. An eosinophil play a role in immune system which is surround a parasite and can consume substances related to infection with a parasite (26). But eosinophilia is not frequent in pulmonary dirofilariasis (27). On the other hand (28) suggested that eosinophilia surges as the fifth larval stage in the pulmonary arteries, it occurs more consistently in infections caused by metazoal parasites, such as fleas, round worm, hookworm, heartworm and lungworm. The major hematological finding have been reported in naturally microfilaremic dogs were a mild to moderate anemia, mild to severe thrombocytopenia, marked leukocyte-osis, moderate to marked neutrophilia, eosinophilia and monocytosis (29). There were marked rise in AST and ALT levels observed in infected animals in present study, (Table, 1) indicate that high disturbed in homeostatic balance. Both ALT and AST were produced inside hepatic cells and can reach the blood flow through a ruptured cell. So an elevated amount these enzymes may indicate rapid damage and death of hepatic cells. Although

great activities have been observed in liver function assay for both enzymes, it differ from; 5-55 in AST and 5-60 in ALT (23), 15-58 in AST and 16-43 in ALT (8), 120.66 ± 4.30 in ALT and 150.0 ± 5.33 in AST (24) 41.60 ± 11.41 in ALT, 80.8 ± 54.66 in AST (25), and from 82 ± 76 , 50 ± 38 in both ALT and AST respectively (10). Serum AST level may be increased with liver disease in all species of animal but neither ALT nor serum alkaline phosphatase determination liver function test (27). Non-significant increased in BUN, and creatinine were observed in natural infected dogs in present study table (2). Similar findings have been observed in creatinine concentration (26). But not to previous studies (24 and 25) in urea and Creatinine 73.92 ± 2.04 , 1.5 ± 0.02 and 9.10 ± 2.88 , 0.78 ± 0.19 respectively. Amount of creatinine (0.5-14) reported by (8). The medical significance for this test concerns their role in the differential diagnosis when the clinical sings were not definitive (10). Through damage to the kidneys by microfilariae a decline in renal blood flow and increased urea nitrogen concentration (11). Protein urine and uremia have been reported in canine dirofilariasis could be related to the pulmonary cardiac and hepatic disorders (9 and 19). It is important to realize that normal values vary among individual laboratories; it may be attributed to the geographic or temporal differences (28). Based on these results of different clinical, blood and biochemical values were concerned with the knowledge of the dogs naturally infected with canine heartworm, it will vary according to the worm burden, their location and get them the interaction of the host immune system. The present study demonstrated the presence of a significant correlation between these changes and the severity of the disease supporting the previous study. In heartworm disease the circulatory system is not the only system affected but also the renal and hepatic systems can be secondarily affected (29). In conclusion, the disease showed no significant clinical signs although many pathological changes in some blood constituent with serum biochemical parameters were observed. More studies are needed for show and confirm the lesions of *Dirofilaria immitis* in natural infected dogs.

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دراسة تأثير الإصابة الطبيعية بداء الخيطيات الكلبية في بعض المعايير الدمية والكيموحيوية

آمال حسين عطية و دنيا عبد الملك العاني
المعهد الطبي التقني، بغداد، العراق.

E-mail: amalhussen@yahoo.com

الخلاصة

هدفت الدراسة إلى التحقق من مقدار التباين في بعض المعايير الدمية والكيموحيوية في الكلاب المصابة طبيعياً بداء الخيطيات. جُمعت عينات الدم من خمسة وستين كلباً بعمر 5-10 سنة ينتمي إلى السلالة المحلية، في قرى قضاء الهندية/ محافظة كربلاء. وقد اعتمدت معايير الصفة التشريحية والدمية والكيموحيوية في تقييم النتائج. أشارت نتائج الدراسة إلى وجود اختلافات في قيم هذه المعايير مقارنة مع القيم المرجعية. حيث يمكن أن نستنتج من هذه الدراسة على الرغم من وجود العديد من التغيرات المرضية في بعض مكونات الدم والمعايير الكيموحيوية في مصل الدم، إلا أنه لم تظهر قيمة معنوية للعلامات السريرية للمرض قبل القتل الرحيم للكلاب.

الكلمات المفتاحية: الدايروفلارياسيس الكلبية، المعايير الدمية، الكيموحيوية.