

Thoracic radiography and oxidative stress indices in heartworm affected dogs

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Abstract

Aim: The aim was to study the pathomorphological changes through thoracic radiography and status of oxidative stress parameters in heartworm affected dogs in Odisha.

Materials and Methods: A total of 16 dogs with clinically established diagnosis of dirofilariasis by wet blood smear and modified Knott's test and equal numbers of dogs as control were included in this study. The present study was conducted in heartworm affected dogs to see the pathomorphological changes through thoracic radiography. Similarly, the evaluation was undertaken for observing any alterations in oxidative stress status in affected as well as non-affected, but healthy control dogs by adopting standard procedure.

Results: Thoracic radiography revealed cardiac enlargement, round heart appearance suggestive of right ventricular hypertrophy, tortuous pulmonary artery and darkening of lungs. Alterations in oxidative stress indices showed a significant rise of lipid peroxidase activity, non-significant rise of superoxide dismutase and a significant although reverse trend for catalase levels in affected dogs in comparison to *Dirofilaria* negative control but apparently healthy dogs.

Conclusions: Radiographic changes, as well as alterations in oxidative stress parameters, may not be diagnostic for heartworm infection, but useful for detecting heartworm disease, assessing severity and evaluating cardiopulmonary parenchyma changes and gives a fair idea about the degree of severity of the disease. It aids as contributing factors in disease pathogenesis.

Keywords: *Dirofilaria immitis*, heartworm, oxidative stress, radiography.

Introduction

Dirofilariasis popularly known as "heartworm disease" is a common and important disease affecting dogs, cats, foxes and wolves in tropics, sub-tropics and temperate regions of the world [1]. The species, *Dirofilaria immitis* is the causal agent of heartworm disease in dogs [2]. Dog is considered to be a definitive host, the mosquito *Culex* being the intermediate host. The disease has a worldwide distribution. Canine dirofilariasis now recognized not only as a veterinary problem, but also a zoonoses in many parts of the world [3,4]. In recent years, there is a conspicuous upsurge in the prevalence of heartworm disease in canine in India. Several factors such as global warming, uncertainty in the seasonal cycle with changes in ever increasing vector's population, rapid and continuous animal movement between different countries play an important role in the high prevalence of this disease [5]. Detection of *Dirofilaria* through wet blood smear and modified Knott's test is the easiest, inexpensive and reliable diagnostic method, but

needs professional expertise to differentiate between *D. immitis* and *Acanthocheilonema reconditum*. The pathophysiological response to heartworm infection is mainly due to the presence of adult worms in the vital organs of the body, that is., in right ventricles of the heart and pulmonary artery [6]. Similarly, the worm burden, host immune response and duration of infection determine the severity of infection by altering the path morphology of several organs, which can be recognized through radiography [7].

Thoracic radiography singularly may not be diagnostic for heartworm infection but useful for detecting heartworm disease, assessing severity and evaluating cardiopulmonary parenchymal changes [8,9]. Post mortem examination and detection of adult worms in the heart, followed by histopathology are very important diagnostic tools in animals that succumb to chronic heart worm infestation. Oxidative stress plays a vital role in the pathogenesis of different diseases and gives a fair idea about the degree of severity of the disease [10-12]. Superoxide dismutase (SOD), lipid peroxidase (LPO) and catalase are the major antioxidant enzymes present in red blood cell (RBC) to counteract the toxic effects of reactive oxygen species such as superoxide radicals and hydrogen peroxides [13], which are also considered as

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indicators of oxidative stress in *Dirofilaria* affected dogs.

In this study, an attempt was made to see the pathomorphological changes through X-ray and image intensifier TV system (C-arm examination) and to study the alterations in oxidative stress indices in heartworm affected dogs in Odisha.

Materials and Methods

Ethical approval

The experiment was carried out according to the national regulations on animal welfare and Institutional Animal Ethical Committee.

Animals

Pet and stray dogs of both sexes with varying ages and breeds were included in this study from several sources viz. Teaching Veterinary Clinical Complex of the College mostly dealing with pet dogs of private owners, Police Dog Squad and Animal Birth Control Program of Bhubaneswar Municipal Corporation at Veterinary Polyclinic, Sahid Nagar dealing with sterilization of stray dogs.

Experiment design

Dogs were screened for dirofilariasis on the basis of clinical signs and blood examinations (wet blood smear and modified Knott's test). In this study, motile microfilariae of more than 10/microscopic field in wet blood smear and more than 5 in modified Knott's test was considered as heavily positive for *Dirofilaria* infestation. Of these, 16 animals were subjected for thoracic radiography and oxidative stress evaluation which were compared against equal numbers of *Dirofilaria* negative but apparently healthy dogs.

Procedures

Radiography was conducted by C-arm examinations and X-ray. A volume of milliliter blood in heparinized test tube was collected from the cephalic vein without giving much stress to dogs for oxidative stress indices study. Three parameters such as LPO, SOD and catalase activity were taken to measure the stress indices. Membrane peroxidation damage in the erythrocyte was expressed in units of malondealdehyde production by modified method of Stock and Dormandy [14] as described by Placer *et al.* [15]. The SOD activity was estimated by methods of Marklund and Marklund [16] with certain suggested modification by Menami and Yoshikawa [17] using diluted nitroblue tetrazolium as substrate. Estimation of catalase activity in 10% RBC hemolysate was done as per the procedure described by Cohen *et al.* [18].

Results and Discussion

Thoracic radiography

Radiographic evaluation through C-arm examination (Figure-1) of affected animals with a heavy load of microfilariae in the blood during screening revealed cardiomegaly, round heart appearance

suggestive of right ventricular hypertrophy, tortuosity of the pulmonary artery and darkening of lungs. Radiographic changes associated with heartworm disease include right ventricular enlargement (Figure-2), increased prominence of the main pulmonary artery segments, increased size and density of the pulmonary arteries, arterial tortuosity. Similar findings were observed by Polizopoulou *et al.* [9], which may be due to the presence of adult worms in right ventricles and pulmonary artery [6] thereby causing certain pathomorphological changes in these organs. Persistence of the parenchyma changes related with severity and chronicity of the disease [6].

Oxidative stress indices

Oxidative stress indices like LPO, SOD and catalase, were done to assess the tissue damage caused by dirofilariasis in canine. For this purpose, 16 samples each from affected and non-affected animals as control were utilized. The LPO value (nm of

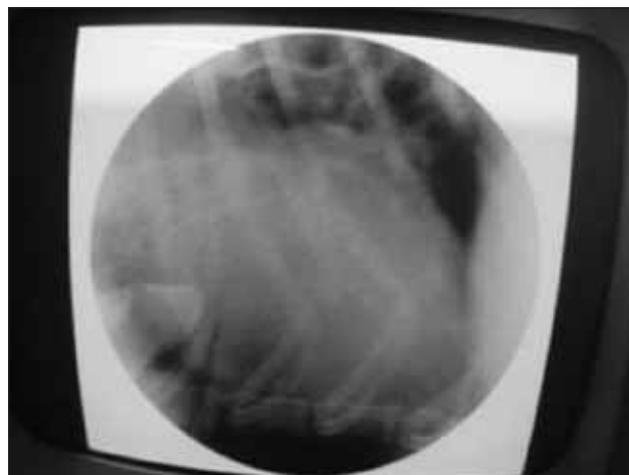


Figure-1: C-arm examination showing cardiomegaly.



Figure-2: X-ray showing cardiomegaly.

malondialdehyde/ml) in average was 33.41 (± 1.01) with a range of minimum 27.54 and maximum 39.21 in the filaria positive dogs which was significantly ($p < 0.05$) higher than the control with an average level of activity at 19.46 (± 1.22) ranging from a minimum of 15.07 to 25.47 (Figure-3). The SOD activity (unit/mg of Hb) varied from 0.99 to 2.72 with mean (\pm SE) at 2.15 (± 0.12). Although this activity was higher than the control counterpart showing an average of 1.51 (± 0.22) of activity, the difference was non-significant (Figure-4). The catalase level (unit/mg of Hb) in the affected dogs, on the other hand, hovered between 0.05 and 0.21 mean (\pm SE) being 0.12 (± 0.01). The control non-affected animals showed the activity

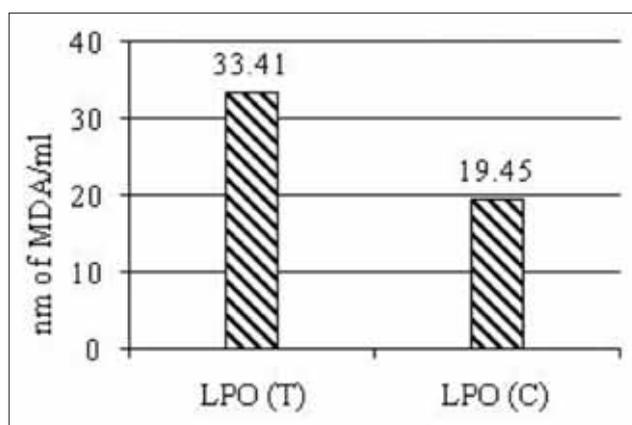


Figure-3: Lipid peroxidase activity level.

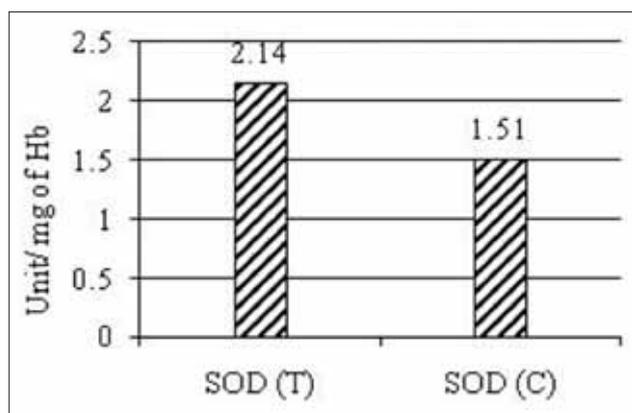


Figure-4: Superoxide dismutase activity level.

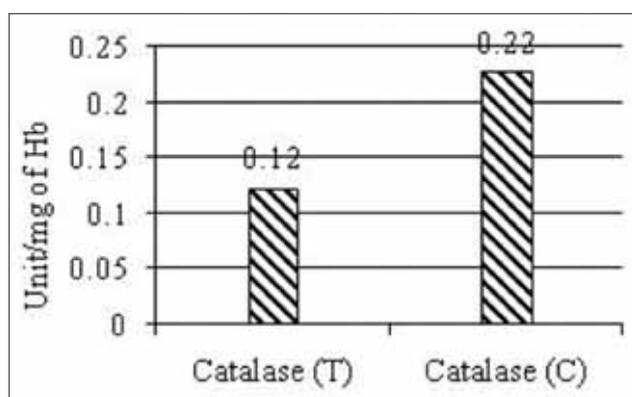


Figure-5: Catalase activity level.

within 0.09 and 0.41 with an average of 0.23 (± 0.03). The difference was significant at $p < 0.05$ (Figure-5). Radiographic changes relating to pathomorphological alterations in heart, lungs and pulmonary artery in this study were in conformity with McCall *et al.* [19]. Dimri *et al.* [20] concluded about a significant rise of LPO activity and non-significant rise of SOD and a significant although reverse trend for catalase levels in affected dogs in comparison to *Dirofilaria* negative, but apparently healthy dogs, which corroborate with the present findings.

Conclusions

Radiographic examination through c-arm and x-ray of the thoracic region showed obvious cardio-pulmonary parenchyma changes owing to the presence of adult worms in these vital organs. Heavy load of adult heartworms and peripheral microfilaria result in certain alterations in oxidative stress parameters directing the disease pathogenesis process to a critical level, which may aid in assessing disease severity in chronic cases.

Authors' Contributions

RCP and IN were involved in the design of the experiment. PKR carried out the research experiment and data collection under the supervision of SKP. PKR and BPM collected materials for the manuscript and prepared the first draft before being revised by all authors. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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