Clinicopathological Studies on the Effect of Filariasis in Egyptian Buffaloes

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Abstract
Filariasis is a vector-transmitted parasitic disease and has a worldwide spread. This work aimed to study the effect of filariasis on hematology, blood chemistry and antioxidant status of adult buffaloes as well as efficacy of ivermectin in disease treatment. The present study was done on fifteen male buffaloes, 2-4 years old, (7 apparently health and 8 suffered from filariasis). The diagnosis was based on the clinical signs and presence of Microfilariae in wet smear. One animal died during treatment. Hematological results revealed hemolytic anemia associated with a significant decrease in erythrocytic count, hemoglobin concentration and packed cell volume. A significant increase in the total and differential leukocytic counts was recorded compared with the normal control. Evaluation of blood chemistry of infested animals showed significant changes in liver and cardiac function, as well as antioxidant parameters. These parameters returned to the normal levels 21 days post treatment with ivermectin. It could be concluded that filariasis induced hemolytic anemia, with alteration in antioxidant enzymes and liver and cardiac functions. Also ivermectin is effective in treatment of filariasis.

Keywords: Filariasis, Buffalo, Antioxidant enzyme, Liver, Blood

Introduction
Filariasis is a vector-transmitted parasitic disease caused by Filarioidea nematode with world wide spread especially throughout the developing countries [1, 2]. The parasites develop inside the vector from microfilariae to infective larvae then migrate to the mouth parts of arthropod and transmitted to the mammalian host during feeding [3]. Filariasis is associated with considerable economic losses to beef and dairy buffaloes as they caused high morbidity and mortality [4]. Filarial parasites succeed to escape from host immunity through the releasing of immunosuppressive materials [5]. Lymphatic filariasis is endemic disease in tropical and subtropical areas [6]. For many years ago, microscopic examination of blood film has been the only tool available for the detection of parasites [7]. Presence of microfilaria in blood smear is a good diagnostic method [8], in addition to the presence of dermatological or lymphatic manifestations. Nowadays ivermectin administration is the major tool in the control of filariasis [4]. Although many investigators studied filariasis in different animals there is a shortage in studying the disease in buffaloes. Also there are many aspects that remain unclear, so this work was aimed to study the effect of filariasis on hematology, blood chemistry and antioxidant status of adult infested buffaloes.

Material and Methods

Animals
Fifteen adult male buffaloes, 2-4 years old, were used in the present study. The animals were sporadic cases admitted to a private clinic and the Clinic of Teaching Animal Hospital, Faculty of Veterinary Medicine, Zagazig University, Shakiya, Egypt. Control animals were selected from the same farms of the diseased animals and nearly within the same age range. One animal died during the treatment. The other fourteen animals were divided into two equal groups, apparently healthy and naturally infested male buffaloes (n=7/each) The diagnosis was based on clinical examination of animals and identification of microfilariae by late night direct smear [8].

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Drugs

Ivermectin (Merial celebrates, Lyon Porte-des-Alpes, France): bottle of 50 ml injected s/c at a dose of 1ml/50kg BW -3 times with 5 days interval [4]. It is used for treatment of filariasis.

Norocillin LA (Norbrook Lab lemitis, station works NEWRY, N. Ireland): bottle of 50 ml used by deep I/M injection at a dose of 1ml/25kg BW twice with 4 days interval [9]. It is used to prevent secondary bacterial infection to the opened nodules.

Blood samples

Blood samples were taken at 0,7,14 and 21 days post treatment from the jugular vein of each animal. The first set of blood was 1 ml received in a clean tube containing dipotassium salt of EDTA and used for hematological examination. The second blood sample (10ml) was taken without anticoagulant in chemical free test tubes, allowed to clot then centrifuged at 3000 rpm for ten minutes for separation of clear serum.

Hematological studies

Erythrocytic count and leukogram were performed [10]. The packed cell volume (PCV) was estimated by the microhematocrit centrifuge [10] and hemoglobin (Hb) concentration was determined using the cyanmethemoglobin colorimetric method [11]. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated.

Serum biochemical studies

Serum total proteins (TP), albumin and globulins were estimated according to Grant et al.,Webster and Doumas& Biggs [12, 13, 14].Aaminotransferases (AST & ALT)bilirubin malondialdehyde and cardiac troponin1 (cTnl) concentration) were determined following to [15,16, 17and 18]. Serum activity of glutathione peroxidase and superoxide dismutase (SOD) was measured after [19, 20]. Lactate dehydrogenase (LDH) and creatine phosphokinase (CK-MB) were also estimated [21, 22].

Statistical analysis

Data obtained from this investigation were statistically analyzed using the one way analysis of variance ; ANOVA [23]. Means at the same row followed by different letters were significantly different and the highest value was represented with the letter (a).

Results

Clinical signs

The main clinical manifestations recorded in affected animals were fever (40-40.6ºC), anorexia, enlargement of one leg or more (Figure 1A) and presence of cutaneous nodules (Figure 1B). These nodules opened spontaneously and produced hemorrhagic exudates (Figure 1C). One animal opened spontaneously and produced hemorrhagic exudates (Figure 1C). One animal died during treatment.

Hematological changes

Evaluation of erythrogram revealed that filarial - infested group had a significant decrease in erythrocytic count, packed cell volume and hemoglobin concentration at Zero and 7 days post treatment (PT) compared with the healthy control. The estimation of MCV and MCHC proved normocytic normochromic anemia before treatment and macrocytic hypochromic 7 days post treatment. The MCH revealed a significant increase before treatment only in the infested group compared with the non infested control. At 14 and 21 days PT, the erythrogram returned to the normal values (Table1).

Regarding the leukogram the results revealed a significant leukocytosis at 0, 7 and 14 days post treatment compared with the normal control. Also a significant eosinophilia, basophilia and lymphocytosis were recorded at 0 and 7 days post treatment. Moreover a significant neutrophilia (day Zero PT) and monocyto (7 days PT) were recorded (Table 1).
The disturbances in the liver functions in filaria infested group manifested by a significant increase in the serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and serum levels of total bilirubin, unconjugated bilirubin (UB) and conjugated bilirubin (CB) at 0, 7 and 14 days post treatment. This elevation was returned to the normal level 21 days post treatment compared to the normal control values (Table 2).

Significant decrease in the activities of glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) with a significant increase in malondialdehyde (MDA) level in the affected animals at 0, 7 and 14 days post treatment compared to the normal control (Table 3). The obtained data revealed that these parameters became non significant with the normal control at 21 days post treatment.
Table 3: Antioxidant enzymes and malondialdehyde of filaria-infested buffaloes compared to normal control (mean±SE)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal group (control)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Glutathione peroxidase (U/L)</td>
<td>26.14±1.01</td>
</tr>
<tr>
<td>Superoxide dismutase (U/L)</td>
<td>11.07±0.29</td>
</tr>
<tr>
<td>Catalase (U/L)</td>
<td>2.53±0.08</td>
</tr>
<tr>
<td>Malondialdehyde (nmol/ml)</td>
<td>0.83±0.05</td>
</tr>
</tbody>
</table>

Means with different superscript letters within the same row are significantly different at p≤0.05.

Alterations in the cardiac markers in filarial-infested animals were manifested by a significant elevation in the serum activities of LDH, CK-MB and serum level of cTnI at 0, 7 and 14 days post treatment (Table 4). This elevation was returned to the normal level 21 days post treatment compared to the normal control values.

Table 4: Cardiac markers in serum of filaria-infested buffaloes compared to normal control (mean±SE)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
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<tbody>
<tr>
<td></td>
<td>Normal group (control)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>cTnI (ng/ml)</td>
<td>0.10±0.01</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>518.43±3.15</td>
</tr>
<tr>
<td>CK–MB (U/L)</td>
<td>281.66±2.95</td>
</tr>
</tbody>
</table>

Means with different superscript letters within the same row are significantly different at p≤0.05.
Discussion

The clinical signs observed in the diseased animals were comparable to those previously mentioned by several investigators as suggestive to bovine flarasis [4,24]. The picture of the erythron mass in the infested group was normocytic normochromic anemia before treatment and macrocytic hypochromic 7 days post treatment. The aforementioned results could be correlated with hemolysis. The significant increase of the mean corpuscular hemoglobin that recorded before treatment supported the presence of hemolysis. As supported by the previously obtained results of Sharma& Joshi and Sarojini, & Senthilkumaar [24,25] who mentioned that microfilariae caused erythrocyte degeneration.

The leukocytosis recorded at 0 and7 days post treatment is due to neutrophilia, eosinophilia, basophilia, lymphocytosis and monocytosis. The lymphocytosis recorded in infested animals may be due to an early antigenic stimulation with an increased demand for transformation of lymphocytes to plasma cells. Such increase in phagocytic cells (neutrophils & monocytes) could be due to tissue destruction [10]. The observed eosinophilia was due to the hypersensitivity resulted from the migration and the foreign protein of the parasite [10]. Nearly similar...
results were recorded previously by Hashem, and Badawy [26].

The significant hypoproteinemia and hypoalbuminemia in infested group at 0, 7 and 14 days PT could be attributed to hypoalbuminemiam which in turn might be due to the decreased feed intake and disturbed metabolism of the liver. The hyperglobulinemia may be due to the reaction to the antigenic stimuli. Albumin/globulin ratio showed a significant decrease in infested group at 0, 7, 14 and 21 days PT. This is due to the hypoalbuminemia and hyperglobulinemia. The obtained results were in harmony with earlier findings of Brzezińska-Slebodzińska [31].

The present study showed a highly significant increase in the serum AST and ALT activities in the infested group. The increased serum activities of aminotransferases (AST and ALT) were associated with hepatocellular damage [27]. This increase in serum aminotransferases activity in infested animals suggested liver and/or myocardial cells degeneration caused by migratory microfilaria [24] or secondary to circulatory disturbance [26]. The hyperbilirubinemia was due to the increased serum levels of conjugated and unconjugated bilirubin. The increased serum conjugated bilirubin level suggested liver dysfunction. While, the increased serum unconjugated bilirubin level may be attributed to the presence of hemolytic jaundice resulted from excessive hemolysis [27].

The antioxidants defense systems play an important role in protection of living organisms from the deleterious effects of reactive oxygen metabolites. Superoxide dismutase; glutathione peroxidase and catalase play an important role. The field of oxidative stress in buffalo’s medicine is still in the early juncture. The evaluations of free radicals damage and body’s defenses have become increasingly important in clinical medicine as complementary tool in the evaluation of metabolic status of the animals [28]. Host immune effector cells such as macrophages, neutrophils and eosinophils release free radicals such as superoxide radical, hydroxyl radicals, singlet oxygen and hydrogen peroxide as defense mechanisms to kill the invading parasites [29]. All living tissues protect themselves from the damaging effects of free radicals by antioxidants and antioxidant enzymes.

The present study showed that serum superoxide dismutase, glutathione peroxidase and catalase enzyme activities were decreased significantly in the infested group. Parasitic infestation causes the release of reactive oxygen species [30]. In this study oxidative stress following the infestation and inflammation caused increased use and weakened protective effect of the antioxidant enzymatic system in the infested animals. Moreover, fever induced oxidative stress [31]. No available data dealing the effect of filariasis on the antioxidants enzymes in buffaloes could be found. Lipid peroxidation is used as an indicator of oxidative stress. Serum malondialdehyde level significantly increased in infested animals compared with the normal health control. This supports the previously obtained result by Doumas and Biggs [4] who recorded a significant increase in serum malondialdehyde level post filarial infestation in buffaloes.

Several biochemical parameters can be used as markers for cardiac function. Among them the myocardial bound creatine kinase (CK-MB) and lactate dehydrogenase (LDH) are important in the diagnosis of heart disease [32]. Troponin I (cTnI) is gold-standard, blood biomarkers with high sensitivity and specificity for myocardial degeneration [33]. Troponin I, lactate dehydrogenase and creatine kinase were increased significantly in the infested buffaloes. So this indicated the presence of myocardial injury. This may be due to necrotic degeneration of myocardial cells caused by migratory microfilaria [24]. The most characteristic finding recorded in the dead animal was severe increase in serum level of troponin I (1.50 ng/ml).

**Conclusion**

It could be concluded that filariasis induced hemolytic anemia, with alteration in antioxidant enzymes and liver and cardiac functions. The ivermectin treatment succeeded to ameliorate the negative impact of parasites on hemato-
biochemical parameters towards the normal control values. These findings support the efficacy of ivermectin for treatment of filariasis in buffaloes. However, increased serum troponinI level to 1.50 ng/ml or more indicates bad prognosis.

**Conflict of interest**

The authors declare no conflict of interest.

**References**


الملخص العربي

دراسات بالباثولوجية الإكلينيكية على تأثير مرض الفلارييا في الجاموس المصري
نصر عبد الوهاب و نصر الدين
قسم الباثولوجيا الإكلينيكية – كلية الطب البيطري – جامعة الزقازيق

مرض الفلارييا هو مرض طبيعي ينتشر بالتوافل، وهو ينتشر في جميع أنحاء العالم. وينتقد هذا الاعمال إلى دراسة تأثير الفلارييا على خلايا الدم، كيمياء الدم والأنزيمات المضادة للأكسدة في الجاموس. تم إجراء الدراسة على خمسة عشر من ذكور الجاموس، عمر 4-7 سنوات، (7 أصحاء و 8 تعاني من مرض الفلارييا). وقد توفي حيوان واحد خلال فترة العزلة. واعتماد التشخيص على الإعراض المرضية ووجود الميكروفلارييا في الدم. وكشفت النتائج عن جود قدر من انحلالي مرتبط بانخفاض عدد الكريات الحمراء وتركز الهيموجلوبين وحجم كرات الدم المنغصTemporal. كما وجدت تغييرات معنوية في كيمياء الدم ووظائف الكبد والقلب مع اضطراب في الانزيمات المضادة للأكسدة. واعادت هذه النتائج إلى المستويات الطبيعية بعد 48 يوم من العزلة. ويمكن أن نستخلص من ذلك أن إصابات الفلارييا بالجاموس تنتج عن قدر من انحلالي، مع اضطراب في الانزيمات المضادة للأكسدة ووظائف الكبد والقلب. أيضا وجدي أن الأفراد الذين علاج مؤثر ضد مرض الفلارييا. وعلاوة على ذلك فإن زيادة الجروبيون في السيرير إلى 1.5 نجم/ملى أو أكثر يدل على عدم جدوى العلاج.