Effect of hepatic dysfunction on serum lipoproteins and macroelements status in sheep fascioliasis

M Ellah, H Ahmed, A Mohamed, A Eltayb, I Ellah, S Elfattah, A Rayan, R Ahmed, N Nagieb, M Luis

Abstract
The present study aimed to evaluate the effect of hepatic dysfunction on serum lipoproteins and macroelements status in sheep fascioliasis. A total number of 13 ewes (2-3 years old) were subjected to study. Ewes were classified into two groups based on precise fecal examination; control group (No.=5) and fasciola infested group (No.=8). From the fasciola infested group; 3 animals showed serum total protein level below 5 g/dl (group1) and the remained animals (No.=5) were classified as group2. Serum triglyceride, cholesterol, HDL, VLDL and calcium levels showed significant decreases in both groups. Serum LDL level was significantly decreased only in group1. In conclusion, hepatic dysfunction due to fasciola infestation in sheep resulted in decreases serum cholesterol, triglyceride, HDL, VLDL and calcium levels.

INTRODUCTION
Fascioliasis usually associated with animals grazing on naturally or artificially flooded areas or around permanent water channels (Urquhart et al., 1994). Liver fluke infestation (Fasciola gigantica) is the most common cause of liver damage (Ayoub, 1983). During migration of young flukes there is direct trauma with necrosis, haemorrhages, and subsequent healing by granulation tissues, which results in cirrhosis of the liver and fibrosis of the bile ducts causing its dilatation, thickening and calcification (Sewell, 1966; Soulsby, 1973; Osuna et al., 1977 and Nwiyi and Chaudrai, 1996).

Normal serum biochemical variables of sheep may vary according to breed, pregnancy, lactation, locality, age and sex. Serum levels of total protein, albumin and globulins in healthy ewes were reported by Balikci et al. (2007) as 7.73 ± 0.88, 3.09 ± 0.43 and 4.65 ± 0.48 g/dl respectively, and by Kaneko et al. (1997) were 7.2 ± 0.52, 2.7 ± 0.19 and 4.4 ± 0.53 g/dl respectively. Serum total cholesterol was reported 73 ± 15 mg/dl by Kaneko et al. (1997) and 56.5 ± 1.31 by Balikci et al. (2007). The latter reported that normal serum triglyceride was 19.3 ± 1.05 mg/dl. Serum biochemistry of infected animals can be a good indication for the degree of damage and the severity of the infection (Otesile et al., 1991). Liver damage upsets the metabolic processes that are vital for normal health and optimum productivity of the animal and performance (Purushothaman and Rajan, 1980 and Rosenberger, 1990 and Rutgers, 1996). Sheep and goats infected with Fasciola sp. exhibit substantial changes both in the amount and distribution of their plasma proteins. These changes generally take the form of a depression in albumin relative to globulins, and take place in two stages. The first stage coincides with the period of fluke migration and is characterized by a progressive hypoalbuminaemia (Thorpe, 1965). The second is associated with the presence of adult parasites in the bile ducts and is attended by further deterioration in albumin. Serum lipoprotein is also may be affected by liver damage (Latimer et al., 2003). The present study aimed to evaluate the effect of hepatic dysfunction on serum lipoproteins and macroelements status in sheep fascioliasis.

MATERIALS AND METHODS
ANIMALS:
A total number of 13 female sheep (2-3 years old) were subjected to study. Animals were divided into two groups based on precise fecal examinations; control group (n=5) and fasciola infested group (n=8). The fasciola infested group was selected from sheep raised at the Veterinary Teaching Hospital, Assiut University, Egypt. The control group was selected from animals raised at private farm.
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(Assiut, Egypt) and was clinically healthy and free from any parasitic infestations.

SAMPLES:

BLOOD SAMPLE:

Blood samples for separation of serum were collected from the jugular vein in plain vacutainer tube according to Coles (1986). Serum samples were used for measuring serum total protein, albumin, glucose, triglyceride, cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), calcium, phosphorus, magnesium levels, and for measuring serum activities of aspartate aminotransferase (AST), gamma glutamyltransferase (GGT) and lactate dehydrogenase (LDH) activities, using commercial test kits supplied by Spectrum Diagnostics (Cairo, Egypt) and by means of Digital VIS/Ultraviolet Spectrophotometer (Cecil instruments, Cambridge, England, Series No. 52.232).

Fecal samples were collected in clean plastic bags from the rectum of all animals under investigations. Fecal samples were examined directly after examination by direct smear, sedimentation and floatation technique according to Coles (1986).

STATISTICAL ANALYSIS

Statistical analysis was conducted using SPSS 16.0 for windows (SPSS, Chicago, USA) and was carried out using one way ANOVA. Data were expressed as Mean ± SD.

RESULTS

Animals belong to the fasciola infested group showed emaciation. The consistency of feces mostly was pasty and some cases showed normal pellets.

LIVER FUNCTION TESTS:

The degree of hepatic dysfunction was assessed based on measuring serum total protein, albumin, globulins levels and serum AST, GGT and LDH activities. From the fasciola infested group; 3 animals showed serum total protein level below 5 g/dl (Group1) and the remained animals (No.=5) were classified as group2. Normal serum total protein level in sheep ranged from 6.0-7.9 g/dl as reported by kaneko et al. (1997).

The results revealed hyperproteinaemia (p<0.01) and hyperglobulinaemia (p<0.01) in group2 and hypoproteinaemia (p<0.01), hypoalbuminaemia (p<0.01) and hypoglobulinaemia (p<0.01) in group1. AST and GGT significantly increased (p<0.01) in both groups. The significant increase in LDH was higher in group2 than in group1. Glucose level showed insignificant changes in both groups (Table 1).

Figure 1

Table 1. Serum biochemical variables in control and diseased sheep

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (No.=5)</th>
<th>Group1 (No.=3)</th>
<th>Group2 (No.=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>6.10 ± 0.75</td>
<td>4.45 ± 0.62**</td>
<td>9.94 ± 2.87**</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.87 ± 0.31</td>
<td>2.51 ± 0.06*</td>
<td>2.50 ± 0.17*</td>
</tr>
<tr>
<td>Globulins (g/dl)</td>
<td>3.73 ± 0.59</td>
<td>1.94 ± 0.65**</td>
<td>7.44 ± 2.86**</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>0.78 ± 0.12</td>
<td>1.41 ± 0.60**</td>
<td>0.39 ± 0.19**</td>
</tr>
<tr>
<td>AST (UI)</td>
<td>22.29 ± 4.57</td>
<td>49.08 ± 16.09**</td>
<td>45.61 ± 10.63**</td>
</tr>
<tr>
<td>GGT (UI)</td>
<td>23.62 ± 2.05</td>
<td>54.52 ± 7.52**</td>
<td>47.34 ± 21.65**</td>
</tr>
<tr>
<td>LDH (UI)</td>
<td>134.22 ± 27.53</td>
<td>199.68 ± 42.68**</td>
<td>304.88 ± 134.77**</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>31.32 ± 10.11</td>
<td>48.08 ± 32.79</td>
<td>31.83 ± 13.47**</td>
</tr>
</tbody>
</table>

Data expressed as Mean ± SD. * Significant (p<0.05), ** Highly significant (p<0.01)

LIPOPROTEINS STATUS

Serum triglyceride (p<0.05), cholesterol (p<0.01), HDL (p<0.01) and VLDL (p<0.05) levels showed significant decreases in both groups. Serum LDL level was significantly decreased (p<0.01) only in group1.

Figure 2

Table 2. Serum triglyceride, cholesterol and lipoproteins levels in control and diseased sheep

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (No.=5)</th>
<th>Group1 (No.=3)</th>
<th>Group2 (No.=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>42.01 ± 11.24</td>
<td>28.08 ± 4.41*</td>
<td>27.05 ± 5.77*</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>63.75 ± 8.56</td>
<td>37.76 ± 6.38**</td>
<td>38.28 ± 6.82**</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>43.87 ± 8.72</td>
<td>23.07 ± 5.51**</td>
<td>19.81 ± 3.62**</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>11.99 ± 2.72</td>
<td>5.07 ± 1.88**</td>
<td>13.0 ± 7.51</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>8.41 ± 2.25</td>
<td>5.61 ± 0.88**</td>
<td>5.41 ± 16**</td>
</tr>
</tbody>
</table>

Data expressed as Mean ± SD. * Significant (p<0.05), ** Highly significant (p<0.01)

MACROELEMENTS STATUS

There were insignificant changes in serum phosphorus and magnesium levels in fasciola infested groups (Table 3). On the other hand, serum calcium level showed a significant decrease in both groups. The corrected serum calcium level was calculated according to Stashak and Adams (2002) as follow; the measured albumin value should be subtracted from the mean albumin value (normal albumin level) and the difference added back to the measured calcium. The
Corrected serum calcium level showed also a significant decrease in both fasciola infested groups (Table 4).

**Figure 3**
Table 3. Serum phosphorus and magnesium levels in control and diseased sheep

<table>
<thead>
<tr>
<th></th>
<th>Control (No. = 5)</th>
<th>Group1 (No. = 3)</th>
<th>Group2 (No. = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>5.94 ± 0.98</td>
<td>5.62 ± 0.74</td>
<td>5.75 ± 0.70</td>
</tr>
<tr>
<td>Magnesium (mg/dl)</td>
<td>2.47 ± 0.27</td>
<td>2.67 ± 1.33</td>
<td>2.38 ± 0.77</td>
</tr>
</tbody>
</table>

**Figure 4**
Table 4. Value of serum calcium and corrected calcium levels (mg/dl) in control and diseased sheep

<table>
<thead>
<tr>
<th></th>
<th>Control (No. = 5)</th>
<th>Group1 (No. = 3)</th>
<th>Group2 (No. = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>9.16 ± 0.75</td>
<td>6.52 ± 0.22**</td>
<td>6.87 ± 0.36**</td>
</tr>
<tr>
<td>Corrected calcium level</td>
<td>7.31 ± 1.38#</td>
<td>7.71 ± 1.41#</td>
<td>7.71 ± 1.41#</td>
</tr>
</tbody>
</table>

DISCUSSION

Total protein concentration in serum is one of the common measurements in clinical laboratory diagnosis. Changes in the amounts of plasma proteins may result from alteration in synthesis, catabolism or from protein losses, so marked hypoproteinaemia in the absence of dehydration usually reflects increased synthesis of gamma-globulins. In addition, hypoproteinaemia can be caused by decreased protein synthesis and is seen in chronic liver disease (Leelewandrowski and Lewandrowski, 1994 and Abd Ellah, 1998). Serum albumin was found to be a sensitive index for the degree of liver damage, also - there is a direct relation between the decrease of albumin level and the degree of liver damage (Abdou, 1976 and Abd-El-Salam et al., 1998). Animals belong to group1 showed hypoproteinaemia, hypoalbuminaemia and hypoglobulinaemia (Mert et al., 2006), which may be attributed to decrease synthesis by the liver (Leelewandrowski and Lewandrowski, 1994; Maclachlan and Cullen, 1995 and Abd Ellah, 1998). Hyperproteinaemia in group2 may be attributed to increase globulins levels (p<0.01) in response to invasion of the bile duct with the parasite (Abd Ellah, 1998).

In the present study, hepatic dysfunction was confirmed by the significant increases in AST, GGT and LDH activities (Table 1); GGT is the most sensitive indicator of liver cell damage in fascioliasis (Anderson et al., 1977 and Mert et al., 2006). Galtier et al. (1986) and Ferre et al. (1995) were reported that the determination of AST, LDH provides information on the passage of young flukes through the liver parenchyma, whereas increase GGT activity indicates penetration into the bile ducts (Abd Ellah, 1998). In the present study, AST, GGT and LDH were significantly increased, which indicated that the hepatic damage was hepatobiliary.

Lipoproteins are synthesized in the liver; the presence of hepatic dysfunction may results in disturbance in their serum levels (Latimer et al., 2003). The decrease in serum cholesterol (p<0.01) and triglyceride (p<0.01) levels may be attributed to decrease synthesis by the liver. It was reported that the majority of serum cholesterol in sheep is circulated as HDL (Latimer et al., 2003). HDL cholesterol in the present study was the major lipoprotein in serum, the decrease in its level may be attributed to decrease serum cholesterol level and to decrease HDL synthesis by the liver. VLDLs export hepatic triglyceride and cholesterol and distribute triglyceride to adipose tissue and striated muscles (Latimer et al., 2003), decrease its concentration may be attributed to injury of the liver and to decrease circulating triglyceride level. Serum LDL was significantly decreased only in group1 and may be attributed to the higher decrease in cholesterol concentration in group1 (33.76 ± 6.38) than in group2 (38.28 ± 6.82).

Hypocalcaemia in the present study may be attributed to hypoalbuminaemia or disturbance in calcium metabolism due to hepatic dysfunction. Similar results obtained in Fasciola hepatica infestation in goats (Mbuh and Mbwaye, 2005). It is important to appreciate that a high proportion of calcium is bound to albumin. However, it is the unbound calcium that is the most important physiologically. For this reason when albumin is low, the total calcium level may be misleading and correction calculation needs to be made (Hamilton and Bickle, 2006). As shown in table 4, the corrected serum calcium level still significantly lesser than the control group, this indicated that the hypocalcaemia may be attributed to hepatic dysfunction. In conclusion, the majority of cholesterol in blood of sheep circulated as HDL. Hepatic dysfunction due to fasciola infestation in sheep resulted in decrease serum calcium, cholesterol, triglyceride, HDL and VLDL levels.

**References**

r-0. Abd Ellah, M. R. 1998. Evaluation of liver function tests in liver disorders in cattle and buffaloes. M. V. Sc. Thesis, Clinical Laboratory Diagnosis, Faculty of Veterinary Medicine, Assiut University, Egypt
Effect of hepatic dysfunction on serum lipoproteins and macroelements status in sheep fascioliasis

r-4. Ayoub, A. A. M. 1983. The interpretation of different tests used for the estimation of parasitic status of Fasciola gigantica in Gharbia Governorate. M. V. Sc. Thesis, Clinical Diagnosis, Faculty of Veterinary Medicine, Cairo University, Egypt.
Effect of hepatic dysfunction on serum lipoproteins and macroelements status in sheep fascioliasis

Author Information

Mahmoud R. Abd Ellah
Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University

Heba M. Ahmed
Final Year Student, Faculty of Veterinary Medicine, Assiut University

Alaa M. Mohamed
Final Year Student, Faculty of Veterinary Medicine, Assiut University

Aamna M. Eltayb
Final Year Student, Faculty of Veterinary Medicine, Assiut University

Ibtesam S. Abd Ellah
Final Year Student, Faculty of Veterinary Medicine, Assiut University

Shimaa A. Abd Elfattah
Final Year Student, Faculty of Veterinary Medicine, Assiut University

Asmaa A. Rayan
Final Year Student, Faculty of Veterinary Medicine, Assiut University

Rania M. Ahmed
Final Year Student, Faculty of Veterinary Medicine, Assiut University

Nevin Y. Nagieb
Final Year Student, Faculty of Veterinary Medicine, Assiut University

Mary K. Luis
Final Year Student, Faculty of Veterinary Medicine, Assiut University