Histopathological study of chronic livers Fascioliasis of cattle in Sulaimani abattoir

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Abstract

Liver fluke disease (fascioliasis) is an important parasitic disease may responsible for morbidity and mortality in most species of animals, like sheep, goat and cattle, as well as other domestic ruminants. The common causative agents are Fasciola hepatica and F. gigantica. The study aimed to detect the gross and microscopic lesions accompanied with chronic bovine liver Fascioliasis of local and Friesian cattle breeds in Sulaimani region and compared it with the same lesions in different species and breeds in other parts of the world according to the available literatures. During March to April 2013, fifty infected bovine livers of different ages were collected from Sulaimani abattoir, and examined grossly. Samples were taken from these livers parenchyma and bile ducts for histopathological examinations. The histopathological examinations illustration presence of different lesions in the liver parenchyma including, hepatocytes swelling, fatty changes, accumulation of bile pigment in bile canaliculi, congestion, neutrophils and eosinophils infiltration, abscess, telangiectasis, pericellular fibrosis, extensive fibrosis and liver cirrhosis. The lesions of bile duct ranged from infiltration of mononuclear inflammatory cells, portal fibrosis, bile duct hyperplasia, papillomatous projection, adenomatous hyperplasia and bile duct metaplasia. We concluded that Fascioliasis had pathological damage or destructive effect on bovine hepatobiliary system.

Key words: Chronic Fascioliasis, cattle, liver flukes, liver lesions, histopathological examination.

دراسة نسجية مرضية في إكيد الأبقار المصابة بداء المتورقات المزمن (فاشيولا) في مجزرة السلمايانية

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الخلاصة

داء المتورقات (متقيبات الكبد) هو مرض طفيلي مهم والذي قد يكون مسؤولا عن الإصابة المرضية والوفيات في معظم أنواع الحيوانات مثل الأغنام والماض والماشية، إضافة إلى الحيوانات الأخرى. أن المتورقات الكبدية والمتوارقة المزمنة هي الأكثر شيوعا من أنواع المزمنة للمرض. تهدف الدراسة إلى التكشف عن الآفات النسيجية والمحجرية السريرية لداء المتورقات الكبدية المزمن في منطقة السلمايانية ومقارنتها مع نفس الآفات في مختلف الأنواع والسلالات في أجزاء أخرى من العالم وفقاً للمصادر المتوفرة. أجريت هذه الدراسة فيهبرة من أرداء و حتى نيسان سنة 2013. جمعت خمسين كبد لأبقار مصابة من مختلف الأعمار من مجهرة السلمايانية وتم فحصها عيانا. تم أخذ عينات من المتن ومن القناة الصفراوية لغرض أجراء الفحوصات السريرية. أوضحت الفحوصات النسيجية عن وجود آفات مختلفة في متن الكبد مثل تورم الخلايا الكبدية، وجود التغيرات الدهنية، تجمع المادة الصفراوية، وجود الاحتكاك، ارتفاع الخلايا العضية و الدوسي، وجود الخراشات، توسع الشعيرات الدموية، تليف محيط الخلايا، تليف واسع و تشع عضوي الكبد. أما آفات القناة الصفراوية فقد لوحظت بين ارتفاع الخلايا أجنبية الدوسي، تليف القناة الصفراوية، فرت تسسيق قناة الصفراوية، نتوافرة حاملة، فرت التسبب في هذه الآفات ناتجة من هذه الدراسة أن الإصابة بداء المتورقات (فاشيولا) يؤدي إلى أضرار مرضية أو تأثير تدمر للجهاز الكبدى الصفراوي.

الكلمات المفتاحية: داء المتورقات (الفاشيولا) المزمن، متقيبات الكبد، الماشية، الأبقار الكبدية، الفحوصات السريرية.
Introduction

Parasitic diseases are the major obstacle in growth and development of animal health (1). Fascioliasis is a disease caused by fluke infestation in the livers and bile ducts of sheep and cattle. However, sheep are more susceptible to the disease than cattle. Horses, deer and goats may also harbor liver flukes and humans too can be infected (2). Two highly infective species are identified as Fasciola hepatica and Fasciola gigantica. F. hepatica survives in a variety of climatic conditions while F. gigantica is generally dominant in tropical areas of many countries of the world. These flukes mainly attack the liver, where they reside and graze on the mucosa of the bile duct and hepatic parenchyma resulting in massive tissue damage (3). Fascioliasis is distributed almost worldwide and parallels that of endemic animal illness. A liver fluke burden can result in deterioration in wool quality, reduced meat and milk production and ill-thrift in young stock. Stock may die from heavy burdens. If flukes are detected in livers at the abattoir, the livers are condemned as unsuitable for human consumption (4, 5). The gross pathological changes of the liver may have an irregular outline, being pale and firm. The ventral lobe is most commonly affected and reduced in size. The liver pathology of chronic disease is characterized by hepatic fibrosis and hyperplastic cholangitis. Several different types of fibrosis may be present and include post-necrotic scarring, ischemic fibrosis and peribiliary fibrosis. Fluke eggs may sometimes stimulate a granuloma-like reaction with obliteration of the affected bile ducts as a consequence. In bovines calcification of bile ducts, enlargement of the gallbladder and aberrant migration of the flukes is more common (6). The aim of the current study is to detect the gross and microscopic lesions which are found in bovine chronic Fascioliasis in local and Friesians breed at Sulaimani region and compare these to the different species and breeds in other parts of the world according to the literature.

Materials and methods

This study was involved collection fifty (50) samples of bovine livers which were showed evidence of infestation with flukes were collected from Sulaimani abattoir / Qeregol region. This study was carried out from March to April, 2013. During that, fifty samples from chronic bovine liver Fascioliasis at different ages (Local and Friesians breeds) were collected after examination of livers grossly, palpation, incision and recorded any changes which observed in the tissue texture of liver parenchyma and bile duct. Specimens from the liver were fixed with 10% neutral buffered formalin for at least 24 hours and then routinely processed. These tissues were embedded in paraffin and sectioned at 5µm thickness, then the sections were stained with the Haematoxylin and Eosin stain then microscopically examined and histological findings were recorded. The histopathological sectioning and staining by H&E stain was performed in research laboratory, department of pathology and forensic pathology, school of medicine, university of Sulaimani.

Results

Gross lesions:

Liver gross lesion showed enlargement in size (hepatomegaly) and pinpoint hemorrhages on the parietal surface of the liver, paleness in some areas which was due to the necrotic or damaged region, congestion, firm whitish areas within parenchyma regarded as fibrosis and abscess with calcification in few cases. Whereas the gross lesions of bile duct included engorgement of bile the duct. By cut section, the swollen and the fibrotic bile ducts were clearly visible and blocked by twisted flukes. The bile ducts were found filled with blackish brown exudate. Variable gross lesions were seen in (Fig. 1).

Microscopic lesions: Microscopically, thirty liver samples (60%) showed cell
Fig. 1: Variable gross lesions as indicated by arrow heads, A. Hemorrhage, B and C. Fibrosis, D. abscess and E. Engorgement of bile duct.

Fig. 2: Histopathological findings in chronic liver Fascioliasis.
Table 1: Histopathological changes of liver accompanied with chronic Fascioliasis.

<table>
<thead>
<tr>
<th>Lesions</th>
<th>No. of samples</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver cirrhosis</td>
<td>22</td>
<td>44</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>Portal fibrosis</td>
<td>26</td>
<td>52</td>
</tr>
<tr>
<td>Pericellular fibrosis</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Extensive fibrosis</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>Bile duct hyperplasia</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>Cell swelling</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Fatty change</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Bile pigment</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Neutrophils infiltration</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>Eosinophils infiltration</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Congestion</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td>Telangiectasis</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td>Abscess</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Infiltration of mononuclear inflammatory cells</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>Immature liver fluke</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>Mature liver fluke</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Bile duct metaplasia</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Adenomatous hyperplasia</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td>Papillomatous projections</td>
<td>10</td>
<td>20</td>
</tr>
</tbody>
</table>

swelling in which hepatocytes increased in size and characterized by opaque cytoplasm with centrally located nuclei as seen in Fig. 2 and Table 1. Two samples (4%) showed fatty change in which clear vacuoles appeared in the cytoplasm with peripherally located nuclei; two samples (4%) showed bile pigment which was accumulated as yellowish brown concretions in the dilated bile ducts or canaliculi (Fig. 3). Ten samples (20%) showed abscesses which included necrotic debris surrounded by a large numbers of inflammatory cells mainly neutrophils, histiocytes, eosinophils and lymphocytes and bounded by fibrous connective tissue capsule (Fig. 4); fourteen samples (28%) showed congestion which was due to enlargement or dilation of central vein and sinusoids which were engorged with a large number of RBCs. Telangiectasis with temporary dilation of sinusoidal capillaries which contained RBCs were noticed in fourteen samples (28%) as shown in (Fig. 5).

Fig. 3: A. Cell swelling; multiple cleared vacuoles are present within the opaque cytoplasm with centrally located nuclei, B. Fatty change: Large clear vacuoles are present within the cytoplasm which pushes the nucleus to the periphery, C. Bile pigment: Yellowish brown materials are accumulated among hepatocytes in bile canaliculi, D. Coagulative necrosis: Eosinophilic cytoplasm with pyknosis and karyolysis (H&E X400).
Fig. 4: A. Large abscess with central calcification, necrotic debris and surrounded by fibrous connective tissue capsule B. Focal micro abscesses (Two arrow heads) consisted of polymorphonuclear leukocytes surrounded by thin layers of fibrous connective tissues (H&E X40).

Fig. 5: A. Dilated sinusoids engorged with RBCs, B. Congestion of sinusoid and engorgement with a large number of RBCs (H&E X400).
Twelve samples (24%) showed immature liver fluke within dilated bile ducts with peri-inflammatory cells and 4 samples (8%) showed mature liver fluke in the lumen of the intra-hepatic bile duct that destroyed the bile duct with heavy infiltration of inflammatory cells in the fibrous connective tissue between newly formed bile ductules (Fig. 6). While twenty four samples (48%) showed infiltration of mononuclear inflammatory...
Figure 8: A and B. Liver cirrhosis in which liver shows dark and light nodules; The dark nodule regarded as regenerative nodule while the light one regarded as fibrosis. C and D. Primary biliary cirrhosis which shows extensive proliferation of fibrous connective tissue around the intra-hepatic bile ductules with peri-infiltration of mononuclear inflammatory cells of the bile duct (H&E X100).

Figure 9: A. Bile duct hyperplasia (Black arrow) appeared as proliferation of the epithelial lining of the bile ducts surrounded by a thin layer of fibrous connective tissue, B. Bile duct hyperplasia (Black arrow) showing proliferation of the epithelial lining of the bile ducts which formed gland-like pattern (H&E X40).
Figure 10: A and B. Adenomatous hyperplasia which shows hyperplastic proliferation of biliary epithelium with the formation of newly formed bile ductules which are forming papillomatous projections toward the lumen (H&E X40). C and D. Bile duct metaplasia of the epithelial lining of the newly formed bile ductules into mucous secretory cells (H&E X100, X400).

Figure 11: A and B. Proliferation of fibrous connective tissue surrounding a group of hepatocytes as indicated by two arrow heads (H&E X400), C. Portal fibrosis showing extensive fibrous connective tissue proliferation in the portal area with infiltration of mononuclear inflammatory cells, D. Extensive fibrosis with infiltration of mononuclear inflammatory cells (H&E X40).
cells with lymphocytes, histiocytes which were observed in the proliferated fibrous tissue among hyperplastic newly formed bile ductules. Sixteen samples (32%) showed neutrophils infiltration and 10 samples (20%) showed eosinophils infiltration (Fig.7). Microscopically bile duct lesions revealed, primary biliary cirrhosis in forty samples (80%) which showed extensive proliferation of fibrous connective tissue around the intra-hepatic bile ductules with peri-infiltration of mononuclear inflammatory cells of the bile ducts; Twenty two samples (44%) showed liver cirrhosis in which liver showed dark and light nodules; The dark nodule was represent as regenerative nodule and the lightly one regarded as fibrosis. The proliferated fibrous connective tissue around the regenerative hepatic lobules was infiltrated with inflammatory cells (Fig.8). Sixteen samples (32%) showed bile duct hyperplasia which appeared as proliferation of the epithelial lining of the bile ducts (Fig. 9); fourteen samples (28%) showed adenomatous hyperplasia which appeared hyperplastic proliferation of biliary epithelium with the formation of newly formed bile ductules; ten samples (20%) showed bile duct metaplasia of the epithelial lining of the newly formed bile ductules into mucous secretory cells with infiltration of eosinophils and papillomatous projections in which hyperplastic proliferation of the epithelial lining of the main bile ducts forming papillomatous projections toward the lumen (Fig.10). This damage was caused by the spiny surface of the flukes and their feeding habits on the hyperplastic bile ducts. Twenty six samples (52%) showed portal fibrosis; twelve samples (24%) had extensive fibrosis and 8 samples (16%) had pericellular fibrosis (Fig.11).

Discussion

The gross pathological changes observed on the liver were enlargement and pin-point hemorrhages on the parietal surface of the liver which is partly due to the inflammatory changes and fibrosis that took place in the liver parenchyma. The affected bile ducts were markedly thickened and there was fibrosis in the duct wall. These findings are congruent with the earlier findings of Velusamy et. al. (7) who proved that thickening of bile duct is due to chronic nature of Fasciola infection and later, this finding is in agreement with a previous studies of Okaiyeto et. al. (8), while the same results were achieved most of the above lesions in chronic Fasciolosis in Black Bengal goats (4). Histopathologically, the chronic hepatic cells showed variable degrees of cell swelling, degeneration and deposition of bile pigment. Similar results were also recorded by MacGavin et. al. (9). Correspondence with study of Talukder et al (4) who reported atrophy, necrosis and fatty changes in chronic Fascioliasis in goat. In our study liver abscess cases is in agreement with study of Sayed et. al. (10) who proved that invasion of the liver by migrating immature liver fluke damages the tissue and provide anaerobic condition, that allowed the germination and proliferation of bacteria that induce hepatocellular necrosis and abscess formation. In present study liver paranchyma showed infiltration of poly morphonuclear inflammatory cells; neutrophil and eosinophil with mononuclear inflammatory cells which were observed in the proliferated fibrous tissue among hyperplastic newly formed bile ductules. Our results are in agreement with two previous studies of Doy et. al. and Wiedosari et. al. (11,12) who proved that the migration of immature liver flukes through the tissue causing hemorrhage and irritation, and brought the cellular inflammatory reactions and this study is partially correlated with the finding reported by Dow et. al. (13) who mentioned the infiltrations of inflammatory cells in experimentally produced Fascioliasis in calves. Reported liver cirrhosis and biliary cirrhosis in our study are in agreement with previous studies who described cirrhosis as portal, multilobular and biliary according to the fibrous connective tissue distribution (14-16). In the present observation, glandular hyperplasia of bile duct walls, which produced a thick and adenomatous picture was marked. It was mostly seen in the main ducts containing many adult flukes. Several
studies detected that the presence of mature flukes within the lumen of intra-hepatic bile ducts which brought a continuous irritation and led to hyperplastic proliferations which were emphasized by papillomatous projections and formation of newly formed bile ductules. This damage was caused by the spiny surface of the flukes and their feeding habits on the hyperplastic bile ducts (17-19).

**In conclusion**

the current study revealed that the most common lesions in chronic Fascioliasis were biliary cirrhosis while in acute cases were cellular degeneration. Opposite our expectations the jaundice cases were less common in our findings.

**References**


