Ultrasonographic and histopathological evaluation of hepatic lipidosis in sheep with cobalt deficiency

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ABSTRACT

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Ultrasonographic and histopathological evaluation of hepatic lipidosis in sheep with cobalt deficiency. Online Journal of Veterinary Research, 12 (2) : 23-32, 2008. The aim of the present study was to describe the ultrasonographic and relevant histopathological changes in Ossimi sheep with hepatic lipidosis caused by cobalt deficiency. A total of 34 animals with illthrift and anemia were studied. Of all, 23 were confirmed to have low serum cobalt and Vitamin B12 levels (0.24 ± 0.09 µmol/l and 0.15 ± 0.07 µg/l, respectively) compared with control (0.640 ± 0.34 µmol/l and 0.31 ± 0.11 µg/l, respectively). Sheep were examined with a real-time ultrasound system using 5-MHz linear and convex transducers. Ultrasound guided liver biopsy, blood and serum samples were obtained from each animal at examination. Ultrasonographic-hepatic changes were recorded in 21 (91.30%) out of 23 sheep with cobalt deficiency. On the bases of histopathological findings, diseased sheep were recorded to have mild (n=5), moderate (n=7) and severe lipidosis (n=11).

Ultrasonographically, severe lipidosis showed diffuse increased echogenicity of hepatic parenchyma. However, focal hyperechoic lesions with various shape, size and position were also visualized in mild and moderate lipidosis. Liver size was increased significantly (P<0.05) in severely affected sheep compared with controls. Histopathologically, macrovaculations, congestion of hepatic sinusoids, biliary hyperplasia and infiltration of portal area with inflammatory cells and connective tissues were recorded in moderate and severe lipidosis. The hematological and biochemical findings supported the diagnosis. Result of the present study demonstrates high a sensitivity of ultrasonography for diagnosis of lipidosis in sheep.
INTRODUCTION

Hepatic lipidosis is one of major metabolic disorders of animals, which develops when the hepatic availability of lipogenic and glucogenic products is imbalanced (Goff and Horst, 1997). In sheep, hepatic lipidosis was found not only to cause liver insufficiency but also result in sudden death (Malone et al. 1984). Cobalt deficiency, vitamin E deficiency, pregnancy toxemia, toxicosis and negative energy balance were recorded as causes of hepatic lipidosis in sheep (Ulvund, 1990; Radostits et al. 2007; Menzies et al. 2004). Cobalt deficiency has been described in sheep to cause fatty hepatic degeneration that has been termed ovine white liver disease (OWLD) (Ulvund, 1990a). The clinical, biochemical and pathological findings of hepatic lipidosis associated with cobalt deficiency had been described in sheep and goats in different localities of the world (Pearson, 1987; Black et al. 1988; Johnson et al. 1999; Johnson et al. 2004).

Ultrasonography has been used successfully to describe the normal hepatic criteria of white Alpine sheep (Braun and Hausammann, 1992). Ultrasonography was found an efficient tool for diagnosis of fascioliasis (Scott et al. 2005) and secondary hepatic changes in sheep with various diseases causing right-sided heart failure (Scott and Penny, 2000; Scott and Sargison, 2001). Ultrasonographic findings of focal and diffuse liver fatty changes in cattle were clearly documented (Iwao, 1987; Mohamed et al. 2004). However, ultrasonographic findings of hepatic lipidosis in sheep have not been described. Consequently, the objective of this report was to describe the ultrasonographic findings of hepatic lipidosis associated with cobalt deficiency in Ossimi sheep under field condition and the associated histopathological and biochemical changes.

MATERIALS AND METHODS

Animals: Thirty-four Ossimi sheep at 13-23-month of age and with 21-27 (24 ± 2.98) kg body weight were studied during 2003-2006. Ten clinically healthy female Ossimi sheep aged 12-17 month and weighed 26- 30 (27 ± 1.68) kg were used as control group.

History and selection of cases: Examined sheep (28 non pregnant females and six males) were raised in small scale flocks at rural areas of kafer El-Sheikh governorate. The feeding system was depending grazing with supplementation of roughage only. Animals with hepatic lipidosis were suspected after obtaining clinical history and performing competent physical examination (Kelly, 1984). Illthrift, anemia and history of poor worm control were constant clinical signs in all sheep and upon which the cases were preliminary selected.

Ultrasonographic examination: Ultrasonographic examination was carried out with computed real-time scanner using 5-MHz linear and convex transducers (Acuson128XP/10, USA). Sheep were examined while they were in standing position following the methods described by Braun and Hausammann (1992) with fixation of time-gain compensation and power output for all examinations. Ultrasound-guided liver biopsy was carried out in all suspected cases using semi automatic fine biopsy needle 16G (Stericut, TSK, Japan).
Hematological and biochemical examinations: Blood samples were collected from each animal under investigation; the first sample was collected on heparin and used for hematological examination. The second sample was collected into plain tube and stored at 4°C overnight, and the serum was separated. Serum was examined for cobalt, vitamin B12, vitamin E, total protein, AST, ALP and glucose. Serum cobalt was estimated with Graphite Furnace atomic absorption spectrometer (640Z) equipped with GTA-100 graphite furnace and PSD-100 auto sampler (Varian, USA). Serum vitamin B12 and vitamin E were determined by using HPLC system (2150, LKB). Total protein, AST, ALP and Glucose were estimated spectrophotometrically following standard methods using commercial test kits (Spinreact, Spain).

Fecal examination: Fecal samples were obtained from each animal under investigation and examined for presence of faciola eggs and or other gastrointestinal parasites.

Postmortem and histopathological examinations: Eleven sheep with severe lipidosis were slaughtered due to poor condition. Samples were obtained for estimation of liver cobalt. All liver specimens that obtained either by biopsy or at postmortem examinations were processed sectioned and stained (Hematoxilin and eosin and Sudan yellow). According to the reported criteria and classification of lipidosis (Cebra et al. 1997; Johnson et al. 2004), livers were classified to have mild, moderate and severe lipidosis.

Liver cobalt estimation: For cobalt analysis of the liver, 10-g aliquots were dryashed at 480°C. The dry-ashed samples were then dissolved in 0.6 mol/L hydrochloric acid. The cobalt concentration of the samples was then determined by absorbance at 240.7 nm with Graphite Furnace atomic absorption spectrophotometer (640Z, USA).

Statistical analysis: Mean and standard deviation for each variable were calculated. Differences between means were calculated by one way ANOVA with post-hoc LSD multiple comparison test using SPSS software statistical program (SPSS for windows ver. 15.00, USA).

RESULTS

23 sheep were confirmed to have low cobalt and Vitamin B12 levels ((0.24 ± 0.09 µmol/l and 0.15 ± 0.07, respectively) compared with control (0.640 ± 0.34 µmol/l and 0.31 ± 0.11 µg/l, respectively). Adjunct to low serum cobalt and Vitamin B12, six cases were confirmed to have low vitamin E levels (1.1 ± 0.27 mg/l versus 2.3 ± 0.34 mg/l). Moreover, fasciola eggs were recovered from three cases.

Ultrasonographic-hepatic changes were recorded in 21 (91.30 %) out of 23 sheep with lipidosis associated with cobalt and Vitamin B12 deficiency only. Six cases were recorded to have low level of vit E adjunct to low serum level of cobalt and Vitamin B12, whereas three sheep were found to be infested with Faciola spp. All cases with severe hepatic lipidosis (n=11) showed diffuse increased echogenicity of liver parenchyma, which appeared in form of hyperechoic dots distributed in the liver parenchyma. In four cases, the liver showed granular appearance in comparison with control (Figure 1, 2). Moderate lipidosis (n=7) was characterized by presence of large focal hyperechoic areas in the hepatic parenchyma in four cases (Figure 3), and small focal areas in three cases. Ultrasonography has yielded positive result in three
out of five sheep with mild lipidosis, which was characterized by presence of focal hyperechoic area(s) distributed in the liver parenchyma. Focal lesions were presented with various shape, dimensions (0.5-3cm) and localization. Liver size significantly (P<0.05) increased in severely affected sheep compared with control. However other ultrasonographic measurements showed no significant variations (Table1). Ultrasound-guided biopsy has provided 100% positive results at histopathological examination.

**Table1.** Mean and SD of ultrasonographic measurements of liver size, diameter of portal vein and caudal vena cava(cm) in healthy sheep and that with hepatic lipidosis.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Liver size (cm)</th>
<th>Portal vein (cm)</th>
<th>Caudal vena cava (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control (n=10)</td>
<td>11.95 ± 0.83a</td>
<td>1.43 ± 0.10</td>
<td>1.65 ± 0.12</td>
</tr>
<tr>
<td>Mild lipidosis (n=5)</td>
<td>12.13 ± 0.71</td>
<td>1.45 ± 0.20</td>
<td>1.67 ± 0.09</td>
</tr>
<tr>
<td>Moderate lipidosis (n=7)</td>
<td>12.60 ± 0.79</td>
<td>1.49 ± 0.07</td>
<td>1.70 ± 0.11</td>
</tr>
<tr>
<td>Severe lipidosis (n=11)</td>
<td>12.92 ± 0.54b</td>
<td>1.47 ± 0.10</td>
<td>1.73 ± 0.08</td>
</tr>
</tbody>
</table>

Values with different superscript in the same column are significantly different at P < 0.0

*Figure 1.* Ultrasound scan of liver of lamb with diffuse fatty infiltration, there is increased echogenicity of the liver parenchyma. L= Liver parenchyma, C = caudal vena cava, Arrows = hepatic veins, G = Gall bladder, Cr = Cranial, Cd = caudal, O = Omasum.
Gross examination of liver was carried out only for 11 cases with severe lipidosis that were slaughtered due to their poor body condition. Grossly, livers appeared pale and friable without changes of the hepatic vasculature or gall bladder. In severe and moderate lipidosis, there was dominance of the macrovacules with congestion of hepatic sinusoids, infiltration of portal area with inflammatory cells and fibrous connective tissues as well as hyperplasia of epithelial lining (Figure 4, 5). In mild lipidosis there was predominance of the microvacules in two cases. Moreover, one case showed combination of macro and microvacules. Mild congestion of the hepatic sinusoids was only recorded in one case.
Erythrocytes showed significant decrease in sheep with mild lipidosis (P<0.05) and in sheep that were moderate to severely affected (P<0.01) compared with control. Furthermore, hemoglobin and PCV% showed significant decrease (P<0.01) in diseased sheep compared with control and in mild cases (P<0.01) compared with moderate and severe cases (Table 2). Differential leukocytic count showed eosinophilia in 18 cases and hypersegmentation of neutrophils in 19 cases without significant changes in the total leucocytic count.

Liver cobalt was estimated only in the slaughtered cases (n=11), the level was 0.014 µg/gDM. Biochemical analysis showed presence of significant changes not only between diseased and control cases but also among the disease cases themselves (Table 2).

### Table 2. Hematological and biochemical findings in Ossimi sheep with hepatic lipidosis associated with cobalt deficiency.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 10)</td>
<td>(n = 5)</td>
<td>(n = 7)</td>
<td>(n = 11)</td>
</tr>
<tr>
<td>RBCs(x10⁶/l)</td>
<td>9.34 ± 2.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.08 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.22 ± 0.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.12 ± 0.59&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HGb(g/dl)</td>
<td>13.20 ± 0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.1 ± 0.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.0 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.1 ± 22&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>33.36 ± 1.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.64 ± 1.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.88 ± 0.83&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.73 ± 0.90&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>TP(g/dl)</td>
<td>6.84 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.70 ± 0.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.65 ± 0.40&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.62 ± 0.36&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>AST(IU/L)</td>
<td>58.00 ± 5.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>111.4 ± 7.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>184.6 ± 9.72&lt;sup&gt;c&lt;/sup&gt;</td>
<td>188.3 ± 11.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALP(IU/L)</td>
<td>100.60 ± 5.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>410 ± 31.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>584.2 ± 22.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>594.9 ± 30.46&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>3.53 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.1 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.06 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.07 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
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</tbody>
</table>

Values with different superscript in the same row are significantly different.

### DISCUSSION

In the present study, ultrasonographic-hepatic changes and relevant pathological findings of hepatic lipidosis in Ossimi sheep with cobalt deficiency under the field conditions were described. Cobalt and vitamin B12 deficiency are usually diagnosed based on the biochemical and pathological findings. However, ultrasonography is not routinely used in sheep for diagnosis of lipidosis. Diseased cases were selected based on the clinical signs and serum level of Cobalt and Vit B12 with exclusion of cases positive for fasciola infestation and Vitamin E deficiency to narrow the differential diagnosis. A sensitivity of 21/23 was recorded for the ability of ultrasonography to diagnose lipidosis in 21 sheep with low serum cobalt and vitamin B12. This result was similar to that recorded in cattle for diagnosis of diffuse liver diseases (Iwao, 1987). However, Acorda et al. (1995) reported that digital analysis had higher sensitivity and specificity for diagnosis of lipidosis in cattle than ultrasonography. Based on histopathological classification (Johnson et al. 2004), all severely affected cases had diffuse increased echogenicity of the liver, which indicate they were in advanced state. This result coincided with that recorded in cattle (Acorda et al. 1995).
On contrary, all cows with liver fatty changes showed diffuse hypoechoic or heterogenic hepatic lesions (Cebra et al. 1997). The moderate and mild cases had focal hyperechoic foci distributed in the liver parenchyma. Focal lesions were presented with various shape, dimensions and localization. Similar finding was recorded by Mohamed et al. (2004) who described the focal fatty liver changes in cattle as unevenly distributed lesion, with geographic boundaries and not associated with displacement of adjacent vessels. Moreover, in human, focal fatty liver changes were presented with various echogenic pattern, shape, dimensions and localization (Caturelli et al. 1992). In the present study, it is suggested that hyperechoic feature of liver with lipidosis may be attributed to changes in the nature of liver tissues which increase the attenuation of the ultrasound beam. This suggestion is supported by Szebeni et al. (2006) who found that in patients with bright liver due to fat deposition, the average attenuation of ultrasound was 1.21+/-0.06 dB/cm per MHz compared with bright liver due to inflammatory reaction (0.80+/-0.03 dB/cm per MHz) and normal liver (0.68+/-0.03 dB/cm per MHz). The authors added that the dominant histopathological finding of bright liver with inflammatory reaction was connective tissues, and lipid deposition due to fatty changes.

The liver size was significantly increased only in the severely affected cases (P < 0.05) compared with control, but we can’t conclude and generalize its use for diagnosis of such condition. Liver size determination provided significant information for diagnosis of an outbreak subacute hepatic fascioliasis (Scott et al. 2005). It is suggested that the liver size has limited information and can’t predict the problem of lipidosis except in severe cases. It is reported that increased liver size is suspected when increase more than 25cm (Braun and Hausammann, 1992). The diameter of the portal vein and caudal vena cava showed no significant changes neither between control and diseased nor among diseased sheep. Contrary to that recorded in cattle, it is found that diffuse fatty changes in cattle were associated with dilatation and stricture of intrahepatic vessels (Cebra et al. 1997).

Ultrasound guided liver biopsy from all of cases confirmed the ultrasonographic findings and provided positive results in 23 cases (100%). This result coincides with that reported in humans (Caturelli et al. 1992). It is suggested that ultrasound guided liver biopsy is important for excluding the disease conditions causing diffuse (Scott et al. 2005), and focal hepatic lesions (Lofstedt et al. 1988). However, in human, ultrasonographic diagnosis of lipidosis was found sufficient and biopsy was not recommended, if no other indications for biopsy exist (Szebeni et al. 2006).

Gross liver examination was carried out only for severe lipidosis in 11 cases that were slaughtered due to their poor body condition. The livers were pale and friable; their histopathological findings were similar to the remaining severely affected cases. Livers of diseases sheep exhibited histopathological findings similar to those from lamb with OWLD and Omani goats with lipidosis (Ulvund, 1990a; Johnson et al. 1999; Johnson et al. 2004; Al-Habsi et al. 2007), which was macrovacule formation of lipidosis was observed in all moderately and severely affected cases. This finding indicates an advanced state of the disease in the examined animals and its presence with connective tissue may support and explain the visualization of hyperechoic lesions on ultrasonographic examination. Biliary hyperplasia was a common finding in cases with moderate and severe hepatic lipidosis. Similar finding was observed in lamb with OWLD (Mcloughlin et al. 1984) and severely affected Omani goat (Johnson et al. 2004). Mild cases varied widely from the moderately and severely affected cases; the finding coincided with that recorded in goat (Braun and Hausammann, 1992).
The histopathological differences among the diseased sheep might reflect the progressive changes associated with lipidosis and degree of liver tissue reaction.

Hematological findings showed results similar to that which were recorded in lamb with OWLD (Ulvund, 1990) and goats with cobalt deficiency (Al-Zadjali et al., 2004). The differential leukocytic count showed eosinophilia in 17 cases. This finding only reflects the status of worm control and it has no direct relation to the cobalt deficiency. Similar result was recorded by Al-Zadjali et al. (2004) who reported that parasitic infestation was found to be contributing factor for a cause of eosinophilia in Omani goat with cobalt deficiency. Moreover, hypersegmentation of neutrophils was recorded in 19 cases of the diseased sheep. A similar result was described by Romero et al. (1999) who recorded that hypersegmented neutrophils were found as constant finding of cobalamin deficiency in human.

Serum total protein showed significant (P<0.05) decrease in moderate and severe cases compared with mild cases. Decrease in the serum total protein may be due to impairment of liver function. Ulvund (1990a) suggested that decrease of serum protein associated with lipidosis might be due to effect of cobalt deficiency on protein metabolism. AST and ALP activities were significantly elevated not only between the diseased and control but also among the diseased cases. The level of enzymes reflects the degree of liver affection. Although AST is not a liver specific enzyme, it has been reported to be elevated in sheep with OWLD (Mitchell et al. 1982) cattle (Cebra et al. 1997), and Omani goat (Al-Habsi et al. 2007) with hepatic lipidosis. Also ALP activity was reported to be elevated in chronic liver disease, hepatic fibrosis, cholangitis, obstruction, cholestasis and most commonly with hepatic lipidosis (Smith, 1996). Glucose was significantly (P<0.01) decrease in diseased sheep compared with control, the finding attributed to the cobalt deficiency and negative energy balance associated with anorexia. Cobalt was found necessary for propionate metabolism and consequently its deficiency could affect the glucose level. A similar finding was recorded by Ulvund (1990a). On contrast, Omani goat with experimental lipidosis didn't exhibit hypoglycemia (Al-Habsi et al., 2007).

The results of histopathological, hematological and biochemical examination of this study could support the ultrasonographic findings and confirm the diagnosis of hepatic lipidosis was due to cobalt and Vitamin B12 deficiency. This study demonstrates the importance of examining liver status via ultrasonography when confronted with illthrift, or hepatopathy in sheep, even when they are receiving sufficient dietary supplementation. It could be also concluded that ultrasound percutaneous liver biopsy is a safe and accurate tool to confirm the ultrasonographic findings. More experimental investigation needs to be done on the developmental stages of hepatic lipidosis and associated ultrasonographic changes to set the appropriate time for intervention.

REFERENCES


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