Ultrasonographic-hepatic changes associated with extrahepatic cholistasis in goats: An experimental study

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Abstract

The aim of the present study was to describe the uktrasonographic hepatic changes associated with extrahepatic cholistasis in goat. For this purpose four goats were used. Experimenta study was carried out by surgical obstruction of the common bile duct. Clinical, Ultrasonographic, clinicopathological and pathological parameters were evaluated. The clinical signs were anorexia, jaundice, dullness, dehydration and sever emaciation. Sonographic appearance of this group revealed enlargement of gall bladder and dilatation of cystic duct, common bile duct and intrahepatic ducts. Serum analysis revealed significant increase in the activity of ALT, AST, GGT and level of total bilirubin, bile acid and total protein persisted until the end of the experiment. Postmortem examination showed distended gall bladder, cystic duct, common bile duct and intrahepatic ducts. Microscopically, the liver biopsies obtained after 7 days revealed some degree of hepatocytic vacuolations, perivascular edema. The biopsies obtained after 14 days revealed hepatocytic necrosis surrounded by peripheral zone of fibroblastic proliferation and fibrosis in addition to congestion and hepatocytic degeneration. The biopsies obtained after 21 days revealed sever hepatocytic vacuolation, excess newly formed bile ductules and coagulative necrosis.

Introduction

Obstructive cholelstasis may be either extra or intrahepatic. In intrahepatic cholestasis, the biliary flow is obstructed in the intrahepatic bile ducts or in the region of hepatic hilus. In extrahepatic cholestasis, the obstruction usually occurs in the duodenal papilla of the common bile duct and seldom in the cystic or hepatic ducts (*Braun et al. 1995*, *Braun, 2003).* the most common causes of biliary obstruction in cattle are fascioliasis, fibrinous or purulent products and solid deposits; other less common causes include gall stones and tissue proliferation (*Rosenbeger and Grunder ,1970; Dirksen ,2002). Moreover, Braun (2005)* pointed out that the cause of cholestasis in cattle may be hepatocellular or obstructive in nature. Hepatocellular cholestasis results from sever impaired liver function, while the causes of obstructive one are mechanical in nature and include mainly fascioliasis, fibrinosuppurative inflammatory products and concretion, and occasionally gall stones or tissue proliferations. The inflammatory products resulting from cholangitis may also lead to impaired bile flow and compression of the main bile ducts by tumors, abscesses or peritoneal lesions is a rare cause of impaired bile flow.*Cable et al. (1997)* reported that cholelithiasis is uncommon in cattle but should be a differential diagnosis in cattle with signs of abdominal pain and serum biochemical evidence of cholestasis and the most common cause of cholecystitis and cholelithiasis in cattle is liver flukes. Cholelithiasis was associated with increase of serum concentration of total bilirubin, GGT, ALP, AST and sorbitol dehydrogenase

activities.Cows with cholistasis were suffering from reduced ruminal contract anorexia, icteric conjunctiva, oral mucous membranes, and the vestibule of vagina and dermatitis solaris which involved the muzzle, teats, udder and vu Cholestasis is also associated with increased serum concentration of bile ac and bilirubin and high activity of AST, GLDH, SDH and GGT (Braun, 19 *Braun, 2005)*.

Asignificant increase in activity of GGT, glutamate dehydrogenase, sorb dehydrogenase and marked decrease in protein concentration in plasma w observed in cattle with ruptured gall bladder (*Braun et al. 2005).Braun (20* mentioned that ultrasonography is avaluable aid in the diagnosis of cholesta and can differentiate between hepatocellular and obstructive cholestasis, wh in hepatocellular cholestasis there is no dilatation of bile ducts; frequently, th are characteristic changes in the liver; such as fatty or congestive changes.

Liver biopsy was recognized as a valuable diagnostic tool in many species animals when altered liver function tests suggested hepatic disease to present. Moreover, the histological examination of liver biopsies allows the t and the extent of many pathological changes to be precisely assessed, t improving the accuracy of the diagnosis and allowing more specific treatmen be undertaken. He also added that liver biopsy has been used routinely for estimation of copper concentration in the liver of sheep and the diagnosis of f liver syndrome in dairy cattle (Simpson, 1985).

Liver biopsy is the definitive means of diagnosing hepatic disease. Histolog examination of the liver provides valuable information regarding etiology severity of the disease process and most cases of liver disease are diffuse the sample will be representative of the disease. He added that samples car obtained blindly, but ultrasonographic guidance decrease the risk complications and the biopsy may not be advised in an animal with clinica clinicopathological evidence of coagulopathy or hepatic abscess beca haemorrhage or contamination of the peritoneal cavity may result (Kahn, 200

To the best of our knowledge, little is known about the cholistasis in goat consequently, the aim of the present study was to evaluate the ultrasonograp hepatic changes associated with extrahepatic cholistasis in goats.

Materials and methods

Animals

Four native breed Zaraibi goats were ued in this study. The experimental st was approaved by the committee of animal welfare and ethics at Alexan University. Experimental extrahepatic cholestasis was carried out at Department of Surgery, Faculty of Veterinary Medicine, Alexandria Univer according to the method described by *Ducharme (1990)*. Animals were fas for 24hours before the operation. Water was allowed for about four hours bef the operation.

A course of antibiotic was given for 4days before the operation and continued successive three days. Penicillin-streptomycin was given as 1ml /25 kg. B weight throught the intramascular route. The operation was performed while animal in lateral recumbent position. The animal was casted on its left side. four legs are fixed to the operating table and the head is rested on the sa table. Sedation was obtained by intramascular administration of xylazine Hc 0.1mg / kg body weight .The operation was carried out under the effect of l

anesthetic associated with a suitable sedative anesthetic. 5-10ml of 2 % lidocaine Hcl was used locally in form of line and in some cases in form of L-shape. The animal was then subjected to routine preparation for a septic surgery by clipping and shaving the area of the operation and animal covering with a sterile towels fixed all over the body except the head. The operation was performed by induction of laboratory wound in the right flank about 3 fingers breadth behind the last rib (fig.5),

incision was carried out in the skin, muscles and peritonieum. Hemostasis was done by pressure and ligation by artery forceps. Dissection was performed carefully to identify and isolate the common bile duct .Duodenal flexure was taken as a guide for isolation of the common bile duct. Ligation was done by using silk N0.1 just before passing to the duodenum via duodenal papilla (fig. 7). Reposition of the protruded viscera was carried out and the abdominal cavity was examined carefully for any haemorrhage. Suspension of penicillin-stretomycin was poured and then the laboratory wound was closed in usual manner. Peritonieum and muscles were sutured in a continuous pattern using chromic cat gut No.1, While subcutis and skin were closed by an interrupted pattern using silk No.2. Animals were kept under observation and daily dressing of wound was considered and stitches were removed after healing within 7-10 days post operation.

Biochemical analysis

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyle transferase (GGT), serum total proteins, total bilirubin and bile acids were measured specrophotometerically using commercial test kits following standard methods.

Ultrasonographic examination and liver biopsy

Ultrasonographic examinations were made by the technique described by *(Braun et al., 1995)* to evaluate normal hepatic structure. The hair was clipped and the skin shaved between the sixth intercostal space and a hand breadth behind the last rib. After the application of transmission gel to the trancducer (6 MHZ linear), the goats were examined caudal to the last rib to the sixth intercostals space.Each intercostal space was examined dorsally to ventrally with the trancducer held parallel to the ribs. The group of goats having experimental extahepatic cholestasis was subjected to hepatic ultrasonography especially the biliary system at interval 1, 4, 7, 11, 14, 18 and 21 days from the experiment while the group of goats with 4-days fasting was subjected to hepatic ultrasonography at 5th day after fasting.The liver biopsy specimen was obtained from each goat using semi-automatic biopsy needle with ultrasonographic guidance according to the technique described by (Braun et al., 1995) at the end of each week for 3weeks in the group (A) and at 5th day after fasting in group (B) (Fig. 8).

The biopsy samples were taken where the parenchyma was thick as possible and there was no major blood vessel. The biopsy needle was introduced at right angle to the body surface and directed towards the liver where it appeared on the screen with a distal shadow (Fig. 9). Then one or two liver biopsy samples were obtained and fixed in 10 % neutral buffered formalin.

Postmortem examination

Liver of each necropsied goat with experimental extrahepatic cholestasis carefully examined by nacked eye for detection of any gross lesions 21days from complete common bile duct ligation.

Histopathological studies

Tissue specimen was collected from liver biopsies of goats with experime extrahepatic cholestasis. Specimens were fixed in 10 % neutral buff formalin solution. After fixation, Specimens were washed in tap water and passed through the routine paraffin embedding technique (dehydratio ascending grades of the ethyl alcohol, clearing in a series of xylene and passed through a series of melted paraffin wax, embedded and put in par blocks). later on, the paraffin blocks were subjected for microtomy to pre paraffin sections of 3-5 microns thickness which were stained with Ma hematoxylin and eosin (**Culling, 1983**) then subjected to the light microscopy

Statistical analysis

Data analysis was carried out using statistical software program (SPSS windows Version 15.0, SPSS Inc. Chicago, USA). The results are presente means ± SD. One-way ANOVA with repeated measures was used to deter significant differences between values at different time points. Sphe assumption and Wilks' Lambda were examined for significance. Whe significant difference was found, Bonferroni *post-hoc* multiple comparison was performed for further evaluation. Differences were considered signifi when $P < 0.05$.

Results

Clinical findings

Goats of this group showed several signs. Partial anorexia which progresse complete anorexia at the end of the period. Jaundice began with slight ye progress to dark yellow cojunctival mucous membrane. Signs of abdominal constipation, dullness and dehydration were also evident. Animals were unable to stand and end with sever emaciation (Figure 1).

Sonographic appearance

Ultrasonography of this group showed sever distention of biliary system b with enlarged gall bladder and cystic duct then progressed to involve all bi tract (common bile duct and intrahepatic ducts) decreasing the area of he parenchyma as a result of compression of the surrounding hepatocytes (Fi 2,3).

Serum analysis

Serum total bilirubin level was significantly ($p < 0.01$) increased one day operative and significant increase in the serum level persisted throughout following days of the experiment until reached the maximum value at the er the experiment. Bile acids level was significantly ($p < 0.01$) increased one post-operative and significant increase in the serum level persisted throug the following days of the experiment until reached the maximum value at the of the experiment. Serum GGT activity was significantly ($p < 0.01$) increased day post-operative and persisted with significant increase until reached maximum value at the end of the experiment. Serum ALT activity significantly ($p < 0.01$) increased one day post- operative and persiste significant increase until reach the maximum value at the end of the experim

Serum AST activity was significantly ($p < 0.01$) increased one day post-operative and persisted in significant increase until reach the maximum value at the end of the experiment.Serum total proteins level was significantly ($p < 0.05$) increased one day post- operative and persist in significant increase until reached the maximum value at the end of the experiment (Table 3, Fig. 44).

Pathological findings

Macroscopically, liver of this group of goats showed areas of yellowish brown colouration, severe distention of gall bladders, cystic ducts, common bile ducts and intrahepatic ducts (Figure 4,5).Microscopically, the liver biopsies of this group revealed different changes according to the time that the biopsy was taken and healthy status of the animals. The biopsies obtained after seven days from experiment revealed some degree of hepatocytic vacuolations, hydropic degeneration, perivascular edema as well as coagulative necrosis of hepatic cells in some animals (Figure 6).The biopsies obtained after forteen days revealed prescence of numerous changes in different animals. Diffuse hepatocytic necrosis with deep bluish pigmentations was seen in some areas (Figure 7).The foci of hepatocytic necrosis appeared to be surrounded with peripheral zone of fibroblastic proliferation and fibrosis (Figure 8). In some other areas; the hepatocytic degenerations (vacuolar and hydropic)and necrosis appeared to be associated with an excess of golden brown bile pigmentation, congestion and haemorrage (Figure 9).The biopsies taken after three weeks revealed sever hepatocytic vacuolation, an excess of newly formed bile ductules in addition to coagulative necrotic hepatocytes (Figure 10).

Table (3): Serum biochemical parameters in goats with experimental complete biliary obstruction.

Parameter	Post-operative (days)						
	Before	1	4	7	11	14	18	21
Bilirubin (mg/dl)	0.50 ± 0.00	3.20 ± 0.33**	3.13 ± 0.13**	13.00 ± 2.93**	13.47 ± 2.50**	13.87 ± 3.32**	15.17 ± 3.87**	18.67 ± 4.37**
Bile acids (Mmol/L)	29.63 ± 6.33	235.33 ± 40.43**	235.33 ± 32.37**	284.33 ± 43.22**	313.00 ± 6.80**	271.00 ± 60.50*	301.00 ± 16.26**	326.67 ± 26.67
GGT (U/L)	35.00 ± 2.86	176.67 ± 44.89**	243.33 ± 65.13**	313.33 ± 93.33**	373.00 ± 96.50**	326.67 ± 91.34*	533.33 ± 44.09**	590.00 ± 49.32**
ALT (U/L)	4.00 ± 0.00	10.00 ± 2.05**	12.33 ± 2.03**	27.00 ± 8.08**	24.33 ± 6.88**	28.67 ± 6.88**	34.00 ± 9.86**	58.33 ± 13.19**
AST (U/L)	7.00 ± 0.00	22.33 ± 4.45**	20.67 ± 5.36**	24.33 ± 3.52**	26.00 ± 2.08**	21.00 ± 3.88**	34.00 ± 7.00**	53.00 ± 6.50**
T.P (g/dl)	5.90 ± 0.26	6.83 ± 0.60*	6.57 ± 0.69	7.77 ± 0.79*	8.17 ± 0.44*	7.67 ± 0.33*	9.00 ± 1.50*	10.90 ± 2.41*

Variables with different superscript letters at the same raw are significantly different at $P< 0.05$

Figure 1. A goat with cholistasis showing yellowish discoloration of mucous membrane

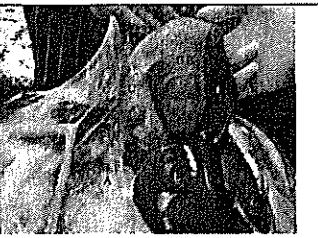

Figure 2. (A) Dilated gall bladder (G.B), cystic d and Common bile duct (C).

Figure 3: Sever adhesion between gall bladder and liver. GB=
Dilated gall bladder, L= Liver, A= Yellowish mesentry.

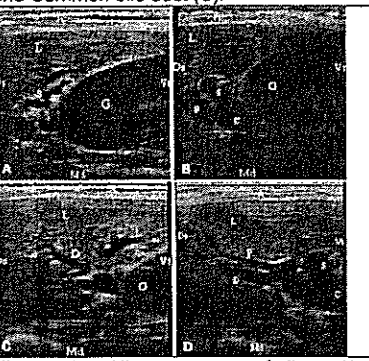

Figure 4a: Ultrasonogram of liver (1 day operative) showing dilated gall bladder (G) and duct (S). The trancducer was placed at ventral right 10th intercostal space. A= Abdominal w Liver, Ds= Dorsal, Vt= Ventral, Md= MedialFig Ultrasonogram of liver (4 days post-operative) s dilated gall bladder (G),cystic duct (S) and c bile duct (C). T; Figure 4c. Ultrasonogram of days post-operative) showing dilated intrahepa (D). ; Figure 4d. Ultrasonogram of liver (11day operative) showing dilated biliary system.

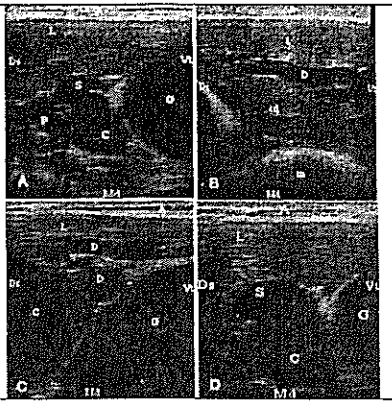

Figure 5 Ultrasonographic progr changes in the liver and biliary s caused by cholistasis at 14 days, 1 and 21 days post-operative. Dilatat cystic duct(A), intrahepatic duct (B,C common bile duct(D)

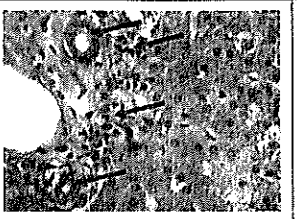

Figure 6: Liver after extrahepatic cholestasis showing mild fatty vacuolation (blue arrows) in addition to sever hydropic degeneration and ruptured hepatocytes (black arrows). (H and E. X 400)

Figure 7. Liver after extrahepatic cholestasis sh an area of diffuse hepatocytic vacuo hydropic degeneration and rupture in addit perivascular edema (arrow).H and E. X 400.

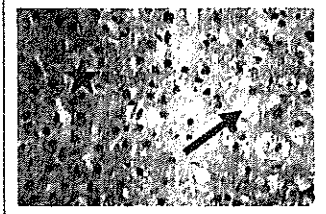

Figure 8: Liver after extrahepatic cholestasis showing an area of coagulative necrosis (asterisks) besides other area of hydropic degeneration (arrow) of the hepatic cells. Hand E. X 400.

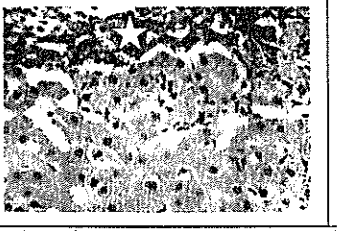

Figure 9: Liver after extrahepatic cholestasis sh an area of diffuse hepatocytic necrosis in assc to an area of deep bluish pigmentations asterisk). H and E. X 400.

Discussion

Clinicaly, goats with extrahepatic cholistasis showed loss of appetite ,gradual decrease in body weight and dehydration. Yellow coloration of the conjunctival mucous membrane appeared in the second day post-operation and increased rapidly in the following days.. This result is attributable to the bile stasis and regurgitation to the systemic circulation. These results agree with that observed in cattle by *Braun et al. (1995*

Ultrasonogram of this group revealed dilatation of the gall bladder and cystic duct followed by the dilatation of the common bile duct and lastly the intrahepatic ducts as a result of distal obstruction. This result was similarly observed in cattle by *Braun et al. (1995) and Braun (2005).* The intrahepatic ducts are normally invisible but when dilated, it became visible and run parallel to the portal vein branches. This result was similar to that obtained *by Banholzer and Weigold (1993).*

The serum levels of bilirubin and bile acids was significantly increased throughout the period of the experiment started in the second day reaching the maximum level in the last day (21th day).This result may attributed to the bile stasis and regurgitation of bile to the liver parenchyma and eventually the systemic circulation. These results agree with that reported in cattle by *Braun et al. (1995), Cable et al. (1997) and Braun (2005).*

The serum activity of gamma- glutamyle transferase (GGT) was in a significant increase throughout the days of the experiment reaching maximum activity in the last day. These results agree with that reported in cattle by *Cable et al. (1997)*

and Braun (2005). The marked increase in the activity of GGT indicate bile ducts were involved *(Braun et al; 1995).*

The serum activity of alanine amino transferase (ALT) significantly i one day post-operative reaching the maximum activity in the last da results agree with that reported in dogs by *Abdel-Raof (1994).* This may be attributed to increased hepatic permeability rather than necros effect of bile on hepatocytes or may be due to surgical trauma of liver et al; 1987). The serum activity of aspartate amino transferase(AST) i one day post-operative reaching maximum activity in the last day. T agree with that reported in cattle by *Braun et al. (1995) and Cat (1997).*This increase may attributed to the effect of bile on the hepatocyt

The serum level of total protein significantly increased throughout the da experiment. These results agree with that of *Braun et al. (1995).*

Regarding to the pathological results:

Macroscopically: this group of goats showed enlarged gall bladder w duct, common bile duct and intrahepatic ducts as a result of retention the biliary ducts.This result as reported *by Abdel-Raof (1994) and Bra (1995).*

Microscopically: the biopsies of liver of this group showed he degeneration (vacuolar and hydropic) in some areas progressed to severly and diffusely distributed in the liver in addition to necrosis in son This may be attributed to continous irritant effect of bile on the hepatocy is in agreement with that of *Abdel-Raof (1994) and Braun et al. (1995).*

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