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RESEARCH ARTICLE

Hepatorenal Syndrome in Dogs with Experimental Extrahepatic Cholestasis

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Abstract

Hepatorenal syndrome (HRS) is a unique form of functional and potentially reversible renal failure secondary to liver diseases. It occurs typically in kidneys that are histologically normal. In this study, the hepatorenal syndrome was observed in a model of extrahepatic biliary stasis. Seven mature male mongrel dogs were subjected to complete surgical ligation of common bile duct at the duodenal region Clinical, ultrasonographic, , histopathological examinations along with biochemical analysis for liver enzymes and urinalysis were performed before and throughout the experimental periods. Clinical observations post-surgery revealed icteric mucosa, abdominal pain, weight loss, dehydration, orange-colored urine, bilirubinurea, and clay feces. Biochemical analysis showed an abrupt increase in the mean values of serum blood urea nitrogen and creatinine from the 3rd day till reaching the peak at the 2nd week post-surgery. A strong positive correlation was detected between BUN and creatinine, and serum total and direct bilirubin. Ultrasonographic findings of liver revealed a progressive dilatation of gallbladder and the common bile duct from the 3rd day post-ligation, with an increase in the echogenicty of the liver, whereas, the kidneys demonstrate normal renal parenchyma and dimensions. Histopathological examination of the kidney revealed hemorrhage, vacuolation, hyaline droplet were observed in renal tubules and edema and yellowish-brown granules were seen in the interstitial tissue, but the significant renal changes that refer to renal failure weren't detected,. In conclusion, the dogs are considered a suitable model for studying hepatorenal syndrome and also there is an association between obstructive jaundice and renal failure.

Keywords: Hepatorenal syndrome, Obstructive jaundice, Dog, Biochemical analysis Pathological features.

Introduction

Hepatorenal syndrome (HRS) is a form of functional and potentially reversible renal failure which occurs in kidneys secondary to liver disease, especially with cirrhosis and ascites, due to the absence of recognized biomarkers, the diagnosis of HRS relies on a combination of clinical and laboratory criteria [1,2].

HRS in human is a life-threatening condition that needs to be diagnosed and treated rapidly in order to improve the clinical outcome [3] There are two clinical types of HRS, type I and type II based on the severity of the condition. Type I HRS is the type develops rapidly with poor prognosis, showing a doubling of the serum creatinine level within two weeks, whereas type II HRS has slower

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rising renal retention parameters. Type I HRS patients have a mortality percent of 50% two weeks after diagnosis, approaching up to 100% within months [4, 5]. The diagnosis of HRS depends upon the presence of hepatic failure; high levels of serum creatinine and the absence of other causes of renal failure such as bacterial infection, shock, and the use of drugs that are toxic to the kidneys with no evidence of renal parenchyma diseases [6].

Patients with obstructive jaundice developed renal failure; similar clinical and pathological features were consistent with those found in HRS. The association of obstructive jaundice and acute renal failure in human has been recognized well since 1843 when a patient with fulminating ascending cholangitis became oliguric and died [7, 8]. The risk of renal failure is increased in obstructive jaundice patients with decreased intravascular volume; the presence of severe hepatic decompensation leads to splanchnic and systemic vasodilatation and decreased effective arterial blood volume to the kidneys. These changes lead to increase renal vasoconstriction and progressive oliguric renal failure [9]. The deeper jaundice the greater the risk of acute renal failure [10].

The major causes of extrahepatic biliary obstruction in dogs are pancreatitis, neoplasia, biliary mucoceles, cholangitis, and cholelithiasis [11]. Cholestatic liver diseases are defined as an accumulation of bile acids in the liver that causes an inflammatory process and liver injury [12]. The most clinical signs of biliary tract diseases in dogs are jaundice, abdominal pain, and ascites [13]

In complete common bile duct obstruction, patients showed pale feces and dark urine. Depending on the development of complications such as gallbladder rupture with leakage of sterile or septic bile the patients can show more severe signs such as fever, acute abdominal pain, and septic shock [14].

The present study aimed to throw the light on the association between obstructive jaundice and renal failure in the absence of significant renal changes in a model of extrahepatic biliary stasis in dogs.

Materials and Methods

The protocol of this study was approved by the Animal Experimentation Ethics Committee of Zagazig University (ZU–IACUC), protocol number ZU – IACUC/2/F/59/2019.

Seven mature male mongrel dogs aging from 6-11 months and weighting (20-25 kg) were included in this study. They were housed indoors in individual cages labeled with dog identifying. They were kept under uniform nutritional regimen, fed *adlibitum* on non-commercial diet (chicken and meat extract) and had free access to water. Dogs were dewormed and vaccinated against viral diseases according to the regulation of veterinary authorities during the one month acclimatization period. After acclimatization periods, all dogs underwent the surgical common bile duct ligation to induce extrahepatic obstructive cholestasis.

Experimental procedures

A more updated protocol for ligation of the common bile duct at the duodenal region was applied (Figure. 1A) according to Kakabadze *et al.* [15]. After the surgery, follow-up and postoperative treatment were applied; Meloxicam ampoules (non-steroidal anti-inflammatory) produced by AMRIYA PHARM. IND. - Alexandria – Egypt in a dose 0.2 mg/kg/24 hours IM for 3-5 days and cefotax as Cefotaxime Sodium (antibiotics) produced by Egyptian Pharmaceutical Company (EPICO) in a dose 20 mg/kg/ 24 hours IM for 5 days, together with daily disinfection of the wound with Betadine 10%. Animals were kept on soft bedding. Closed observation to all examined dogs was done at regular intervals (3^{rd} day, 1^{st}, 2^{nd}, 3^{rd}, 5^{th} and 8^{th} week post-surgery).

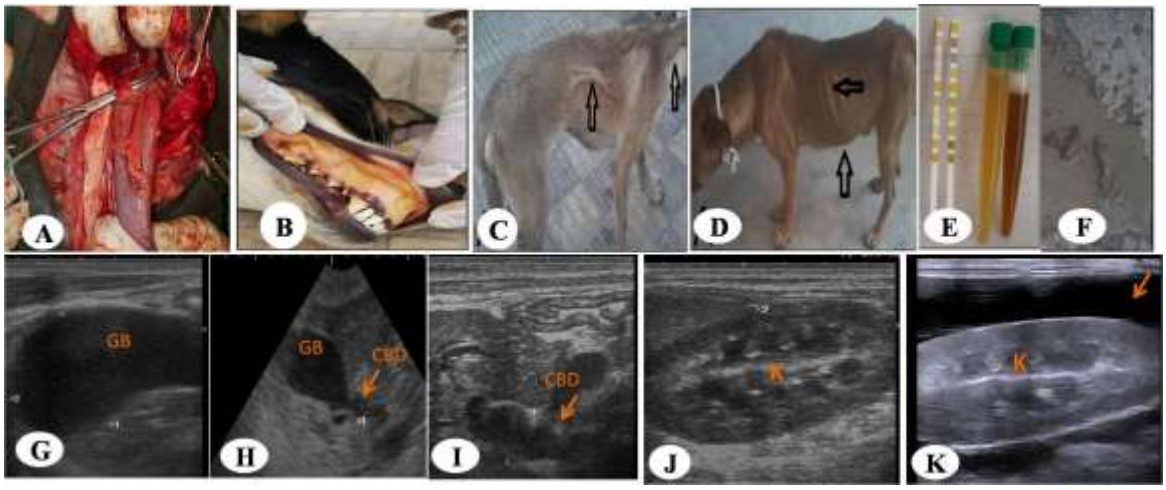

Figure 1: (A) Surgical ligation of common bile duct, (B) Icteric oral mucosa, (C) Dog showed emaciation and severe dehydration (skin fold test, black arrow). (D) dog showed severe emaciation, ribs (arrow), lumber vertebrae, pelvic bones were evident and abdominal distension (arrow).(E) Urine samples examination using urine strip test, color (orange & yellow) and (F) Clay diarrhoea. (G) Ultrasonography of the gallbladder (GB) at the ^{2}nd week post-surgery (64.1x39.2 mm), (H) Gall bladder (GB) was (60 x 22.4 mm) and comon bile duct (CBD) was 7.9 mm at the 8t^{h} week. (I) CBD was16.3 mm at the 2nd week. Ultrasonography of the kidneys demonstrates (J) normal renal parenchyma post-surgery and normal kidney with ascites (K).

Clinical examination

Clinical examination was thoughtfully performed before and during different experimental periods (0 day, 3rd day, 1st, 2nd, 3rd, 5th and 8th week post-surgery). The preliminary general examination was applied; general appearance, behavior, state of appetite, skin tent test, sunken eyes, urine, and fecal characters were noted and body weight was recorded. Special attention was paid to visible mucous membranes (conjunctival and oral mucosa) and sclera. The examination of mucous membrane was applied in good day light, in some cases, artificial light was used. Closed observation of all cases during the experimental period was done and any clinical alterations were recorded [16].

Ultrasonographic examination

Abdominal ultrasound (liver parenchyma, gall bladder, common bile duct and kidneys) was performed using a high definition ultrasound system equipped with 5 MHz micro convex and 9 MHz linear transducer Sonoscape Ultrasound (A5V, China) during different experimental periods.

Biochemical analysis

Five ml of blood was collected via cephalic vein puncture into a clean dry centrifuge tube without anticoagulant for serum separation at zero day, and during the different experimental periods; 3rd day, 1st, 2nd, 3rd, 5th and 8th week post-surgery. Serum liver enzymes were determined according to the methods described by Reitman and Frankel [17] for Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Tietz [18] for serum gamma glutamyl transferase (GGT) levels. Serum total protein and albumin were estimated according to the method described by Doumas *et al.* [19] and Henary *et al.* [20] respectively. Serum total and direct bilirubin levels were estimated according to Malloy and Evelyn [21]. Serum blood urea nitrogen (BUN) and creatinine were estimated by kinetic method using test kits according to the method described by Patton and Crouch [22] and Houot [23] respectively.

Urinalysis was applied at zero day, and during the experimental period; 3rd day, 1st, 2nd, 3rd, 5th and 8th week post-surgery. Samples were obtained by spontaneous urination or by catheterization. Physical examination of urine samples including color, volume, transparency, sediment, and odor was applied. Comburg multiple test strips (Boehringer Monnheim, Germany) were used for rapid urinalysis.

Postmortem and histopathological examinations

Postmortem examinations were performed for all dogs (dead or euthanized). The different lesions were recorded and samples were collected. Tissue specimens (liver and kidney) were taken and fixed in 10% buffered formalin. The histopathological slides were prepared and reviewed according to Jorge *et al.* [24].

Statistical analysis

Data were statistically evaluated by a software program (SPSS, version 16) using One- way ANOVA to compare between the findings during the experimental periods and at 0 day. Results were considered statistically significant at $P < 0.05$ [25]. The correlation coefficient and significance of the correlation between liver and kidney function tests were evaluated by Pearson correlation analysis according to Feldman, *et al.* [26].

Results

Clinical examination

Table, 1 and Figure1 (B, C, D, E and F) summarize the observed symptoms and their onset of appearance post-surgery. No postoperative complications were recorded, all dogs showed jaundice, orange colored urine and clay feces from the 3rd day post-surgery. The normal color of fecal samples restored gradually from the 3rd week and the feces were normal by the 8th week. Abdominal pain, excessive emaciation, dehydration, and reduced appetite were also recorded. It is worthy to mention that 2 of 7 cases showed depression, recumbency, and they become oliguric at the 9th day post-surgery and death follow in the 2nd week, one of them showed severe ascites. All dogs showed a significant ($p< 0.05$) decrease in their body weight from the 1st week post-surgery (17.20± 1.16 kg) till the 3rd week (16.67± 1.76 kg), after that an improvement in their weight was recorded from the 4th week (18± 1.53 kg).

Table (1): Clinical findings according to the onset of appearance after complete surgical ligation of common bile duct in morngerl dogs.

Symptoms	Onset of appearance	Onset of disappearance	No. of affected /total.	%
Icteric m.m	3rd - 5th day	4th -6th week	7 of 7	100
Yellowish skin	3rd - 5th day	4th -6th week	7 of 7	100
Orange color urine	3rd- 7th day	3rd -5th week	7 of 7	100
Clay feces	3rd- 7th day	3rd -5th week	7 of 7	100
Diarrhea	4th day	3rd week	1 of 7	14
Abdominal pain	5th day	2nd -6th week	7 of 7	100
Polyphagia	6th day	Euthanasia (8th week)	1 of 7	14
weight loss and reduced activity	7th day	3rd -6th week	7 of 7	100
Dehydration	7th day	Death (2nd week)	2 of 7	28
Ascites	7th day	Death (2nd week)	1 of 7	14
Reduced appetite	9th day	3rd -6th week	6 of 7	86
Depression and recumbency	9th day	Death (2nd week)	2 of 7	28
Death	2nd week	2nd week	2 of 7	28

Ultrasonographic findings

The cystic, hepatic, lobar and interlobular ducts could not be identified on pre-surgical scans. While normal gallbladder was seen as anechoic, round structure lies in the right paracostal region between right medial and quadrate lobes. The normal bile duct was small 2-3 mm in diameter. Progressive distension of gallbladder and common bile duct started from the 3rd day post-surgery reaching the peak at the 2nd week, and a slight reduction was recorded after there with some increase in echogenicty of the liver (Figure 1 G to I). Ultrasonography of the kidneys before and during experimental periods revealed a normal picture, that renal medulla is the least echogenicty, followed by the renal cortex, and then the renal sinus with normal dimensions in different periods (Figure 1 J and K).

Biochemical analysis

Biochemical findings were illustrated in (Figure 2); regarding the liver function tests, a significant increase in serum liver enzymes AST, ALT, and GGT, and total and direct bilirubin started from the 3rd day post-surgery till reaching the peak at the 2nd week. While, their levels began to decline from the 3rd week post-surgery and the lowest levels were recorded at the 8th week. Meanwhile, a significant reduction in serum total protein and albumin were recorded from the 3rd day until the end of the study without significant changes in serum globulin. Regarding to kidney function tests, there was a significant

increase ($p< 0.05$) in the mean value of serum BUN and creatinine starting from the 3rdday post-surgery (47.67± 6.40 and 1.09± 0.11 mg/dL, respectively), 55.25± 7.17 and 1.25± 0.05 mg/dL at 1st week, till reaching the peak at the 2ndweek post-surgery (60.99± 0.99 and1.33± 0.01 mg/dL), then their levels showed gradual reduction from the 3rdweek (49.02± 1.17 and1.17± 0.15 mg/dL), 42.39± 3.04 and 0.75± 0.10 mg/dL at 5th till reaching the lowest level at the 8thweek (36.14± 1.05 and 0.72± 0.02 mg/dL) (Figure 2 D and E).

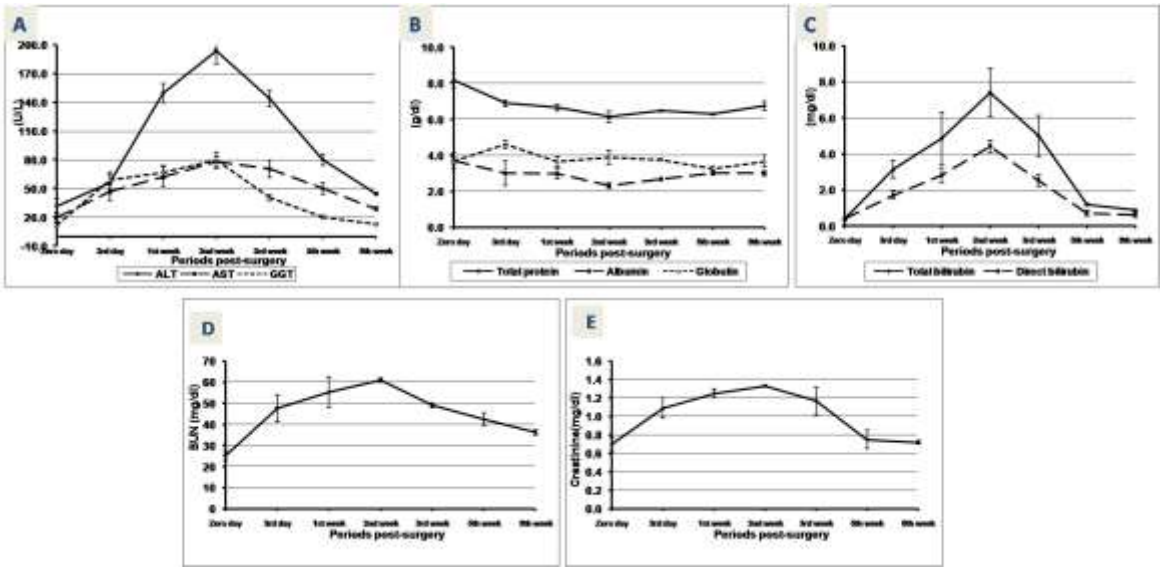

Figure 2: The mean values of liver function tests after complete surgical ligation of bile duct in mongrel dogs; A) Serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT), B) **Serum total protein, albuin and globulin, C)** Serum total and direct bilirubin**, and kidney function tests D) Serum urea and E) Serum creatinine from zero day till the 8th week post-surgery.**

As presented in Table (2) there was correlation between kidney function tests including BUN and serum creatinine and liver function tests as ALT, AST, GGT, total bilirubin and direct bilirubin. BUN were significantly correlated in a positive mode with serum ALT (r= 0.89, $p<0.01$), AST(r= 0.94 , $p<0.002$), GGT(r= 0.91, $p<0.003$), total bilirubin (r= 0.92, $p<0.001$) and direct bilirubin(r= 0.95, $p<0.001$). Also, strong positive correlation were recorded between serum creatinine and serum ALT (r= 0.87, $p<0.01$), AST(r= 0.88, $p<0.01$), GGT(r= 0.95, $p<0.001$), total bilirubin (r= 0.97, $p<0.001$) and direct bilirubin (r= 0.95, $p<0.003$).

Table (2): Pearson correlation coefficients between liver and kidney function tests after complete surgical ligation of bile duct in mongrel dogs.

	ALT	AST	GGT	Total protein	Albumi	Total bilirubin	Direct bilirubi	BUN
AST	0.94**							
GGT	0.79*	0.80*						
Total protein	-0.67	-0.79*	-0.50					
Albumin	-0.82*	-0.88*	-0.68	0.90*				
Globulin	-0.07	0.06	0.47	0.12	-0.11			
Total bilirubin	0.94**	0.92**	0.91**	-0.60	-0.83*			
Direct bilirubin	0.94**	0.89*	0.92**	-0.57	-0.81*	0.99**		
BUN	0.89*	0.94**	0.91**	-0.80*	-0.88*	0.92**	0.91**	
Creatinine	0.87*	0.88*	0.95**	-0.52	-0.73	0.97**	0.95**	0.92**

Correlation is significant at *$p<0.05$, and **at $p<0.01$. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: gamma glutamyl transferase and BUN: Blood urea nitrogen.

Urinalysis revealed orange colored urine by the 3rd day till the 2nd week post-surgery, then the intensity of color regress and appeared nearly similar to the normal color by the 3rd week. Reduction in the volume of the voided urine was observed from 9th day till 2nd week post-surgery. All samples were clear and neither sediment nor abnormal odor was observed at different times of examination. Bilirubinuria (7of 7dog) were recorded from the 3rd day post-surgery and their levels increased gradually till reaching the maximum by the 2nd week, then regress gradually from the 3rd week. Proteinuria was recorded in 3 of 7 dogs.

Post-mortem examination

Postmortem examination was applied in 2 dogs that died at the 2nd week post-surgery, after that euthanasia was applied to 3 dogs at the 5th week and to 2 dogs at 8thweek post-surgery. Out of seven dogs, four were severely emaciated, 6 dogs had icteric mucous membranes and 5 had yellowish staining ribs, intestine and mesentery. Intra-abdominal fluid effusion (about 10 L) was recorded within the abdominal cavity (1 of 7 dogs) with fibrinous peritonitis that covering liver and kidney (Figure 3 A and B), enlarged gallbladder, common and hepatic bile ducts (6 of 7 dogs). Paleness of the kidney was observed in 2 of 7 dogs that died in the 2ndweek post-surgery (Figure 3 C).

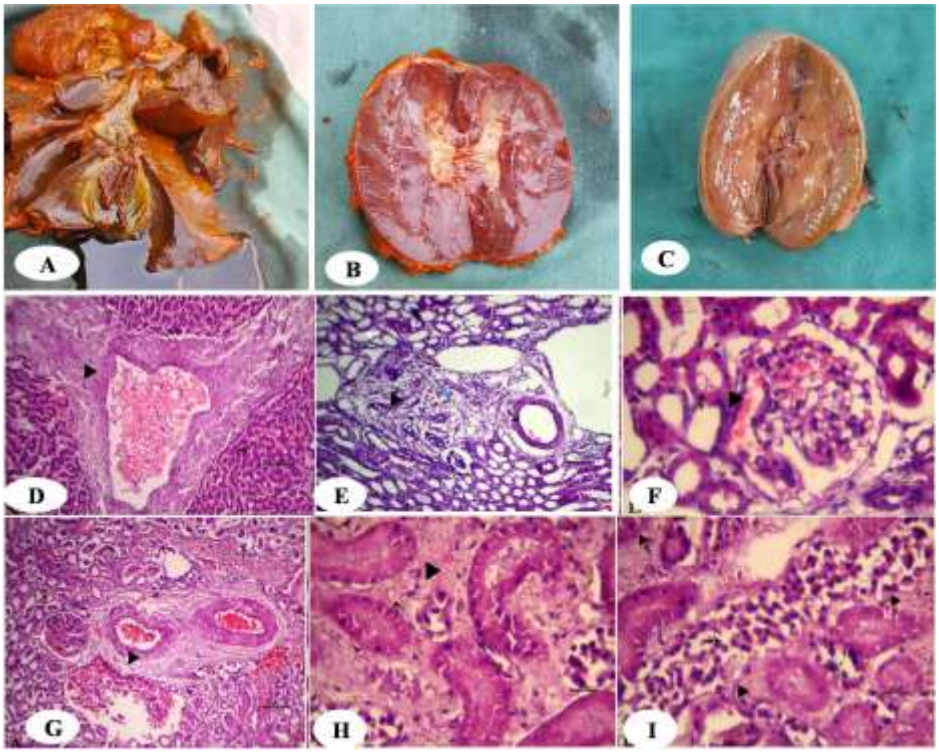

Figure 3: A) liver of dog suffered from ascites and peritonitis, B) kidney of the same dog, C) Kidney of dog with acute renal failure in the 2nd week showed paleness .D)The portal area of liver showing congestion of portal vein and cirrhosis characterized by proliferation of fibrous tissue replaced bile duct (arrow head) with congestion of hepatic sinusoids (arrow) (H&E, 300X). E) The kidney showing interstitial lymphocytic infiltrations and mild fibrous proliferation (arrow head) with hemorrhages (blue arrow) and dilated blood vessels (black arrow) in the interstitial tissue, some renal tubules showing hydronephosis (white arrow) (H&E,300 X). F): the kidney showing hemorrhage and vacuolation in the epithelial cells of glomuleri (arrow head) and hyaline droplet in the lining epithelium of some renal tubules (arrow) (H&E,1200 X). G) the kidney showing congestion of blood vessels with thickening and hyalinization in its wall and perivascular proliferation of fibrous tissue (arrow head) with epithelial cast inside lumen of some renal tubules (black arrow) and hemorrhage inside the glomeruli (white arrow, H&E,300 X) H): the kidney showing edema (arrow head) and yellowish brown granules of bile pigment (arrow) in the interstitial tissue (H&E, 1200 X). I): the kidney showing interstitial lymphocytic infiltrations (arrow) with yellowish brown granules of bile pigment (arrow head) (H&E, 1200 X).

Histopathological Findings

Liver showed congestion of central vein with vacuolation in some hepatocytes and yellowish-brown granules of bile pigment in hepatocytes were also detected. The portal area of liver showing congestion of portal vein and cirrhosis characterized by proliferation of fibrous tissue replaced bile duct with congestion of hepatic sinusoids (Figure 3 D). The kidneys showed hemorrhage, vacuolation in the epithelial cells of the glomuleri, hyaline droplet in the lining epithelium of some renal tubules, edema and yellowish-brown granules of bile pigment were seen in the interstitial tissue (Figure 3 E to I). Such findings in the kidney were clear in 1 dog that died in the 2nd week and 2 dogs that euthanized in 5th week post-surgery and less clear in the remaining dogs.

Discussion

In this study, the severity of the condition is often evidenced by the degree of jaundice. All dogs showed jaundice from the 3rd day post-surgery. Jaundice was attributed to the increase in the concentration of bilirubin in the blood owing to impairment of bile flow after common bile duct ligation and, the deposition of such substance on tissues mainly skin and mucous membranes. These results are similar to those obtained by Wang and Wei-Feng [27]. Abdominal pain, excessive emaciation and reduced appetite recorded in this study could be attributed to impaired bile flow in the extrahepatic biliary tree and the retention of bile salts in the liver with severe damage of hepatocytes and hepatic dysfunction occurred, these findings agreed with other studies [28-30].

Ascites was recorded in one dog might be owed to excessive accumulation of unconjugated bile acids in the blood. Unconjugated bile acids are cytotoxic and induce tissue inflammation and alter the permeability of vascular structures within the peritoneum leading to transudation of fluid into the peritoneal cavity, this results were previously mentioned by Owens *et al.* [31].

Orange colored urine and bilirubinuria could be attributed mainly to the blockage of enterohepatic circulation of bilirubin after common bile duct ligation. So the kidney becomes the main route for its elimination leading to impairment of kidney function [32, 33].

Increase serum creatinine levels with no or minimal proteinurea and decreased urine volume are characteristic to HRS. Clay-colored feces could be attributed to biliary obstruction, so bilirubin cannot be discharged or discharged in a small amount into the intestines, consequently fecal pigment reduced and clay-colored feces observed. This result is inconsistent with the findings of previous studies [33, 34]. As recorded by Utkan *et al.* [35] the reduction in body weight was attributed to hepatocellular dysfunction secondary to the impaired enterohepatic circulation of bile, which results in insufficient protein synthesis and gluconeogenesis, malabsorption of fats and steatorrhea and poorly absorbed fat-soluble vitamins..

In the present study, ultrasonography plays an important role in the early diagnosis of extrahepatic cholestasis. In contrast, it is of low value in diagnosing HRS. While, Duplex-Doppler ultrasound of intralobar arteries is a simple, non-invasive and effective method that enables the early detection of renal hemodynamic disturbances before renal dysfunction becomes clinically evident [36].

The main laboratory finding of HRS is an acutely elevated serum BUN and creatinine after ligation from the 3rd day and reaching the peak in the 2nd week post-surgery. It is worthy to mention that about 3 and 4 folds increase in serum BUN and creatinine were recorded in 2 of 7 dogs that died in the 2nd week.

There are no specific clinical findings in diagnosing of HRS, the more important aid is the biochemical findings. Significant increase in the level of serum BUN and creatinine which parallel with the marked increase in the level of serum total and direct bilirubin post-surgery from the 3rd day and the peak was at the 2nd week, then decrement began from the 3rd week. Our results coincided with Heinrich *et al*. [37] who recorded that complete common bile duct ligation in mice resulted in an immediate increase in bilirubin which remained constant during 14 days after ligation. Also, Crema *et al.* [38] recorded that

common bile duct ligation in dog resulted in an increase in serum total bilirubin concentration one week post-operative. Serum bilirubin rises in this study secondary to biliary tract obstruction. Bile is eliminated in the gastrointestinal tract but when the normal elimination is impaired, the serum concentration of bilirubin will elevate and hyperbilirubinemia occurs.

In cholestasis, the hepatic ability for metabolizing and excreting toxic substances is lost, causing renal overload. Therefore, the kidneys become the major route for elimination of toxic metabolites in the body, leading to increased exposure to nephrotoxic substances and disorders as kidney failure and hepatorenal syndrome [39, 40]. Hyperbilirubinemia has additionally been shown to attenuate the development of angiotensin II and induced arterial hypertension by reducing the production of superoxide and sodium reabsorption in the thick ascending loop of Henle [41].

The histopathological examination revealed loss of the normal histological architecture of the hepatic tissue after common bile duct ligation. This change was attributed to retention of the bile in hepatocytes with subsequent liver damage after biliary stasis. Awad *et al* [42], Aller *et al* [43], Panqueva [44] recorded similar consequences. Intrahepatic biliary obstruction includes the presence of bile within hepatocytes and canalicular spaces, and bile duct proliferation that may progress to cellular death and hepatic cirrhosis [45, 46, 47]. Also, hemorrhage and vacuolation in the epithelial cells of the glomuleri and yellowish-brown granules of bile pigment that were seen in the interstitial tissue could be attributed to the toxic effect of bilirubin on renal tissue. Van Slambrouck *et al*. [48] reported similar findings. It is worthy to mention that histopathological alteration of the renal tissue was not grave enough to cause such retention of urea and creatinine.

Finally, a fundamental question remains to be answered; why a considerable improvement occurs after the 2nd week post-surgery? We are currently unsure of the exact answers, so, there is an unmet need to understand such mechanism. In this study, the complete common bile duct ligation was performed surgically using Vicryl (polyglactin 910) suture material- Ethicon. The suture material holds its tensile strength approximately for two to three weeks in the tissue and is completely absorbed by hydrolysis within 56 to 70 days (8-10 weeks). So all biochemical parameters reached the peak in the 2nd week post-surgery and declined from the 3rd week with slight improvement in all dogs. All parameters reached the lowest level in the 8th week.

We can assume that the two dogs that died in the 2nd week might be suffered from HRS type that is highly fatal. Meanwhile, 5 of 7 cases showed clinical improvement parallel to the reduction in total and direct bilirubin.

Conclusion

The dog is considered a suitable model for studying HRS. In addition, obstructive jaundice and high total and direct bilirubin are the contributing factors for HRS.

Conflict of interest

None of the authors have any conflict of interest to declare.

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الملخص العربي

المتلازمة الكبدية الكلوية في الكلاب عقب جراحه تجريبية بوقف مسار العصارة الصفراوية

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تعرف المتلازمة الكبدية الكلوية بأنها نوع فريد من أنواع الفشل الكلوي الوظيفي نتيجة أمراض الكبد ويحدث عادة في الكلى التي تفقد وظيفتها مع بقاء النسيج محتفظا بشكله الطبيعي ،وفي هذه الدراسة تم ملاحظة متلازمة الكبديه الكلوية في نموذج تجريبي لوقف مسار العصارة الصفراوية بعد الكبد.حيث خضع سبعة ذكور من الكلاب الضالة الناضجة للربط الجراحي الكامل للقناة المرارية عند منطقة الاثني عشر.. تم إجراء الفحوصات الإكلينيكية والموجات فوق الصوتية والنسيجية والتحليل الكيميائي الحيوي لانزيمات الكبد وتحليل البول قبل وأثناء الفترات التجريبية.وقد كشفت الملاحظات الاكلينيكيه للحيوانات بعد الجراحة عن وجود اصفرار شديد في الأغشية المخاطية ، وآلام في البطن ، وفقدان الوزن، وجفاف الجلد ، و تغير البول إلى اللون البرتقالي والبراز الطيني من اليوم الثالث بعد الجراحة.وأظهرت نتائج التحليل البيوكيميائي للسيرم عن وجود زيادة حادة في قيم نيتروجين اليوريا في الدم والكرياتنين بداية من اليوم الثالث بعد الجراحة لتصل إلى الذروة في الأسبوع الثاني بعد الجراحة ومع وجود معامل ارتباط قوي بين قيم نيتروجين اليوريا في الدم والكرياتنين و البليروبين الكلي والمباشر. وكشفت نتائج الفحص بالموجات فوق الصوتية عن وجود زيادة تدريجية في حجم الحويصلة المرارية والقناة الصفراوية اعتبارا من اليوم الثالث للربط الجراحي ، مع زيادة ملحوظة في كثافة النسيج الكبدي ، وبينما أظهرت نتائج الفحص بالموجات فوق الصوتية عن خلو الكلي من اى تغير غير طبيعي في النسيج الكلوي وكذلك في الأبعاد .وكشفت نتائج الهستوباثولوجي للنسيج الكلوى عن وجود انزفة وفجوات وترسيبات من الهيالين داخل الانابيب الكلوية و كذلك لوحظ ترسيب حبيبات بنية صفراء اللون من صبغة البليروبين داخل الخلايا الكلوية ولكن هذه التغيرات لا تشير بوضوح لوجود فشل كلوي. في الختام ، تعتبر الكلب نموذجًا مناسبًا لدراسة متلازمة الكبد ويوجد ارتباط بين اليرقان الانسدادي والفشل الكلوي.