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Laparoscopic liver biopsy through cauterization in dogs

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ABSTRACT

Liver biopsy was collected through laparoscopy with simultaneous cauterization in the present study, whose principal aim was to evaluate the efficacy of the applied method and to study its effects in the liver of healthy dogs. Furthermore, we tried to verify the main haematological and biochemical profile alterations related to the liver function, and to investigate the viability of the fragments collected by histopathology. To attain this objective, 18 clinically healthy dogs were submitted to hepatic biopsy with forceps connected to the cautery, applying frequency energy at 45 watts performed cautery. Thirty-six liver biopsies through laparoscopy were conducted in the animals. In group 1, one hepatic fragment per animal was collected, in group 2, two hepatic fragments per animal were collected and in group 3, three fragments per animals were collected. Haematocrit and alanine-aminotransferase measurements were conducted to evaluate the animals at the pre biopsy period, at 1 and 2 h and day at 1, 3 and 7 post biopsy. The results revealed that the procedure was safe and effective for liver biopsy in dogs. There were no clinical and haemato-biochemical alterations related to the technique. The fragments collected were viable for histopathology. Liver biopsy through laparoscopy with simultaneous cauterization was effective and useful in dogs. This technique was successfully applied in other small species of animals.

Key words: Biopsy, Cauterization, Dogs, Laparoscopy, Liver

Laparoscopy permits precise and accurate site localization of the various internal organs of which most reported is liver (Fantinatti *et al.* 2003). It provides an excellent means for visualizing the surface of the liver and to detect changes in colour and texture (Tams 1996). One of the distinct advantages of laparoscopy is capability of biopsy of selected areas of the liver under direct visual control (Twedt 1990, Maiti *et al.* 2004, 2007).

Keeping these all in mind, an attempt was made in this study to evaluate the viability of the liver biopsy through laparoscopy by cauterization, considering the quality of the collected samples, as well as to study the subsequent clinico-haemato-biochemical and histopathological changes related to liver biopsy in normal dogs.

MATERIALS AND METHODS

This study was performed in 2 phases: In the first phase, the laparoscopic technique for liver biopsy by simultaneous cauterization was standardized in normal clinically healthy dogs. In second phase, this laparoscopic technique was applied to other small species of clinically healthy animals (sheep and goats) and laboratory animal (rabbits) for collection of liver biopsy.

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Clinically healthy 18 mongrel adult dogs (12 males and 6 females) weighing between 12 to 18 kg were used in this study. Biopsies (36) through laparoscopy were collected in these 18 animals, distributed in 3 groups of 6 animals each. In the group 1 one hepatic fragment per animal was collected. In the group 2, 2 hepatic fragments were collected, whereas in group 3, 3 hepatic fragments were collected per animal. The fragments were collected in the right lateral lobe, side by side, with intervals of 2 cm between them.

All the animals were kept in similar environmental condition with identical diet. They were housed in the kennel 1 week before the experimentation. All the animals were fasted for 24 h before anaesthesia. They were premedicated with atropine sulphate @ 0.04 mg/kg subcutaneously. After 15 min, general anaesthesia was achieved with combination of xylazine hydrochloride @1 mg/kg and ketamine hydrochloride @10mg/kg intramuscularly. The animals were submitted to the oro-tracheal intubation (in order to volatile anaesthetic halothane diluted in oxygen). The exploratory laparoscopy (minimal invasive surgical technique) for collection of liver biopsy was conducted in the epigastric region, in the midline and under the inferior margin of the diaphragmatic muscle. Mid-ventral abdomen, 2 cm posterior to the xyphoid was prepared aseptically. A 1 cm long incision was made through the skin of the prepared site, followed by blunt dissection through the muscle layers to the peritoneum.

Table 1. Mean±SE of serum alanine transaminase (U/L) recorded in animals of 3 groups

Groups	Before biopsy	After biopsy				
		1 h	2 h	1 day	3 days	7 days
1	20.67	26.00	28.00	48.67^{ab}	50.33	23.33^{ab}
	±0.67	±3.06	±5.29	±8.99	±15.90	±3.53
2	18.33	22.33	27.67	36.00b	30.33	16.33^{b}
	±3.28	±1.67	±2.96	±2.52	±9.87	±3.84
3	22.67	25.00	26.33	45.33^{ab}	40.33	21.33^{ab}
	±10.17	±8.50	±5.36	±4.67	±8.37	±7.42

Means bearing different superscripts (a, b) differed significantly between the groups (P<0.05).

Table 2. Mean±SE of serum aspartate transaminase (U/L) recorded three groups

Groups	Before biopsy	After biopsy				
		1 h	2 h	1 day	3 days	7 days
1	21.33^{bc}	26.67^{ab}	38.00^{a}	42.67	22.00^{b}	20.00^{bc}
	±2.91	±4.37	±1.15	±1.76	±5.03	±3.06
2	23.00^{bc}	21.00^{b}	30.67^{ac}	48.33	25.67^{b}	22.67^{bc}
	±2.65	±1.73	±1.76	±4.33	±2.19	±1.45
3	21.00^{bc}	22.00^{b}	23.67^{bc}	40.33	30.33^{ab}	22.67^{bc}
	±3.61	±2.31	±2.19	±2.19	±3.48	±2.40

Means bearing different superscripts (a, b, c) differed significantly between the groups (P <0.05).

Veres needle was introduced through the peritoneum into the abdominal cavity and connected with CO_2 insufflator to establish capnoperitoneum at a pressure of 10 mm of Hg. A 5 mm optical safety trocar with cannula was then inserted through the same puncture, after removal of Veres needle. Safety trocar was removed and a rigid 5 mm telescope (30 degree oblique) attached to the halogen light source by a flexible fiber optic cable and endocamera was guided through the cannula into the insufflated abdominal cavity. It was then progressed in a right cranial direction to visualize the free edge of right liver lobe. A second port was created through the skin over the corresponding site of right liver lobe under the guidance of telescope. Biopsy forceps with sharp and concave borders and connected with an electrocautery unit cable was inserted through the second port. Electrocautery was adjusted to cut and clotting with the intensity of 45 W of the potency of monopolar current to haemostasis. The hepatic tissue fragments/biopsies were collected from liver lobe (Fig.1) and simultaneously cauterized at the biopsy site (Fig.2) as per requirements in different groups, with dimensions proportional to the collection compartment of the applied biopsy forceps. After completion of the biopsy collection, each incision site was closed with a single mattress suture using non-absorbable suture material. Broad-spectrum antibiotic and analgesics were administered for 3 days post-laparoscopy.

Table 3. Mean±SE of serum alkaline phosphatase (U/L) recorded in animals of different groups

Groups	Before biopsy	After biopsy				
		1 h	2 h	1 day	3 days	7 days
1	20.39^{ab}	23.14	23.53^{ab}	24.51^{ab}	23.53^{ab}	24.11^{ab}
	±2.18	±5.16	±3.35	±1.61	±3.35	±0.90
2	25.10^{a}	23.92	26.67^{a}	29.21^{a}	21.57^{ac}	25.10^{ac}
	±2.89	±3.21	±4.15	±3.15	±5.37	±3.63
3	17.65^{b}	22.16	17.06^{bc}	17.45^{cde}	19.80^{bcd}	12.94^{e}
	±1.56	±2.07	±3.45	±3.14	±2.21	±1.48

Means bearing different superscripts differed significantly between the groups (P < 0.05).

Heart rate (beats/min), respiratory rate (breaths/min) and rectal temperature (°F) were recorded before and 1 h, 2 h, 1 day, 3 days and 7 days post biopsy.

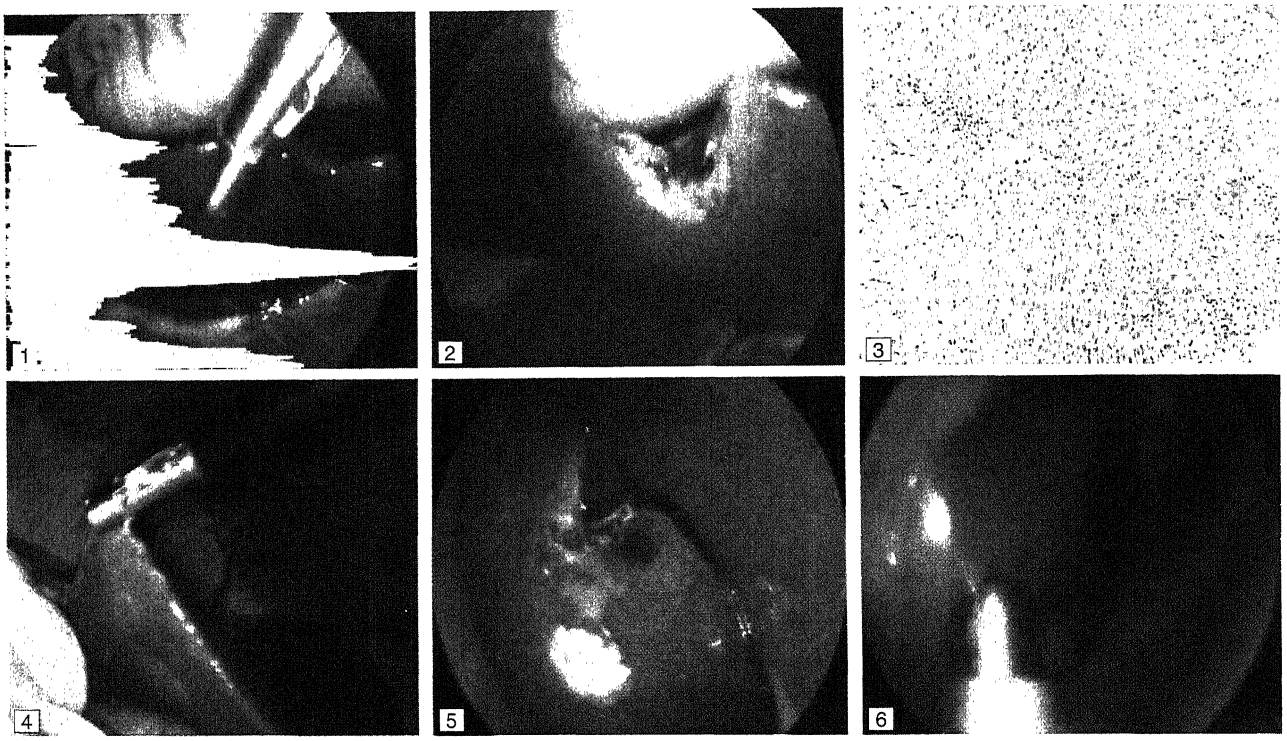
Haematological parameters such as total leukocyte count (TLC) and differential leukocyte count (DLC) were recorded before surgery and 1 h, 2 h, 1 day, 3 days and 7 days post biopsy using standard procedures. Plasma was separated from heparinized blood collected before and 1 h, 2 h, 1 day, 3 days and 7 days after biopsy collection for the estimation of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and bilirubin.

Intraoperative observations such as hemorrhage at the biopsy site at the time of surgery and the presence of any adhesions on 7th, 15th, 30th and 60th post-biopsy days of laparoscopy were assessed by scores of 0 (no adhesions), + (mild adhesions), ++ (moderate adhesions) and +++ (severe adhesions). The entire biopsy specimen obtained by laparoscopic technique was preserved in 10% formal saline solution for histopathological examination. Parameters such as relation between microscopical and gross lesions and tissue viability for histopathology in relation with the size of the biopsy samples were recorded.

The data were subjected to analysis of variance (ANOVA) as per standard statistical methods (Snedecor and Cochran 1989), using SPSS (1996) software package. The mean values at different time intervals were compared with base values using Student's 't' test.

RESULTS AND DISCUSSION

The animal presented good clinical condition. Systemic or local alterations that could be related to surgical complications were not observed. During the operative period, visualization of the epigastric region of the abdomen permitted the study of some anatomical elements includes liver, gall bladder, spleen, stomach and pancreas. Alterations on shape, colour and localization of liver were not verified.



Figs 1–6. **1.** Laparoscopic liver biopsy in a dog **2.** Laparoscopic liver biopsy after cauterization in a dog; **3.** Histopathological evaluation (normal liver architecture) of laparoscopic liver biopsy sample of dog. **4.** Laparoscopic liver biopsy in a goat. **5.** Laparoscopic liver biopsy after cauterization in a goat. **6.** Laparoscopic liver biopsy in a rabbit.

No postoperative complications were found in any of the animals. Three animals (1 in group 2 and 2 in group 3) presented bleeding in 3 biopsy sites after collection and cauterization of the liver fragment, becoming necessary to repeat the procedure of cauterization. This repetition was enough to eliminate the hemorrhage at the biopsy sites. This small amount of bleeding did not mean an increase in the risk factor of the technique, because a second electrocauterization a few seconds after the collection of the sample was enough to stop the bleeding. An equivalent procedure was also adopted by Fantinatti *et al.* (2003) with similar result.

The respiratory and heart rates in all 3 groups reduced significantly ($P<0.05$) during first 2 h after biopsy procedure, however, they returned to their base level after recovery from anaesthesia, and all the variations were within the normal range. Rectal temperature of all the animals of 3 groups did not show any significant variation between intervals of observation. Cardiac depression for first 2 h could be related to the parasympathetic effect of the vagus nerve on heart (vagal inhibition) owing to the increased intra-abdominal pressure caused by capnoperitoneum (Wolf and Stoller 2004).

Total leucocyte count (TLC) in the animals of 3 groups did not show any significant variations at different time intervals and the values remained within the normal range. The differential leucocyte count (DLC) showed a transient but nonsignificant change in all the 3 groups in first 3 days post biopsy.

Alanine aminotransferase (ALT) levels in the animals of 3 groups showed significantly ($P < 0.05$) higher values on first 3 post-biopsy days and thereafter it reduced and returned to the base level on day 7. However, all the values were in the normal range. Alanine aminotransferase (ALT) is the major liver specific enzyme in canines. Significant increase of ALT (within normal limits) could be related to hepatic injury and to a some extent skeletal muscle tissue injury. In hepatic biopsy, increase in ALT activity was observed as the degree of increase in ALT activity in serum is correlated with the number of hepatocyte damaged (Valentine *et al.* 1990, Bain 2003). As this liver specific enzyme was showing only negligible alterations after taking one or more biopsy samples in these animals, laparoscopic technique can be considered as one of the reliable and precise minimal invasive surgical techniques for collection of liver biopsy to diagnose different liver diseases in animals.

Asparate transaminase (AST) levels in all animals of 3 groups showed significant ($P<0.05$) increase on the first day post-biopsy. But all the values varied within the normal range. The values returned to base level in all groups on third post-biopsy day. Asparate transaminase (AST) is another reliable but less specific marker than ALT for hepatocellular injury and necrosis (Gilbony 2005). There was a little effect on this enzyme by biopsy methods and number of biopsy specimens collected in this study. However, these results further added

the importance of laparoscopic liver biopsy technique in diagnosing liver diseases.

Serum alkaline phosphatase (ALP) levels did not show any significant variation at different intervals. A nonsignificant increase was noticed in all the 3 groups on first and third day post-biopsy. But all the values were within the normal range. Serum alkaline phosphatase (ALP) is considered to be a useful parameter in diagnosing hepatobiliary diseases. However, it has a low specificity and a good sensitivity in these diseases owing to its numerous isoenzymes (Ettinger and Feldman 2000). The hepatic isoenzyme of ALP (L-ALP) is considered to be a drug induced or cholestatic enzyme (Bain 2003). In the present study, the changes observed in 3 groups were in the normal limit and so negligible that it cannot be significantly attributed to the effect of biopsy.

Blood urea nitrogen (BUN) values in animals of all the groups remained more or less same at different intervals of observation and remained within the normal range. It is a parameter to assess hepatic as well as renal function.

Serum bilirubin levels in animals of 3 groups fluctuated inconsistently on first 3 days post-biopsy. All the values were elevated nonsignificantly from normal range (1.7–10.3 µmol/L) however it returned to normal range on 7th post-biopsy day. Serum bilirubin is an important indicator of liver function rather than hepatic injury. It has been increased in different liver or biliary tract diseases, but relatively non-specific. Type of biopsy and number of biopsy sample taken by laparoscopic technique has no effects on serum bilirubin values.

The biopsy site was examined on 7th, 15th, 30th and 60th postoperative days using laparoscope. On 15th post-biopsy day, the wound in the liver was completely healed with scar tissue. On day 60, the wound was completely filled by hepatic tissue by regeneration. No adhesions at the biopsy site in any animals underwent laparoscopic surgery were observed on any of these days (score 0). This result showed the importance of laparoscopic biopsy with electrocauterization of the biopsy site in post-biopsy regeneration without any complications. The advantage of laparoscopic biopsy in precise identification of desired biopsy site was also established in this study based on intraoperative observations.

All the biopsy samples obtained from different groups were proven to be suitable and viable for histopathological analysis since all the samples maintained normal tissue architecture (Fig.3). The mean±SE of size of biopsy specimen obtained from animals of different groups are as follows: length (mm)–5.36±0.22; breadth (mm)–3.82±0.15 and thickness (mm)–2.82±0.18. The specimen obtained from laparoscopic biopsy method varied largely in size due to its control over the biopsy size. In laparoscopic technique, as the tissue could be taken mainly from the margins of liver lobes contained more of connective tissue of Glisson's capsule.

In the second phase of this study, this standardize laparoscopic technique in dogs was successfully utilized for collection of liver biopsy (26) from other species of small animals-sheep and goat (Figs 4, 5) and laboratory animal–rabbit (Fig.6), without any intraoperative or post biopsy complications.

The present study underlines the diagnostic utility of laparoscopy in hepato-biliary diseases in small animals. This technique of laparoscopic liver biopsy caused minimal stress to the animals as not much variation was seen in clinico-haemato-biochemical parameters. The use of electro-cauterization in a frequency energy at 45 watts, combining clotting, and a monopolar equipment assure the hemorrhage control, that was obtained in a few moments, establishing a dry liver surface, with a minimum of tissue necrosis. The technique is considered safe and effective for liver biopsy in small animals.

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