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Original Article

Assisted laparoscopic splenectomy: current concept for treatment of splenic hemangiosarcoma in dogs

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Abstract

Background: Hemangiosarcoma (HSA) is a common devastating malignant splenic tumor with high mortalities in dogs. Laparoscopic splenectomy (LS) is safe and gold standard technique for diseases required splenectomy. However, LS operation is a sensitive approach, which requires a restricted control for the vascular sealing procedures. **Aims:** We modified the LS technique through the application of hemostatic clips assisted-LS as an improved approach for the treatment of splenic HSA. **Methods:** Twelve dogs were admitted to the Veterinary Teaching Hospital, Zagazig University, Egypt for investigation of splenic HSA. Abdominal ultrasonography reveals the presence of large hypoechoic splenic masses with no further abnormalities within the peritoneal cavity. Thoracic radiographs, hematological assays, tumor necrosis factor-α (TNF-α) and histopathological investigations were done. **Results:** Hemostatic clips assisted-LS showed no major intra or post-operative complications. Laboratory analysis revealed anemia with an increase in TNF-α. Significant increase in platelets count with a slight increase in TNF-α was detected post-operative. **Conclusion:** Application of the improved hemostatic clips-assisted-LS is cost effective, with shorter operative time, feasible method for vessel sealing, as well as having few complications during the HAS treatment in dogs.

Key words: Assisted laparoscopy, Hemangiosarcoma, Hemostatic clips, Splenectomy, Ultrasonography

Introduction

Hemangiosarcoma **(**HSA**)** is an aggressive malignant neoplasm of the endothelial cells of the vascular system. It is more prevalent in dogs compared with other domestic animals (Spangler and Culbertson, 1992). It leads to a grave prognosis if left untreated due to the rupture of the blood vessels causing internal hemorrhage, collapse, and death (Kim *et al*., 2015). Splenic HSA has been classified into three stages; stage I where the tumor is confined to the spleen only, stage II where the mass ruptures and spreads to the regional lymph node, and stage III where an involvement of distant lymph nodes or other tissues (Martins *et al*., 2013). HSA could metastasize to different tissues such as lungs, heart, liver, brain, kidney, skin, skeletal muscles, and bones (Cole, 2012). The clinical signs of HSA are considered non-specific and generally associated with episodes of weakness. Hematological analysis may help diagnose (Ngetich *et al*., 2017). Tumor necrosis factor-α (TNF-α) is considered one of the parameters which known to activate the endothelial cell growth and movement. Thus, it can be used to correlate with tumor metastasis and disease progression (Clifford *et al*., 2000).

Complete splenectomy is a procedure performed to treat splenic abnormalities (Slatter, 2003). It is commonly performed in dogs with splenic tumors, especially malignant neoplasia or hemoabdomen (Stedile *et al*., 2009). Splenectomy was performed through laparotomy or laparoscopy (Gelmini *et al*., 2006). Generally, laparoscopic splenectomy (LS) has gained wide acceptance due to the minimum post-operative pain, decreased hospitalization time, and less medicament use (Aryazand and Ashegh, 2015). Although LS is considered a safe and convenient technique compared with open splenectomy, it is still more sensitive approach as it requires a restricted control in the splenic blood supply (Bulus *et al*., 2013).

Several methods were commonly used for hemostasis in LS, such as intracorporeal ligature, vessel sealing device, and harmonic scalpel; however, most of them are considered expensive tools (Richter, 2006; Stedile *et al*.,

2009). Ligature slippage might also occur as ligature technique outcome (Rodgerson and Hanson, 2000). Conventional bipolar electrosurgery were also used in open surgeries of splenectomy and multiple incisions laparoscopic surgeries (Stedile *et al*., 2009). Other methods of vascular sealing such as laser, ultrasonic coagulation, as well as modern plasma pulses have been developed (Collard *et al*., 2010). Hemostatic clips have been tested for vessel sealing due to their ease application in different laparoscopic techniques (Erridge *et al*., 2019), however, it has not grasp the attention to be applicable in LS in dogs.

Therefore, this experiment attempts to study the application of hemostatic clips in assisted-LS technique during the treatment of splenic HSA in dogs. We focus on the proposed clips as a new hemostatic technique to be incorporated to control the vascular bleeding during the LS operation. Several investigations were used to diagnose of splenic HAS and to assess the efficiency of the technique itself, such as ultrasonography, radiography, hematological analysis, TNF-α assay, and histopathology.

Materials and Methods

Animals

Twelve mixed-breed dogs were admitted to the Veterinary Teaching Hospital, Zagazig University, Egypt, during the period between January 2017 to January 2019 with a history of depression, anorexia, and weight loss. Dogs were investigated for splenic HSA utilizing inclusion criteria, which include disease history, clinical findings, laboratory investigations, ultra-sonographic scanning, and radiographic examination. Another twelve apparently healthy dogs belonging to the Faculty of Veterinary Medicine, Zagazig University, were used as control dogs without any surgical intervention. All procedures and protocols were strictly followed the institutional guideline for animal care and use. All animals were complied with the local animal welfare laws and regularly checked by specialists. All the applied procedures and protocols were strictly followed the guideline for animal care and use according to the National Institutes of Health (NIH publication No. 85-23, revised 1996).

Clinical examination

The clinical examination was applied under control and the diseased dogs were subjected to the evaluation of the vital parameters, including respiratory rate, heart rate, and body temperature. Examination of the gum, lips mucous membrane, and lymph nodes such as submandibular, cervical, and periscapular lymph nodes was done. Abnormal movements, spinal reflexes, dog's appetite were measured. Nervous system status such as behavior, mental status, posture, coordination, gait, and postural reactions were also evaluated.

Ultrasonographic examination

Abdominal scanning was performed on the body organs and soft tissues by ultrasound according to (Braun, 2009). The ultrasound machine (SonoScape A5V, China) was connected with 3.5 and 6 MHz convex and linear transducers. Post-operative ultrasonographic examination was applied at the 10th, 30th, 90th days of the examination for post-operative evaluation and monitoring of the health status of the dogs.

Laboratory investigation

Blood sampling

Blood samples were collected from the cephalic vein of each dog. Two milliliters of venous blood was taken on EDTA-containing tubes at the time of admission for the hematological examination. Five milliliters of the blood samples were collected in plain tubes to separate serum and centrifuged at 1,957 × g for 10 min. Clear serum was obtained via disposable Pasteur pipettes from the supernatant, then transferred to 1.5 ml sterile dry microtubes for further analysis.

Hematological analysis

Analysis of the hematological parameters was applied using automated cell counter F-820 (Sysmex Co. Ltd., Kobe, Japan). The hematological parameters such as red blood cells (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and platelets count (PLT) were measured.

TNF-α assay

The concentration of TNF-α was measured in the collected serum samples using quantitative sandwich ELISA kits (Canine TNF-α ELISA Kit, R&D Systems, USA). The final reaction was measured according to the intensity of the produced color. The optical density (OD) of each well was measured spectrophotometrically using 2HB 96-well microtiter Immunolon plate (Thermo Scientific, USA) at 450 nm wavelength. All measured samples were run in duplicate, and the results were reported in pg/ml.

Radiographic examination

Thoracic radiography was performed as the most common test for screening for veterinary cancer patients. Radiography was done on the 10th, 30th, 90th days post-operatively for all dogs in the study using an X-ray machine (Toshiba, RotanodeTM). Radiographs were taken with extended neck and legs and with 75 kVp 100 MA, and 6.3 mAS thoracic exposure factors.

LS technique

Admitted dogs suffering from splenic HSA underwent assisted-LS. Restriction of oral feeding was done for 12 h prior to the induction of general anesthesia. Animals were premedicated with 2% xylazine HCL (ADWIA, 10th of Ramadan City, Egypt) at a dose of 0.5 mg/kg body weight (BW) by intramuscular (I/M) injection. General anesthesia was induced with intravascular (I/V) injection of thiopental sodium 2.5% (Thiopental Sodium, EIPICO, 10th of Ramadan city,

Egypt) at a dose of 25 mg/kg BW and maintained with isoflurane in oxygen and the dog positioned in dorsal recumbency. CO_2 pneumoperitoneum was established to 10 mmHg, insufflators with 20 L/min gas flow (SOPRO-COMEG, GmbH, Tuttlingen, Germany). A laparoscopic telescope, 5 mm diameter (Karl Storz, Germany) connected to a camera, control unit, monitor, and light source with light cable (SOPRO-COMEG, GmbH, Tuttlingen, Germany) was inserted through a 5.5 mm cannula through the umbilicus (first portal).

These dogs were rotated to their right side, and the second port was created in the midline 5 cm caudal to the first port. In 50% castrated males, 33.3% spayed females, and 16.7% entire animals, the second port was created a few centimeters to the midline. A closed non-traumatic grasping forceps was introduced through the second port, and the splenic tail was elevated to allow observation of the ventral aspect of the spleen. Another port was created 5 cm lateral and caudal to the first port in the left flank. A 5 mm grasper forceps entered through the third port and the splenic vessels were caught (Fig. 1A). The third port was widened to be 5 cm in length. Six Med/Large double hemostatic clips in total were used for each dog. LIGACLIP Endoscopic Rotating Multiple Clip Applier (33 cm length, 10 mm diameter) in double raw were applied to seal the Hilar vessels and short gastric vessels without any tension for hemostasis and transection of the splenic vessels and the gastrosplenic ligament (Fig. 1B). Each hemostatic clip closes two blood vessels. The distal hemostatic clip was placed as distal as possible to the spleen and the proximal ones, 1 cm proximal to the distal clip. All splenic vessels were double clipped in the same way the conventional open splenectomy was formed. Dissection of splenic attachments started at the caudal aspect of the spleen then completed cranially (Figs. 1C-1 and C-2). The abdominal incisions were then routinely closed.

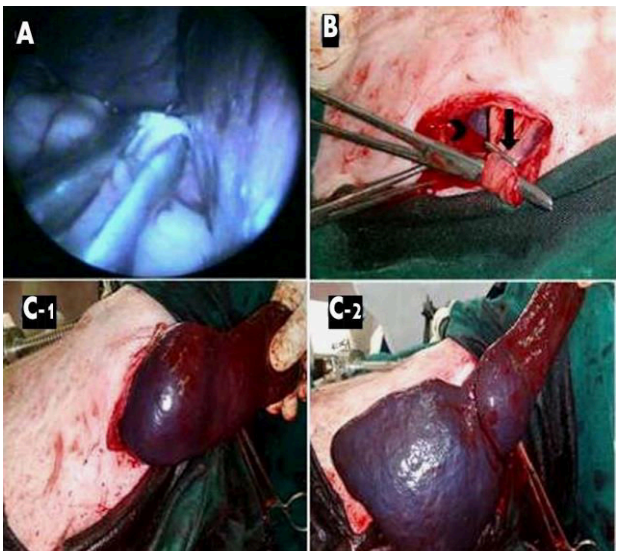

Fig. 1: Admitted dogs underwent assisted-LS. (**A**) Ventral aspect of the spleen and splenic vessels with grasping forceps, were introduced through the third portal, (**B**) A 10 mm hemostatic clips after its exteriorization from the third laparoscopic port. (**C-1**) Spleen appears through the incision, and (**C-2**) Exteriorized spleen through the small abdominal incision of the third laparoscopic port

Post-operative care was done where dogs were closely monitored 1 h after recovery from anesthesia. Medexaflam (Global Pharmaceutical Industries, Egypt) 0.2 mg/kg BWt IM Injection was used as analgesic daily for 2 successive days post-operatively. Antibiotic was injected IM 1 h prior to the operation and then twice daily for 5 successive days using 30 mg/kg BWt Ceftriaxone (Pharco International, Egypt).

Histopathological examination

Samples from the spleens were collected for histopathological examination. The biopsy specimens were fixed in 10% solution of neutral buffered formalin at room temperature for 24 h and then dehydrated in ethyl alcohol from 70% to 100%, cleared in xylene, and embedded in paraffin. After paraffin embedding, 5 µm sections of the samples were stained with hematoxylin and eosin (H&E) staining for microscopic examination (Suvarna *et al*., 2018).

Statistical analysis

The results were compared done using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 17.0. Analysis of variance was done with Duncan's post-hoc test for one-way ANOVA test to determine the significance. The results were expressed as means±standard error (SE) and estimated at $P<0.05$.

Results

Clinical findings

Twelve mixed breed dogs were examined for splenic HSA and admitted for assisted-LS operation. The mean age of dogs at the time of examination was 8 years old with an average BW of 30 kg. The clinical signs showed the mean respiratory rate of 24.36 beats/min, the mean heart rate of 106.27 beats/min, and the mean body temperature of 38.5°C. There were no detected abnormalities before and after operation in the lymph nodes of the examined dogs. Minimal intra-operative hemorrhage with no major intra or post-operative complication was detected. There was no slippage of the clips, and no need for extra ligation as the hemostatic clips provided adequate hemostasis without the association with other forms of vascular sealing. So there was no need to stop the procedure or convert to an open approach. The mucous membrane was pale before the operation, and then it turned pink 90 days post-operatively. The dogs also showed an increase in body weight after the operation. Dogs with splenic HSA showed depression pre-operatively. Gradual improvement in the appetite was shown in the post-operative dogs that started to eat. Meanwhile, no overweight was detected; the weight gain was clear at 90 days post-operatively. Post-operative dogs did not return to normal weight in comparison to the control dogs.

Ultrasonographic findings

Preoperative abdominal ultrasonographic findings in all cases were consistent with large hypoechoic splenic masses that were about 10 mm width and 8 mm length. Large sizes spleens (splenomegaly) were seen in all examined dogs that ranged from (22 mm to 25 mm in width). No other significant abnormalities within the peritoneal cavity were detected (n=11) (Fig. 2A). Hemorrhagic peritoneal effusion was detected in only one dog out of twelve. Post-operative abdominal ultrasonographic findings (Fig. 2B-1) revealed the presence of small amount of anechoic exudates on the 10th day post-operatively. There was no excess exudate in the abdominal cavity with minimal fibrinous hyperechoic areas on the 30th day post-operatively. The gastrosplenic ligament at the site of spleen dissection appeared as a hyperechoic line with commit tail artifacts under the hemostatic clips (Fig. 2B-2). On the 90th day post-operatively, there was neither anechoic exudate nor hyperechoic fibrinous adhesions (Fig. 2B-3).

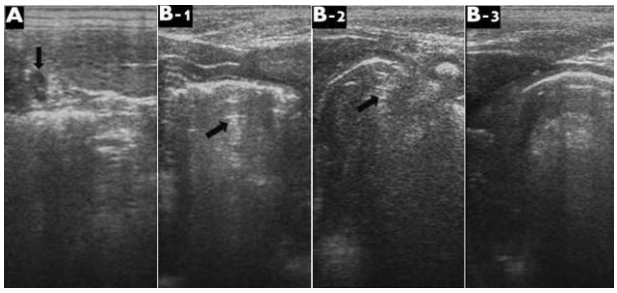

Fig. 2: Ultrasonographic image of a 2.5 mm sized hypoechogenic mass (arrow) in the spleen of a dog using 6 MHz linear transducer (**A**), Ultrasonography of the gastrosplenic ligament on the 10th day (**B-1**), 30th day (**B-2**), and 90th day post-splenectomy (**B-3**). Gastrosplenic ligament appeared as hyperechogenic line, and the comet tail artifact (arrows) under the hemostatic clips attached to the gastrosplenic ligament

Laboratory findings

The RBCs count and Hb concentrations showed a significant decrease ($P<0.01$) pre- and post-operatively compared with the control group. The value of the PCV was significantly decreased ($P<0.01$) pre-operative and on the 30th day post-operative. Meanwhile, the PCV returned to the control level on the 90th day post-operative (Fig. 3A). The MCV and MCHC levels revealed a non-significant change compared with the control reflecting that animals suffered from normocytic normochromic anemia (Fig. 3B). The Platelets count showed a non-significant decrease pre-operative and at 30th day postoperative with a significant increase ($P<0.01$) at the 90th day post-operative (Fig. 3C). TNF-α showed a highly significant increase ($P<0.01$) in diseased cases pre-operative, and its level was decreased at the 30th and 90th day post-operatively when compared with the control group (Fig. 3D).

Radiographic findings

Pre-operative thoracic radiographs were performed on the diseased dogs and revealed no evidence of metastasis tumor cells into internal organs on the 30th and 90th day postoperatively.

Surgical care and outcome

Intraoperative care showed successful assisted-LS which was performed on twelve dogs without any major intra or post-operative complications and the wounds healed uneventfully. Two h post-operatively, all dogs were ambulatory. One day after the surgery, dogs were comfortable. The clinical findings after the surgery showed an improvement when compared with the health condition of the dogs before surgical operations. The post-operative clinical findings of the eleven dogs on the 10th, 30th, and 90th day were nearly normal compared to the control.

Histopathological findings

The histopathology was performed on spleen biopsies collected before the LS operation and showed the proliferation of endothelial cells forming small neoplastic vascular space and large irregular vascular channel with mild thickening of splenic capsule (Fig. 4A). Small neoplastic vascular space lined and surrounded by elongated, plump, anaplastic endothelial cells, and the nucleus was enlarged ovoid to elongate with a mitotic figure and brown pigment of hemosiderin (Fig. 4B). The spleen showed cleft-like spaces lined by neoplastic endothelial cells with necrosis of lymphocytes in the white and red pulp (Fig. 4C). Cleft-like spaces lined by pleomorphic nucleus with mitosis, brown pigment of hemosiderin, and degeneration in the wall of center arterioles were detected (Fig. 4D).

Discussion

Hemangiosarcoma (HSA) is a malignant tumor that has been reported in dogs. The most prevalent splenic mass arises from the vascular endothelial cells, and the spleen is the predilection site of the primary incidence. The overall prevalence is reported 0.3-2.0% of all tumors in dogs (Bojrab and Monnet, 2010). Ultrasound or radiographic examination has been commonly used to diagnose the splenic HAS as a splenic mass. These techniques provided a comprehensive understanding of the involved organs and the state of the abdominal cavity (Ngetich *et al*., 2017). Ultrasonographic guides biopsy to confirm the presence of splenic tumors and provides some information that directs to the surgical removal of the spleen (Ngetich *et al*., 2017). The pre-operative diagnostic imaging is very important to complete the abdominal exploration, which cannot be performed during LS (Wright *et al*., 2016). Post-operative abdominal ultrasonographic findings confirm the complete healing without intra-abdominal adhesions.

The non-specific clinical signs of HSA require hematological techniques to diagnose and regular screening (Ngetich *et al*., 2017). Clinical and hematological findings, including anemia and non

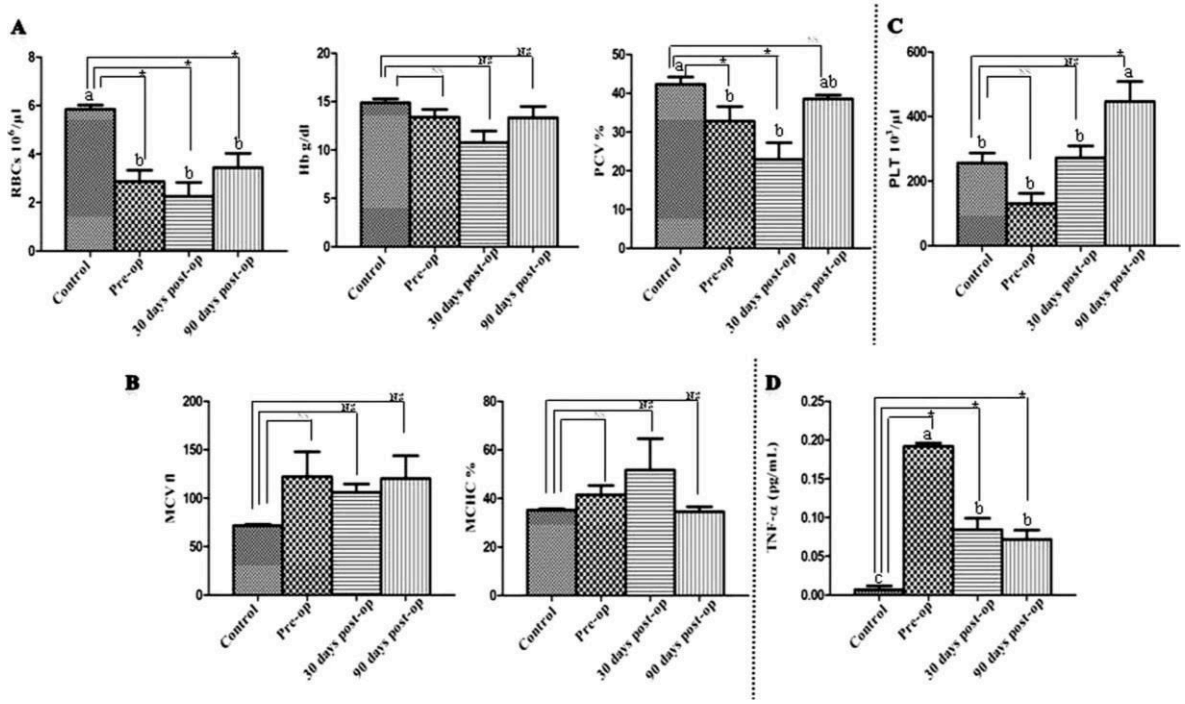

Fig. 3: Hematological and TNF-α assay. (**A**) Red blood cells (RBCs) level, hemoglobin (Hb) concentration, and packed cell volume (PCV), (**B**) Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), (**C**) Platelets (PLT) concentration, and (**D**) Tumor necrosis factor-α (TNF-α). Values were represented as mean±SE. The level of significance was detected at $P<0.05$. Different letters $^{a, b, c}$ means significant changes. The graphpad prism 7.0 software was utilized to create our graph

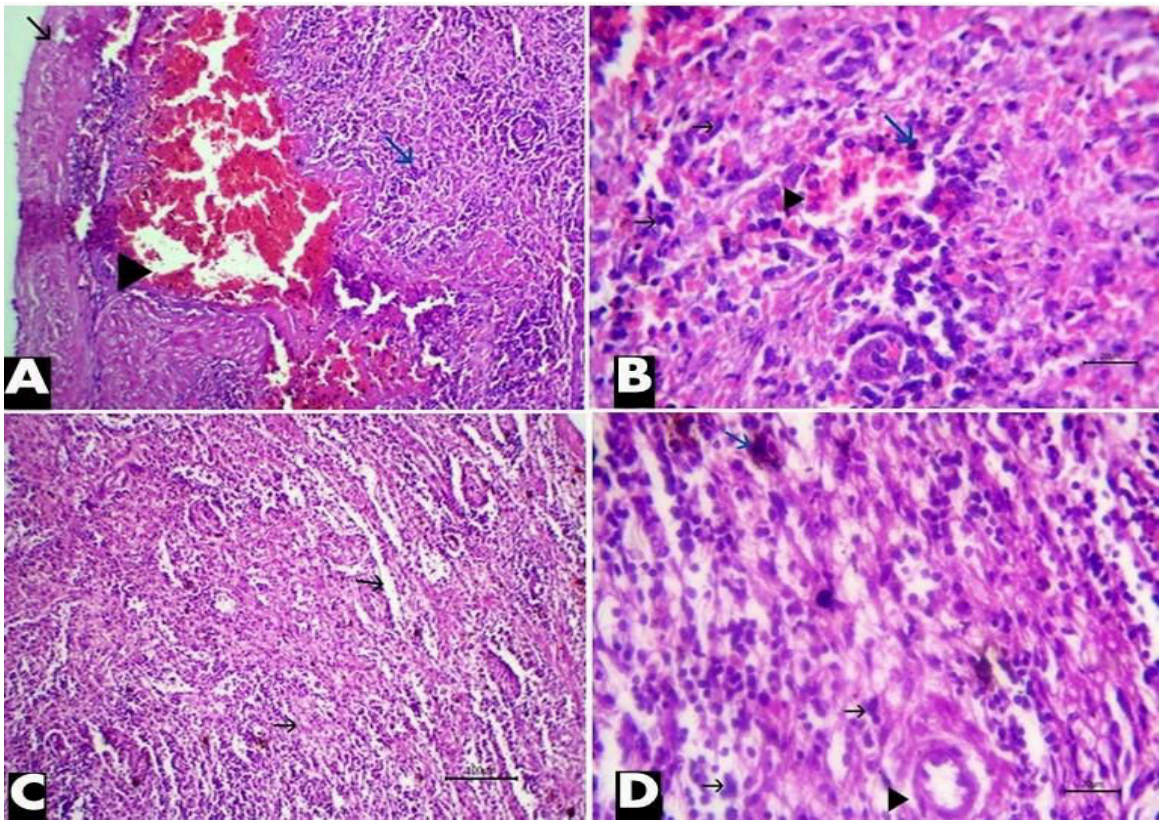

Fig. 4: Spleen tissue stained with H&E showing (**A**) Vascular space (blue arrow), and vascular channel (arrowhead) with mild thickening of the splenic capsule (black arrow), ×300, (**B**) Small neoplastic space (arrowhead), and enlarged nucleus with mitotic figure (black arrow), and hemosiderin pigment (blue arrow) ×1200, (**C**) Cleft-like spaces with necrosis of lymphocytes (arrow), ×300, and (**D**) Pleomorphic nucleus with mitoses (black arrow), hemosiderin pigment (blue arrow), and degeneration of center arterioles (arrow head), ×1200

significant thrombocytopenia were previously reported as the diagnostic marker of HSA in dogs that the decreased erythrocytic counts resulting in regenerative anemia (Martins *et al*., 2013). Many cases of the reported regenerative anemia were normochromic normocytic (Hodges and Christopher, 2011). They have been reported by the oncologist corresponding to internal organs` neoplastic changes (Clifford *et al*., 2001). Other reported signs that could be attributed to the total splenectomy include persistent decrease in the RBCs count with significant increase in the platelets count post-operatively after total splenectomy (Chaudhry *et al*., 1997).

The level of TNF-α could help evaluate the operated cases pre- and post-operatively. It has been used as a marker for metastasis of the disease on the 30th and 90th days post-operatively. The serum TNF-α concentration was markedly decreased during the cancer treatment suggesting that its level could be an indicator for cancer prognosis. It is reported to be important cytokine associated with tumor regression (Clifford *et al*., 2001; Wang and Lin, 2008). Microscopically, the spleen showed proliferation of endothelial cells forming small neoplastic vascular space or vascular cleft lined, and surrounded by pleomorphic endothelial cells. The nucleuses were enlarged ovoid to elongate with the mitotic figure extensive hemorrhage and necrosis of lymphocytes inside white and red pulp.

Although the LS techniques have been reported to use different devices for induction of hemostasis, this study is considered an early one in dogs that proved the feasibility of hemostatic clips in assisted-LS of neoplastic spleen without dissection of hilar vessels before stapling. Hemostatic clips through assisted-LS in dogs were applied in this study to enhance the advantages of LS technique as it decreases the excess time for tissue manipulation with less morbidity and minimum post-operative pain. There was no need to increase the length of the incision by more than 5 cm as it occurs in the traditional open surgery. Thus, it leads to an easy approach to the splenic hilum and therefore reduces the risk of hemorrhage compared with regular laparoscopic technique. Therefore, assisted-LS was completed successfully without increasing the size of the incision and the short operation time without intra-operative hemorrhage.

Still, some researchers advised the use of hemostatic clips in dogs only with non-neoplastic splenic diseases or for small splenic masses (Collard *et al*., 2010). Here, we have applied three hemostatic clips in the current study aiming to seal the hilar vessels and short gastric vessels close to the spleen without any tension for hemostasis plus the transection of the splenic vessels and the gastrosplenic ligament. Thus, the hemostatic clips provided adequate hemostasis and stopped the vascular bleeding without the association with other forms of vascular sealing.

Dissection of splenic attachments was started at the caudal aspect of the spleen, where making the dissection of the spleen's tail was easier and facilitated the excision of the spleen outside the abdominal wall gradually from caudal to cranial till complete detachment. Accordingly, the direction of dissection, the mean operative time, and wound length were found to be closely near to that in three portal laparoscopic technique used for LS (Collard *et al*., 2010) and (Khalaj *et al*., 2012). Where the wound increased, the spleen could be exteriorized from the abdomen to avoid its rupture due to the relatively large size of the spleen, flexibility, and the fragility of the capsule.

Collectively, assisted-LS using hemostatic clips could be performed successfully in dogs suffering from splenic HAS. This technique has several advantages as it provides proper hemostasis, decreases the operative time, safe and trustable method for vessel sealing without any reported complications. Therefore, hemostatic clips and assisted-LS approach are considered of great value to be used in dogs for the surgical treatment of neoplastic splenic diseases.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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