수의학석사학위논문

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Laparoscopic Excision of Bilateral Medial Iliac Lymph Nodes Using a 3-Portal Technique in Dogs

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수의학과 임상수의학 전공

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이 논문을 수의학석사학위논문으로 제출함

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Laparoscopic Excision of Bilateral Medial Iliac Lymph Nodes Using a 3-Portal Technique in 12 Dogs

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ABSTRACT

The medial iliac lymph node (MILN) is one of the most common lymph nodes in which the metastasis of a number of neoplasia is identified, particularly anal sac adenocarcinoma**.** MILNs can be challenging to biopsy pre-operatively considering their intimate location to large vessels. If minimally invasive excision of lymph nodes is found to be feasible, it could

make biopsy of regional lymph nodes easier to perform in dogs in need of differential diagnosis for metastasis of neoplasia. This study was designed to describe a simple technique for laparoscopic excision of bilateral MILNs using 3-portal technique in dogs. Dogs were positioned in dorsal recumbency, and 3 transperitoneal portals were established. Bilateral MILN dissection was performed with vessel-sealing devices while tilting the surgical table to contralateral side of the target MILN. The position of the surgeon was maintained in the left side of the dog for the entire procedure. The median surgical time was 9.75 minutes for each MILN, and 21 minutes for bilateral MILNs. There was no major complication to be repaired with conversion to open surgery. All MILN samples were evaluated histologically, and found to be suitable for histopathological diagnosis for metastasis. Obtaining feasible histologic samples was successfully achieved using this 3-portal technique, which offers the advantages of a short operative time, simple procedure, and less postoperative pain. Developing this procedure can make MILN biopsy procedure easier to access for obtaining more detailed staging on microscopic disease status and get positive prognostic effect in case of metastatic MILNs.

Keywords: laparoscopy, medial iliac lymph node, minimally invasive surgery, biopsy, dog

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CONTENTS

I. Introduction

Minimally invasive procedures for small animals have been described in many veterinary studies as feasible techniques, associated with less pain and rapid recovery. (Devitt et al., 2005, Culp et al., 2009) While there have been many studies of laparoscopic biopsy of lymph nodes in human medicine, (Buist et al., 2003, Kamprath et al., 1997, Papadia et al., 2004, Pijpers et al., 2004, Possover et al., 1998) only a few studies have been conducted in veterinary medicine. (Steffey et al. 2015) A recent study reported the technique of laparoscopic extirpation of the medial iliac lymph nodes (MILNs) in dogs with a lateral approach. (Steffey et al., 2015) The dogs were medium- to large-breed dogs whose body weight ranged between 15 and 25 kg, and access to bilateral MILNs was limited without changing the dog's position and establishing additional laparoscopic cannulas. Establishment of 3 laparoscopic cannulas on each lateral side can be considered a rather invasive method and can induce a great deal of postoperative pain in small dogs, which is a matter of concern considering the large population of small-breed dogs weighing under 15 kg in the Republic of Korea. (Shim SH, 2009)

Assessment of the MILN is thought to be important in patients with neoplasia in these anatomic locations, as it receives lymph vessels from the caudal abdomen, pelvis, and pelvic limbs. (Howard E, 2013) and is considered to be one of the most common lymph nodes in

which the metastasis of a number of neoplasia is identified, (Robat et al., 2013, Chaffin et al., 2002, Bennet et al, 2002, Williams et al.2003, Hobson et al, 2006, Polton et al, 2007) particularly anal sac adenocarcinoma. It has been reported that more than 50% of dogs with anal sac adenocarcinoma display metastasis to regional lymph nodes at diagnosis. (Bennet et al., 2002, Williams et al., 2003, Hobson et al., 2006, Polton et al., 2007).

However, the MILN is located deep in the retroperitoneum and adjacent to large blood vessels, including the aorta, caudal vena cava, and external iliac arteries, and the surgical technique for biopsy of the MILN with laparotomy is thought to be a relatively invasive and aggressive diagnostic technique. Although abdominal ultrasonography is a useful diagnostic tool for evaluating the morphology and echogenicity of the MILN, it is possible that there may be no lymphadenomegaly or change of echogenicity in cases of micrometastasis of neoplasia, in which case ultrasonography would not be useful for the assessment. (Anderson et al., 2015, Llabres et al., 2004)

If the procedure of laparoscopic excision of bilateral MILNs in small dogs with neoplasia could be less invasive and simpler, clients and veterinarians would be more likely to consider MILN biopsy as a useful diagnostic tool for evaluating lymph node metastasis and developing the treatment plan. Hence, we carried out this study with the purpose of (1) developing a short and simple procedures using a 3-portal technique and ventral approach

for laparoscopic excision of bilateral MILNs in dogs weighing under 15 kg, (2) reporting the surgical time and complications of this technique, and (3) describing the quality of the biopsy specimens obtained.

II. Materials and Methods

This study was approved by institutional animal care and use committees of Seoul National University. (SNU-151230-2)

1. Animals

Twelve healthy intact male purpose-bred research dogs, weighing 9.5 to 11.5 kg, were included in this study. None of these dogs had previously undergone abdominal surgery. Each dog underwent a complete physical examination and hematologic evaluation including a complete blood count and serum chemistry.

2. Surgical Techniques

All surgical procedures were performed by the same surgeon. All the dogs were premedicated with acepromazine (0.01 mg/kg, intravenously [IV], Sedaject®, Samu Medican Co. Ltd, Korea). Anesthesia was induced with alfaxalone (5 mg/kg, IV, Alfaxan®, Jurox Inc., Kansas, MO), and maintained with inhalant isoflurane (Ifan®, Hana Pharm Co. Ltd., Republic of Korea) in oxygen. Tramadol (5 mg/kg, IV, once before surgery, and 5 mg/kg, orally, twice daily for 14 days after surgery) was administered for peri-operative analgesia.

The dogs were positioned in dorsal recumbency. The hair was widely clipped from the xiphoid process to the pubic region. Urine in the urinary bladder was voided using an 8-Fr urinary catheter. A Veress needle (Karl Storz Veterinary Endoscopy, Goleta, CA) was inserted through the right lateral abdominal wall, and CO_2 pneumoperitoneum (maximal pressure, 10–15 mmHg) was established and maintained using a pressure-regulating mechanical insufflator.

In all dogs, a 3-portal technique was used; all dogs had 2 working portals and a single camera portal with 6-mm laparoscopic cannulas (Ternamian endotip cannula, 6.5 cm, Karl Storz Veterinary Endoscopy, Goleta, CA) established after pneumoperitoneum was achieved. In the right upper abdominal region, 3 cm craniolateral to the umbilicus at the level of the

last ribs, an approximately 1.5-cm skin incision was made with No.10 blade, and the first cannula (Ternamian endotip cannula with a multifunctional valve, 6 mm × 6.5 cm, camera portal, Karl Storz, Veterinary Endoscopy, Goleta, CA) was inserted into the abdomen at a 45° angle in the caudomedial direction. After exploring the entire abdomen with a 5 mm × 29 cm 0° laparoscope (Karl Storz, Veterinary Endoscopy, Goleta, CA) to evaluate the organs for iatrogenic damage, the second cannula (Ternamian endotip cannula, 6 mm × 6.5 cm instrumental portal, Fig 1) was inserted at a point equidistant from the midline and the camera portal on the contralateral side of the animal under laparoscopic guidance, taking care to prevent trauma to the abdominal organs (particularly the spleen). The surgical table was tilted by up to 15° towards the Trendelenburg position (head down). The third cannula was established in the caudal region, the third of the distance to the left caudal wing of the ilium, using the same method as that used for the second cannula (Fig 1).

A laparoscopic probe (Palpation probe, 5 mm × 36 cm, Karl Storz, Veterinary Endoscopy, Goleta, CA) and laparoscopic tissue grasping forceps (Kelly forceps or Bobcock forceps, 5 mm × 36 cm , Karl Storz, Veterinary Endoscopy, Goleta, CA) were introduced through the second and third cannulas, respectively, and an inspection of the caudal abdomen was performed.

For dissection of the right MILN, the surgical table was tilted to the left within 30° to allow the organs to displace towards the dependent aspects of the peritoneal cavity. After identification of the right external iliac artery, right deep circumflex iliac artery, and the silhouette of the MILN behind the retroperitoneal tissue, a laparoscopic vessel-sealing device (Ligasure-Dolphin tip, 5mm-37cm, Covidien, Inc., Mansfield, MA) was introduced through the second cannula. The retroperitoneum was incised at a point caudal to the right deep circumflex iliac artery and between the right external iliac artery and the testicular vessels. The tissue surrounding the retroperitoneum and the small vessels attached to the lymph nodes were dissected using blunt dissection and sealing/transection by the vessel-sealing device, respectively. During the procedure, the lymph node was retracted away with laparoscopic Bobcock forceps from the large vessels, including the right external iliac artery and right deep circumflex artery. The dissection was performed in the cranial-to-caudal direction and toward the dorsomedial side of the MILN in order to completely separate the MILN from the surrounding tissues and vessels. The excised lymph nodes were withdrawn using the third cannula. For dissection of the left MILN, the surgical table was tilted to the right, and the procedure was performed on the opposite side in a similar manner.

The portal sites were sutured in a simple interrupted pattern using a monofilament absorbable suture (3-0, PDS II, Ethicon, Cincinnati, OH) in the abdominal wall and the

subcutaneous tissue, and monofilament nylon (4-0, Nylon, Ethicon, Cincinnati, OH) was used to suture the skin layer. All dogs recovered from anesthesia.

Portal sites, surgery time, anatomic landmarks, any physiologic changes during table-tilting, and peri-operative and post-operative complications including abdominal hemorrhage or inflammation or infection of the incision site were recorded.

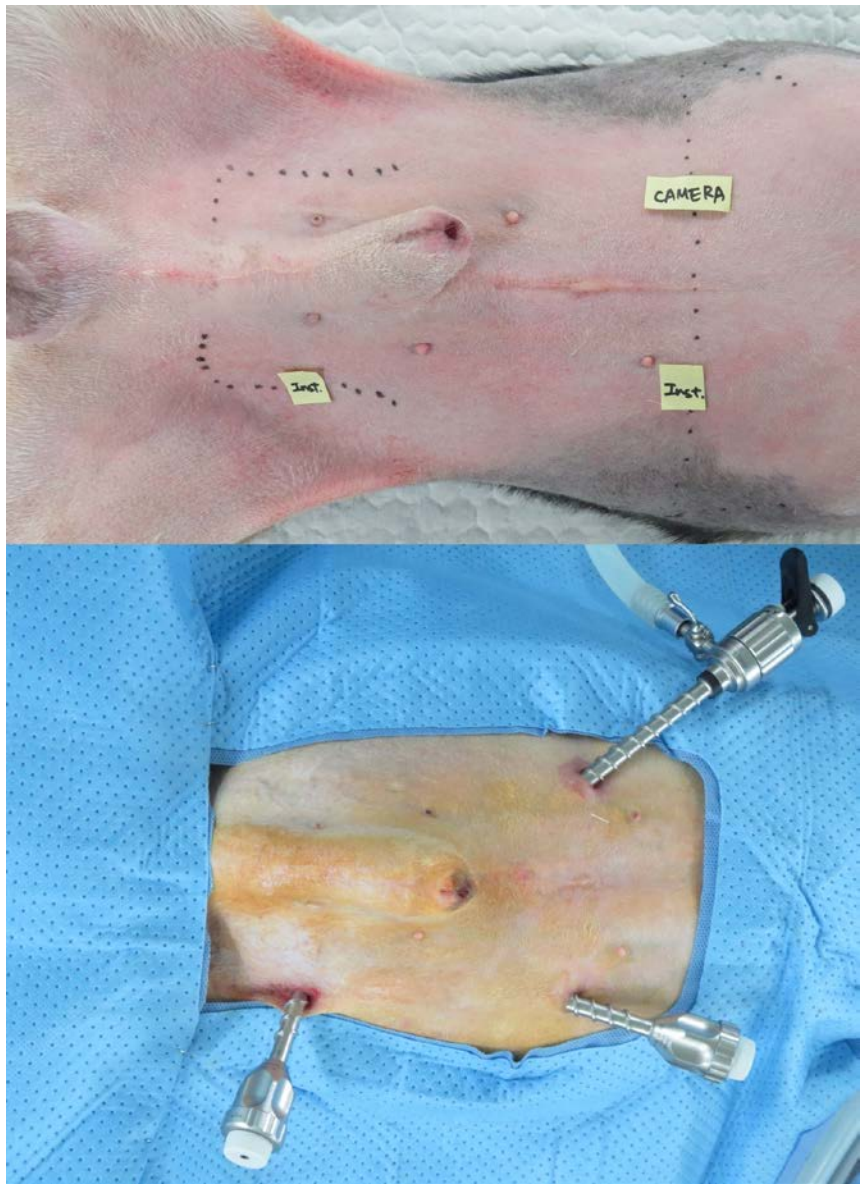

Fig 1. Operative images. Positioning of Laparoscopic Ports.

3. Sample Evaluation

Every excised MILN was immersed in 10% buffered formalin and bisected longitudinally. Hematoxylin and eosin staining was performed for histologic evaluation of the mechanical trauma caused during dissection. Both halves of the cut surface of the bisected MILNs were evaluated and scored using previously described criteria. (Steffey et al., 2015) Histologic artifacts were characterized as central (pinch/pressure/crush artifacts affecting larger central areas of the lymph node) or peripheral (pinch/pressure/crush artifacts affecting the peri-nodal tissue and the capsular and superficial cortex/paracortex of the lymph node). The histologic artifacts were scored according to the following criteria: 0 = no artifact, 1 = minimal/marginal deformation, 2 = mild crush, 3 = moderate crush, 4 = severe crush. When different areas within a central or peripheral region of a given lymph node received a range of scores, the worst score received was assigned to that lymph node as the overall score.

III. Results

The mean age of the dogs was 5 years (range, 4.5–5.5 years). The mean body weight was 10.3 kg (range, 9.5–11.5 kg). The body condition scores ranged between 4/9 and 5/9. All dogs were determined to be healthy on the basis of the physical examination and hematologic evaluation.

1. Surgical Findings

MILNs were successfully identified and excised using the ventral approach without major perioperative complications. The use of the 3-portal technique and tilting of the table by up to 30° in the direction opposite to the target MILN on the basis of the horizon level for bilateral observation were useful to identify bilateral MILNs without changing the surgeon's position.

Right MILNs were located lateral to the right external iliac artery and right ureter, caudal to the right deep circumflex iliac artery, and medial to the right testicular vessels (Fig 2). Left MILNs were located symmetrically with respect to the right MILNs (Fig 3).

Observed intraoperative complications included splenic hemorrhage caused by insertion of the laparoscopic cannula, capillary hemorrhage from peritoneal fat and vessels efferent to the lymph node, and tearing of the capsule of the lymph node.

The dogs were positioned in the Trendelenburg position and rotated in both directions within 30°. There were no abrupt changes in respiratory rate, heart rate, peripheral capillary oxygen saturation (SpO_2), or end-tidal carbon dioxide ($EtCO_2$) after tilting the table.

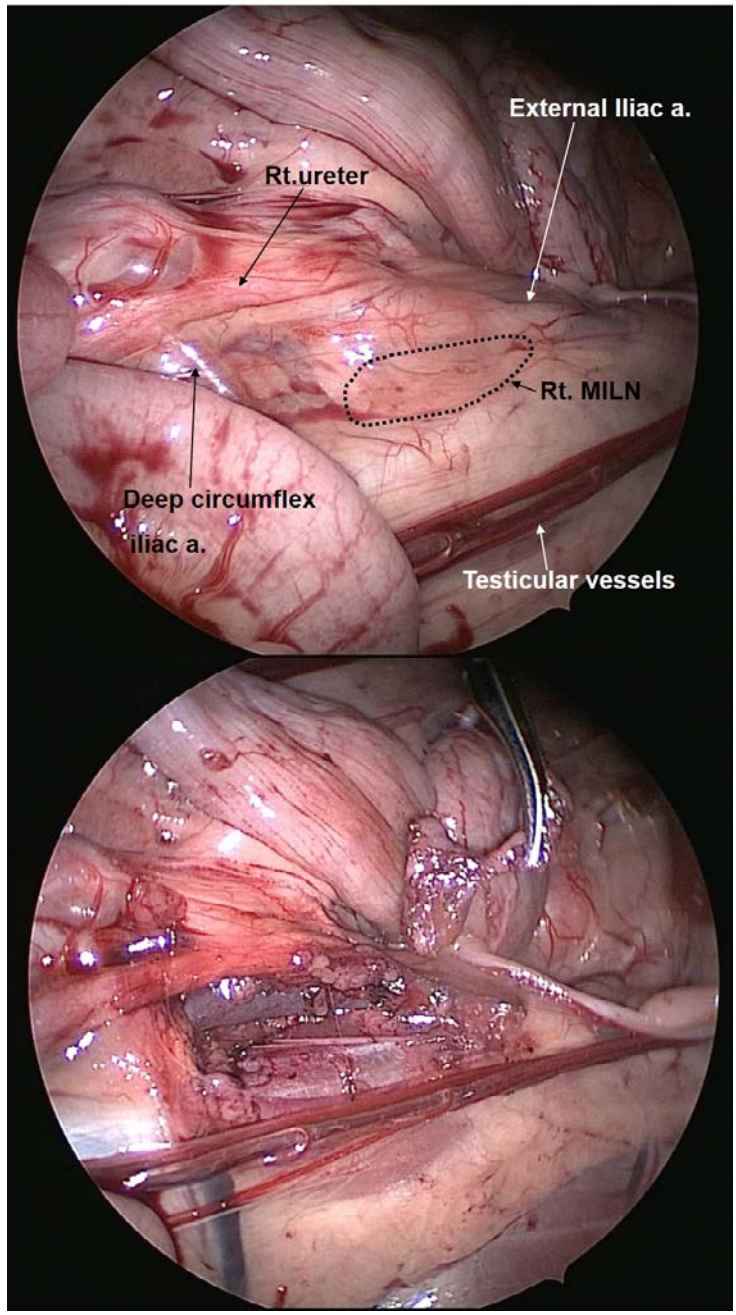

Fig 2. Laparoscopic images of the pre-dissection and post dissection regional anatomy of the right MILN.

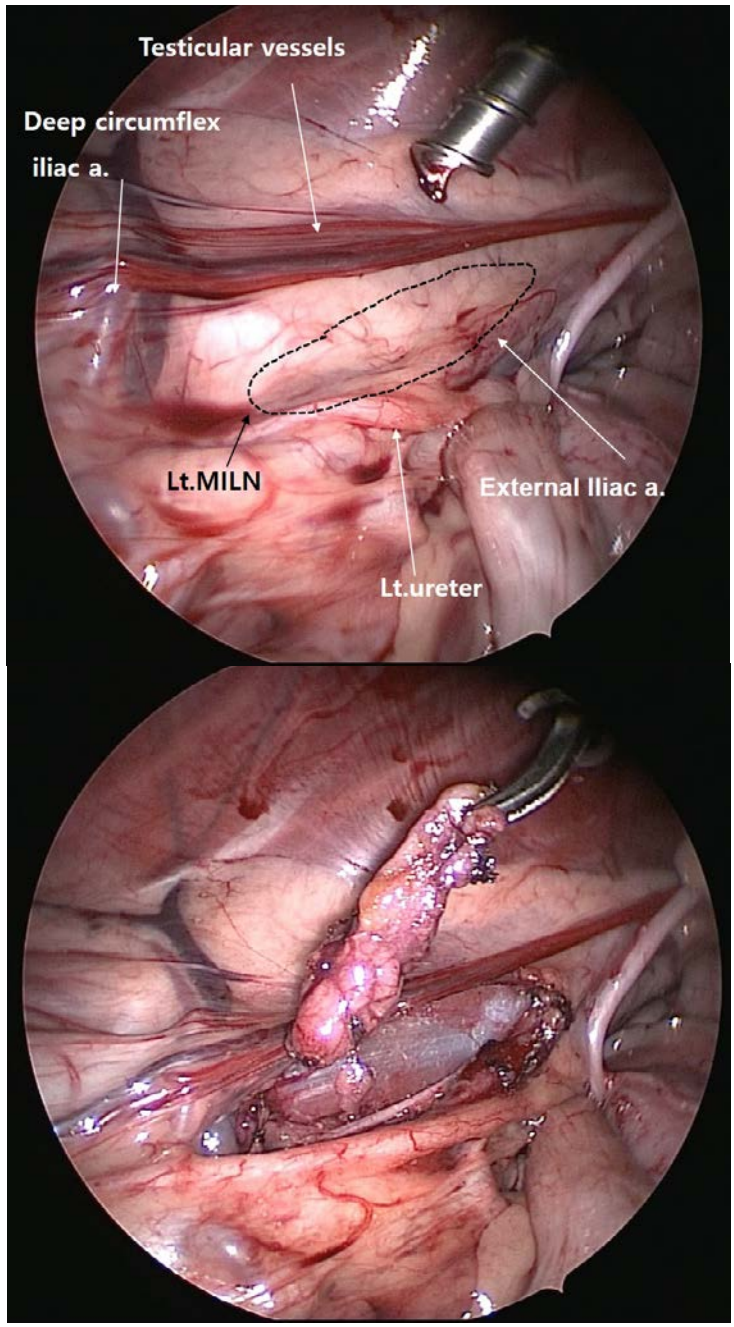

Fig 3. Laparoscopic images of the pre-dissection and post dissection regional anatomy of the left MILN.

2. Postoperative Management

The vital signs of all the dogs remained within the normal range during the entire perioperative period, and they all recovered from anesthesia uneventfully. Postoperative pain was evaluated as mild using the visual analogue scale with clinical judgment; all dogs could stand and walk within 2–3 hours after the procedure and showed good appetite and activity within 2 weeks after surgery. No clinical symptoms were observed indicating postoperative complications, including abdominal hemorrhage or inflammation or infection of the incision site during the monitoring period.

3. Surgical Time and Gross Evaluation of Samples

The median number of identified MILNs was 2 (range, 2–3). There were additional MILNs in 2 dogs, on the left side in both. The median dissection time was 9.75 min (range, 6–18 min) for unilateral and 21 min (range, 12–30 min) for bilateral MILN dissection. The median total time for surgery was 52 min (range, 40–72 min). The median measured length of the excised MILNs was 16.2 mm (range, 5–28 mm), and the median width was 6.8 mm (range, 3–14 mm).

The median dissection time was 9.5 min (range, 6–18 min) for right and 10 min (range, 6-15 min) for left MILN dissection. The median measured length of the excised right MILNs was 17.9 mm (range, 12–28 mm), and the median width was 7 mm (range, 4–10 mm). The median measured length of the excised left MILNs was 14.5 mm (range, 5–23 mm), and the median width was 6.6 mm (range, 3–14 mm).

4. Histologic Evaluation of Samples

The samples collected were histologically confirmed as lymphoid tissue in all 12 dogs, and categorized as MILNs considering the anatomic location. All samples were found to be histologically suitable for diagnosis. There were some peripheral artifacts caused by mechanical trauma in all samples, while artifacts in the central portions were relatively fewer. The median central artifact score for all MILNs was 0 (range, 0–2), while the median peripheral artifact score was 1 (range, 1–4). For all MILNs, the median percentage of the surface area of the dissected MILNs affected by histologic artifacts was 5% (range, 5-30%).

IV. Discussion

The sentinel lymph node (SNL) is the first node in the lymphatic system that drains the primary tumor site via specific lymphatic channels. If the sentinel node is not affected by tumor metastasis, all other lymph nodes should be free of disease. (Borgstein et al., 1998) Based on this concept, evaluation of the SLN, even when it appears unremarkable on palpation or normal in appearance on imaging, is recommended to check for microscopic metastatic disease in a number of human cancers. (Cochran et al., 2008) Thus, assessment of the sentinel lymph node status is thought to be the standard of care for surgical or therapeutic planning in the treatment of a number of human cancers. (Buitst et al., 2003, Kamprath et al., 1997, Knapp et al., 2007, Papadia et al., 2004, Pijpers et al., 2004, Possover et al., 1998, Rob et al. 2005) This evaluation can provide information for predicting the prognosis, planning the extent of surgery, assessing the need for adjuvant therapy, and understanding the biological mechanisms of lymphatic metastasis. Although its use in veterinary medicine is not as common as in human medicine, the importance of this procedure has been recently reported in veterinary studies. (de Araujo et al., 2015, Worley et al., 2014)

The MILN belongs to the iliosacral lymph center. It is consistently observed in situ as a large lymph node located between the deep circumflex iliac and the external iliac arteries.

Usually, single MILNs are present, but 2 MILNs on 1 or both sides may be observed. This lymph node receives efferent lymph vessels from the skin and muscles of the caudal abdomen and the pelvic limbs, as well as from organs located in the caudal abdomen and pelvis, including the colon, rectum, anus, vagina, prostate gland, ureter, bladder, and urethra. (Howard E, 2013) While the MILNs have not been definitively demonstrated to be the true SLNs for all dogs with perineal neoplasia such as anal sac adenocarcinomas, they are currently the most commonly assessed lymph nodes for evidence of metastasis. (Robat et al., 2013, Chaffin et al., 2002, Bennett et al., 2002, Williams et al., 2003, Hobson et al., 2006, Polton et al., 2007, Anderson et al., 2015, Llabres et al., 2004, Steffey et al., 2015)

Especially in case of dogs with anal sac adenocarcinoma, the extirpation of metastatic MILNs showed a positive effect on the prognosis in 2 studies. In most of these cases, extirpation of lymph nodes was peformed after lymphadenopathy or lymphadenomegaly was found in the iliac region on ultrasound images, (Hobson et al., 2006) and ultrasound-guided fine needle aspiration was performed for metastasis evaluation. (Polton et al., 2007) If excisional biopsy of MILNs can be performed with primary tumor resection in the early stage of metastasis, when the lymph node is normal in appearance on ultrasound images, it would facilitate not only exact staging of the disease but also improvement of the patients' prognosis.

Biopsy of the bilateral MILNSs can be helpful for evaluating the exact stage of neoplasia and developing treatment plans, considering that most cases of lymphadenopathy in dogs have been found on both MILNs, with similar sizes in lymphoma and anal sac adenocarcinoma. (Llabres et al., 2004) It has also been reported that optimal bilateral SLN detection was found to substantially decrease the false negative rate of SLN ultrastaging and increase the rate of detection of micrometastasis in human cervical cancers. (Cibula et al., 2012)

Laparoscopic lymphadenectomy at similar anatomical locations, performed for staging and therapy of gynecological cancers, has been reported in a previous human medicine study. (Possover et al., 1998) Using the 4-portal techique with a ventral approach, surgical excision of several lymph nodes on the bilateral sides was successfully achieved in this study.

In this study, access to bilateral MILNs was available with laparoscopy using a ventral approach in a manner similar to that used in the above-mentioned study. (Possover et al., 1998) Compared to a previous study, (Steffey et al., 2015) this study involved less effort in biopsying bilateral MILNs. The need to change the position of the dog or the surgeon to explore both sides of the peritoneum was obviated by tilting the table to access both sides. This could reduce the operation time and minimize the effort required from the surgeons. None of the dogs showed a remarkable change in the physiological status during tilting of

the table during the operation, and no anesthetic complications were observed. In the previous study, the 3-portal technique was used with the lateral approach on the ipsilateral side of the MILN. To approach the side contralateral to the MILN, establishment of additional laparoscopic cannulas is inevitable. In this study, use of the ventral approach made the 3-portal technique for one side feasible for the entire procedure. It reduced the number of cannulas required, resulting in lesser postoperative pain. (Case et al., 2011) Moreover, as the procedure is simpler than conventional open surgery, it can be helpful for reducing the total surgical time when performed in conjunction with primary tumor resections.

The observed MILNs were located lateral to the external iliac arteries, caudal to the deep circumflex iliac arteries, and medial to the testicular vessels. The mobility of the ureter could make the appropriate positioning for incision of the retroperitoneum difficult; it was more feasible to access the lateral side of the ureter than the medial side without compromising the ureter.

While exploring the caudal abdomen, efforts were made to identify other lymph nodes of the iliosacral lymph center, including the hypogastric lymph nodes and sacral lymph nodes. However, these lymph nodes are too small in normal dogs, and the organs of the caudal abdomen and pelvis (i.e., the urinary bladder and colon) made the exploration unsuccessful.

With the 6-mm laparoscopic cannula, appropriate access to the abdominal cavity was achieved, and insertion of the laparoscopic instruments was successful. In this study, all the MILN samples were normal in size and small enough to enter the 6-mm laparoscopic cannula. However, if an MILN is enlarged because of metastasis or inflammation, a commercial laparoscopic specimen retrieval bag, a wound retractor, or the finger of a sterile surgical glove can be utilized for removal. (Steffey et al., 2015, Naan et al., 2013)

Intraoperative complications were controllable without conversion to laparotomy; however, considering the anatomic intimacy of the MILN and large blood vessels, care should always be taken not to tear or damage the vessels. Capillary hemorrhages of the peritoneal fat and vessels were successfully controlled using the vessel-sealing device. As the capsule was easily torn by even minimal direct manipulation with laparoscopic Bobcock forceps, retraction and dissection should be performed with manipulation of the perinodal tissues.

In this study, splenic hemorrhage occurred in 2 dogs while establishing the first cannula (camera portal) in the right upper abdomen to prevent trauma to the spleen. However, the tail of the spleen was extended to the right upper abdomen in these 2 dogs. It is thought that splenic congestion could be induced by injection of pre-anesthetic drugs including acepromazine during the preanesthetic period. (O’Brian et al., 2004) Splenic trauma caused by insertion of a Veress needle or laparoscopic cannula is a common complication in

laparoscopy, and has been reported in a few studies in human medicine. (Prian et al., 1974, Audebert et al., 2000) In these studies, splenic congestion was also observed. Therefore, blind insertion of the Veress needle and the first cannula should be performed with extreme care considering the possibility of splenic congestion in the anesthetized state.

There were a few limitations to this study. The dogs were all young, medically fit, and healthy; there was no variation in body size and no excessive fat in the caudal abdomen. In cases of old, obese patients with tumors, the identification and dissection of MILNs may be more challenging because of the presence of excessive retroperitoneal fat, additional vascularization, and a more fragile lymph node capsule. Identification of other sublumbar lymph nodes could not be achieved because of the small size of the lymph nodes, and because of the organs and fat within the caudal abdomen.

V. Conclusion

To summarize, identification and dissection of bilateral MILNs were performed in a small cohort of normal dogs weighing under 15 kg, with laparoscopic excision using 3-portal technique and a ventral approach. It was successfully achieved to obtain histologic samples suitable for biopsy with this 3-portal technique, which offers the advantages of a short operative time, simple procedure, and less postoperative pain. Developing this procedure can help clients of small dogs and veterinarians consider MILN biopsy procedure easier to access for obtaining more detailed staging on microscopic disease status and get positive prognostic effect in case of metastatic MILNs with normal appearance in ultrasound images performing with primary tumor resection.

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VII. 국문초록

개에서 3-Portal Techniuqe을 이용한 양측 내측 장골 림프절의 복강경 절제술

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내측 장골 림프절은 깊은엉덩휘돌이동맥과 바깥엉덩동맥 사이에 위치해 있어 하복부 및 골반, 후지에 분지하는 림프관으로부터 림프를 수용하는 주요 림프절로, 해당 부위에 종양이 발생한 개, 특히 항문샘 선암종이 발생한 개

에서 주로 전이가 발생하는 것으로 알려져 있다. 종양 환자에서 전이 여부를 평가하기 위해 생검을 통한 평가는 중요한 진단 단계이나, 내측 장골 림프절의 해부학적 위치로 인한 생검의 위험성이 존재한다. 본 연구의 목적은 복강경을 이용한 최소 침습 수술을 통하여 개에서 양측 내측 장골 림프절의 생검 방법을 고안하고자 하였다. 15 킬로그램 미만의 12 마리의 건강한 수컷 비글견을 대상으로 실험을 진행하였으며, 모든 개는 등쪽 횡와위로 위치 후 양측 상복부 및 좌측 하복부에 각각 1개씩, 총 3개의 복강경 포트가 장착되었다. 개는 등쪽 횡와위 자세를 유지한 상태로 수술대를 머리쪽으로 15도 이내로 기울여 트렌델렌버그 자세를 취한 후, 절제하고자 하는 내측 장골 림프절의 반대쪽으로 수술대를 기울인 후 혈관 결찰 기구를 이용하여 양측 림프절을 절제하였다. 양측 림프절을 분리하는 전체 과정 중 술자의 위치는 개의 왼쪽으로 유지되었다. 각 림프절의 절제 소요 시간은 평균 9.75분이었으며, 양측 림프절의 절제 소요 시간은 평균 21분이었다. 수술 과정 중 주요한 복합증은 발생하지 않았으며, 모든 개는 마취에서 안전하게 회복하였다. 절제한 림프절은 10% 포르말린에 보관 후 H&E 염색을 통하여 조직학적인 평가가 진행되었으며, 모든 샘플에 대하여 종양성 병변에 대한 조직병리학적인 평가가 가능한 샘플로 확인되었다. 위 결과를 종합하였을 때, 복측 접근을 통한 복강경을 이용한 양측 내측 장골 림프절의 절제술이 기존의 생검 방법에 비하여 술후 통증 반응의 감소, 수술 시간의 단축 및 술자

의 수술 과정의 간소화가 가능하므로 종양이 의심되는 개에서 양측 내측 장골 림프절로의 전이 여부를 평가하기 위한 생검 방법으로 유용하게 이용될 수 있을 것으로 평가된다.

주요어 : 복강경, 내측 장골 림프절, 최소 침습 수술, 생검, 개

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