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Puli Vishnu Vardhan Reddy
Ph.D. Scholar, Department of Veterinary Surgery and Radiology, Madras Veterinary College, Chennai, Tamil Nadu, India

Pushkin Raj H
PG Scholars, Department of Veterinary Surgery and Radiology, Madras Veterinary College, Chennai, Tamil Nadu, India

Mohamed Shafiuzama
Professor and Head, Department of Veterinary Surgery and Radiology, Madras Veterinary College, Chennai, Tamil Nadu, India

Chandra Sekhar M
Professor and Head, Resident Veterinary Office Section, Madras Veterinary College, Chennai, Tamil Nadu, India

Ramesh S
Professor, Department of Veterinary Pathology, Madras Veterinary College, Chennai, Tamil Nadu, India

Saran S
PG Scholars, Department of Veterinary Surgery and Radiology, Madras Veterinary College, Chennai, Tamil Nadu, India

Sindhu R
PG Scholars, Department of Veterinary Surgery and Radiology, Madras Veterinary College, Chennai, Tamil Nadu, India

Corresponding Author:
Puli Vishnu Vardhan Reddy
Ph.D. Scholar, Department of Veterinary Surgery and Radiology, Madras Veterinary College, Chennai, Tamil Nadu, India

Sentinel lymph node diagnosis in mammary tumors of canines using computed tomography: Indirect lymphangiography

Puli Vishnu Vardhan Reddy, Pushkin Raj H, Mohamed Shafiuzama, Chandra Sekhar M, Ramesh S, Saran S and Sindhu R

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Abstract
Computed tomography indirect lymphangiography was performed on six canines with mammary tumours. Peri-tumoral injection, Iomeprol was injected to detect the sentinel lymph node. Tumor in the inguinal mammary gland had primary draining lymph node as inguinal lymph node, cranial and caudal abdominal mammary glands also had inguinal lymph node as primary draining lymph node. After injection of the contrast agent, the mean values after one, five and ten minutes were 28.43HU (−52.4 to 65.7) in the periphery and 35.64 HU (−53 to 84) in the center, 28.43HU (−52.4 to 65.7) in the periphery and 35.64 HU (−53 to 84) in the center and 36.7HU (−47.3 to 70.1) in the periphery and 48.25HU (−38.2 to 77.3) in the center of the node, respectively.

Keywords: Mammary tumor, computed tomography indirect lymphangiography, iomeprol

1. Introduction
Dogs are more prone than other domisticated animals to develop mammary gland tumours, which are frequently found in the inguinal or caudal abdominal mammary glands due to their larger volumes of glandular tissue. (Lana *et al*., 2007) [7]. Tumor metastasis to other organs mostly results in a fatal outcome for canine mammary neoplasia patients (Klopfleisch *et al*., 2011) [6]. Neoplastic spread mainly occurs by lymphatic invasion (Soultani *et al*., 2017) [11], less commonly by hematogenous dissemination (Mandara *et al*., 2007) [9]. Although biopsy is the gold standard procedure for identification of the metastatic invasion of the Sentinal Lymph Node (SLN), Computed Tomography (CT), Ultrasonography, Magnetic Resonance Imaging (MRI), and lately
Computed Tomographic indirect Lymphangiography (CT-LG) have also been used in women with breast cancer for the identification of sentinal lymph nodes and for the differentiation of metastatic from non-metastatic SLNs (Barrett *et al*., 2006) [1]. In the process of cancer staging in humans, the detection of sentinel lymph node (SLN) metastasis is pivotal and has a particularly high prognostic value, because the SLN is the first node to receive lymphatic flow from the primary tumor site (Gipponi *et al.*, 2004) [3]. Indirect lymphography is defined as the deposit of a contrast medium in the periphery of lymphatic vessels in the view of being absorbed and drained by the lymphatic system (Brissot and Edery, 2017) [2].

2. Materials and Methods
Study was conducted on bitches that were presented to the SAC-OP- surgery unit of Madras Veterinary College Teaching Hospital with the mammary tumors (Fig:1). Computed Tomography indirect lymphangiography, performed after peri-tumoral injection of contrast agent Iomeprol and scans were performed immediately, 5minutes and at 10 minutes interval. Parameters evaluated are location of tumor, TNM (Tumor Node Metastasis) classification, Haemato-biochemical parameters and Computed Tomography findings Fine Needle Aspiration Cytology (FNAC).

Fig 1: Right cranial and caudal abdominal mammary tumor

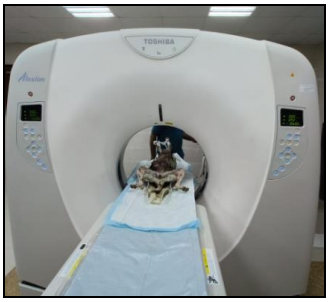

Fig 2: Positioning of animal ventro -dorsal in gantry

Fig 3: Peri-tumoral injection of Iomeprol

The dogs were subjected to CT indirect lymphangiography by injecting Iomeprol into mammary gland after induction of general anaesthesia.

3.1 Pre CT scan check up

Complete blood count, serum biochemistry and thoracic radiographs were performed for all the cases to check the physiological status of the patient for computed tomography scan as they were subjected to general anaesthesia.

3.2 Anaesthesia and patient preparation

Food was withheld for eight hours before anaesthesia. All the animals were premedicated using Inj Butorphanol & Diazepam@ 0.2 mg/ kg body weight I/V, followed by induction with Inj propofol @ 4 mg/ kg body weight I/V titrated to effect and maintainance with vaporizer setting of 2.5% Isoflurane in oxygen.

3.3 Computed tomography scan

All the dogs were placed in dorsal recumbancy by extending the fore limbs cranially and hind limbs caudally (Fig:2). Computed tomographic scan was obtained with a 16 slice Toshiba scanner, Alexion machine, helical scanner by employing a slice thickness of 1mm, pitch=0.6, 120 kVp and 150 mAs from thorax to sacral bone. Peri-tumoral injection of 1 ml iomeprol in four symmetrical sites (CT-LG) was injected in order to identify the location of the sentinel lymph node (SLNs), corresponding to the neoplastic glands (Fig: 3). CT imaging was performed before, 1, 5 and 10 minutes after the administration of contrast agent. Sagittal, coronal reformatting, MPR and 3D reconstructions were then performed.

Using Soft tissue (abdominal) window (window level = 40, window width = 400) contrast-enhanced-CT images were obtained after 1, 5, and 10 minutes of iomeprol administration. Using a 0.5 mm^2 region of interest, degree of post-contrast enhancement was measured in Hounsfield unit (HU) in the center and in each of their peripheral quadrants (a total of five measurements of the nodes). Means were calculated for centre and periphery of each node, separately.

4. Results

Out of six dogs, two had inguinal mammary tumor, three had caudal abdominal and one involving cranial, caudal and inguinal mammary glands (Figure 1). FNAC findings includes fibrosarcoma in two cases, inflammatory carcinoma in two cases, adenocarcinoma and round cell tumor in one case (figure2), these include mostly the malignant tumors but none had metastasis to thorax. On TNM classification two dogs had stage II, IV and one dog had stage I and III.

Table 1: Breed, location, FNAC findings, TNM classisfication

S.No	Breed	Age	Body weight(kg)	Location	FNAC findings	TNM classification
1.	Golden Retriever	6 Years	25	Left Caudal inguinal	Fibrosarcoma	Stage II
2.	Labrador	8 Years	35	Right cranial, caudal abdominal and caudal inguinal	Adenocarcinoma	Stage IV
3.	Labrador	9 Years	29	Left caudal inguinal	Round cell tumor	Stage I
4.	Spitz	9 Years	13	Right caudal abdominal	Mammary carcinoma	Stage II
5.	Non descript`	6 Years	18	Right caudal abdominal	Inflammatory carcinoma	Stage III
6.	Doberman	7 Years	29	Right caudal abdominal	Fibrosarcoma	Stage IV

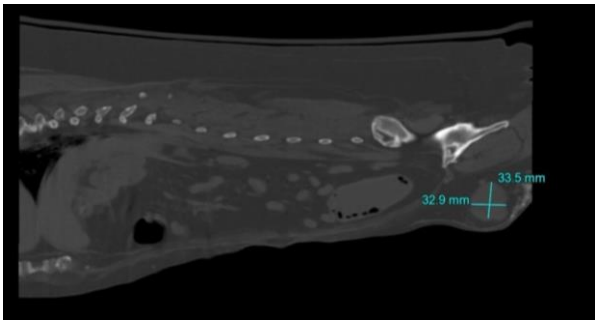

Fig 4: Measurement of size of mammary tumor in computed tomography

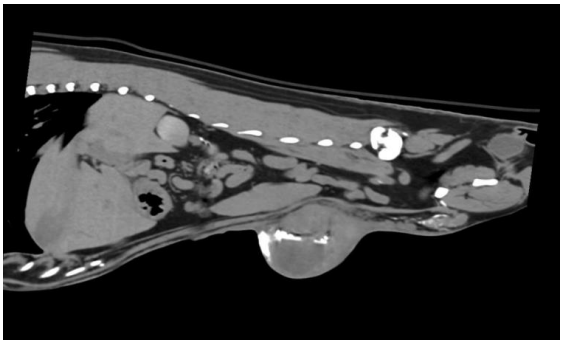

Fig 5: Passage of contrast agent to inguinal lymph node

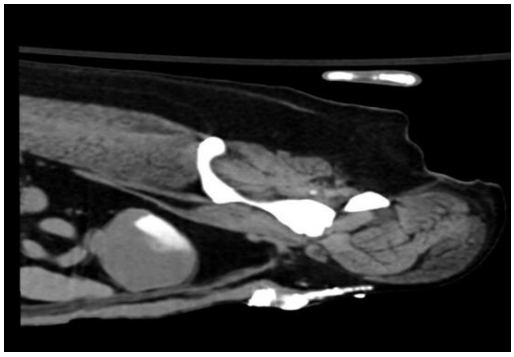

Fig 6: Passage of contrast agent to inguinal lymph node and bladder

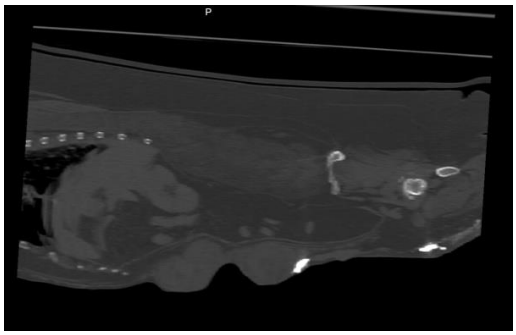

Fig 7: Passage of contrast agent to inguinal lymph node from cranial and caudal mammary glands

4.1 Computed tomography findings
In three cases, injection related side effects (small bleeding at the injection site, hematoma, etc.,) were noted, no allergic reaction to the contrast agent were seen in any these cases.

4.2 Before the contrast injection
The mean absolute density value of the sentinal lymph node was found in the center to be 25.75 HU (−68.8 to 59) and12.3HU (−58.6 to 62.8) in the periphery.

4.3 One-minute after contrast injection
Out of six cases passage of iomeprol to lymph node noted in five cases and in one case with inguinal mammary tumor passage of contrast agent was not noted. One minute after injection of the contrast agent, the mean values were found in the center to be 35.64 HU (−53 to 84) and 28.43 HU (−52.4 to 65.7) in the periphery of the SLN.

4.4 Five-minute after contrast injection
Passage of contrast agent noted in five cases. Five minutes after injection of contrast agent the mean values were found to be 38.64 HU (−53 to 84) in the center and 30.43HU (−52.4 to 65.7) in the periphery of the SLN.

4.5 Ten minutes after contrast injection
After ten mintues only five cases showed passage of contrast. Ten minutes after injection of contrast, the mean values were found to be 36.7HU (−47.3 to 70.1) in the periphery and 48.25HU and (−38.2 to 77.3) in the center of the SLN.

Tumor in the inguinal mammary gland has primary draining lymph node as inguinal lymph node, cranial and caudal abdominal mammary glands also had inguinal lymph node (Fig:7) as primary draining lymph node. In ten minutes post contrast two dogs showed dye passage in to kidneys and bladder indicating extensive vasculature of the tumor.

5. Discussion
Technically, the peri-tumorally injected CT-contrast agent technique described here was feasible with few side effects. The majority of patients had a set of easily distinguishable draining lymph nodes and lymphatic vessels, to the subsequent CT-lymphography for primary draining lymph node mapping. Neovascularization in neoplastic processes involves both lymphangiogenesis and angiogenesis. It has been demonstrated that tumour lymphangiogenesis results in the development of lymphatic vessels that may bypass the initial draining node. (Karaman and Detmar, 2014) [5].

After 1 minute following local injection of contrast agent, sentinel lymph nodes were found to be easily distinguishable, frequently in conjunction with the lymphatic vessels similar to (Rossi *et al*., 2017) [10].

The CT lymphangiography results in this study did not correlate with the metastatic status of the SLNs. In previous study (Majeski *et al*., 2017) [8] found that a reduced enhancement at 1 minute and heterogenous opacification pattern of the SLN were more likely to indicate metastasis which was contrary in our study that all normal lymph nodes displayed a homogeneous pattern.

Prior to CT lymphangiography intravenous contrast was given (Brissot and Edery, 2017) [2]. Increase in the Hounsfield units within lymph nodes were noted after administration of intravenous contrast (Grimes *et al*., 2019) [4]. Despite this rise, the Hounsfield units measured within the node increased significantly as a direct result of the peri-tumoral injection of contrast during CT lymphangiography. The overall Hounsfield unit values measured in the SLN were comparable to values reported in the earlier study by (Soultani *et al*., 2017) [11] despite the absence of intravenous contrast administration in this study.

6. Conclusion
Identification of primary draining lymph nodes using the peritumorally administered contrast agent technique and subsequent CT-lymphography was an efficient approach. This technique should currently only be viewed as a supplement to appropriate lymph node staging because sampling of anatomical lymph nodes would still be necessary if they do not enhance after the application of locoregional contrast.

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