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COMPARISON OF BENIGN AND MALIGNANT MAMMARY TUMORS IN DOGS THROUGH RAMAN SPECTROSCOPY: TWO CLINICAL CASES

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Abstract

This paper reports on two clinical cases where Raman spectroscopy has been employed for diagnostic in dog mammary tumour surgery. The comparison between histopathology and SERS results proves that the spectral markers can be used to distinguish between malignant or normal tissue. By comparing results from histopathology and Raman spectroscopy we set up a fast and affordable diagnostic tool using the marker-like Raman bands of carotenoids and respectively interfacial water. Application of the described technique is useful in comparative studies of canine and human mammary cancer.

Keywords: *Raman spectroscopy, markers, mammary tumor, interfacial water, carotenoids*

INTRODUCTION

One of the most frequently diagnosed neoplasm in intact female dogs is the mammary tumor. Canine mammary tumors have greatly increased in recent years, thus demanding rapid diagnosis and effective treatment in order to determine the animal survival (Andrade et al., 2010).
Surgery is the basic treatment of canine mammary tumors and is the most effective for disease regional control (dos Santos Horta et al., 2014).
The aim of the surgery is the complete tumor removal while saving as much healthy tissue as possible. One of the main challenges in mastectomy is to achieve margins free of cancerous cells in order to prevent possible recurrences (Sebastian et al., 2015).
A number of methods are currently used in oncology to assess the surgical margins of tumors. Histopathology is the gold-standard method used in the margin evaluation. Its only drawback stays in being not an intraoperative procedure and consequently in its impossibility to tell in real time whether all cancerous cells have been removed at the time of surgery (Liptak, 2013).
Newly emerging intraoperative diagnostic techniques adding to histopathology encompass Raman Spectroscopy. Raman Spectra contains fingerprints of each molecule in a sample, i.e. band characteristic to their vibration modes (Kneipp et al., 2015).
The spectra results from inelastic scattering of light on the sample. Energy is exchanged between light and samples with energy and momentum conservation. Surface enhanced Raman scattering (SERS) is a special Raman technique that uses metal nanostructured surfaces to amplify the Raman signal (many orders of magnitude). The mechanism relies on surface plasmon resonance. As working principle, the system photon-bio-sample-metal gives the photon sufficient momentum for efficient interaction. Coupling to local waves (a

plasmon is a quantum or quasiparticle associated to local oscillations of charge density) in the metal-biological sample enhances the scattering cross-section thus allowing detection of even single molecules (Micsa et al., 2016). Best metals for SERS are gold and silver.

MATERIALS AND METHODS

An intact 3-year-old German Shepherd female and a sterilized 12-year-old Cocker Spaniel female, were brought in by their owners at the Faculty of Veterinary Medicine Clinic of Bucharest.

Clinical examination revealed in both cases some nodular mammary masses.

The German Shepherd, named Cora, had two tumors, one on the fourth right mammary gland and one on the fifth left mammary gland. The owner discovered the lumps but only after 3 months decided to request a physical examination.

She was also previously diagnosed with Sticker tumor and had been receiving chemotherapy treatment for it.

The Cocker Spaniel, named Kisha, had one tumor, on the second right mammary gland. Kisha was brought 4 months after the owner discovered them.

At the age of 5, she was diagnosed with pyometra and had an ovariohysterectomy.

Both females were sent for thoracic X-rays and their results were the same, their pulmonary X-rays were not showing any visible modifications (Figure 1, 2).

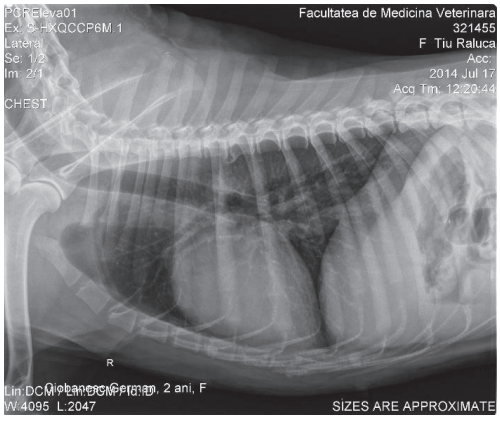

Figure 1. Cora's thoracic X-ray showed no modifications

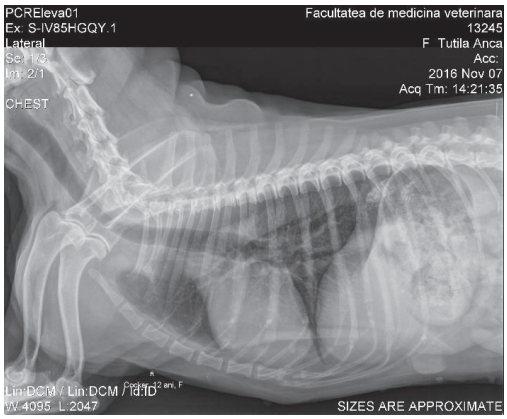

Figure 2. Kisha's thoracic X-ray showed no modifications

The biochemical parameters of both females were in normal limits.

Considering that the test results did not show any changes that could put their lives in danger if they went under anesthesia, they were scheduled for surgery.

The protocols for anesthesia and analgesia were elected in accordance with the ASA status of the patients (ASA2). Cora was premedicated with acepromazine 0,03mg/kg and ketamine 5mg/kg, and Kisha was premedicated with midazolam 0,2 mg/kg and butorphanol 0,2 mg/kg. Both dogs were induced with propofol and maintained with isoflurane gas. Butorphanol 0,2 mg/kg was used for analgesia during the surgery.

The surgeon chose for Cora to remove the last three mammary glands on both sides and for Kisha only the affected mammary gland (Figure 3, 4).

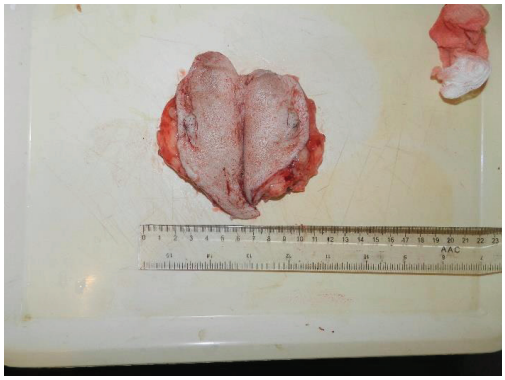

Figure 3. Part of Cora's mammary glands including the tumor

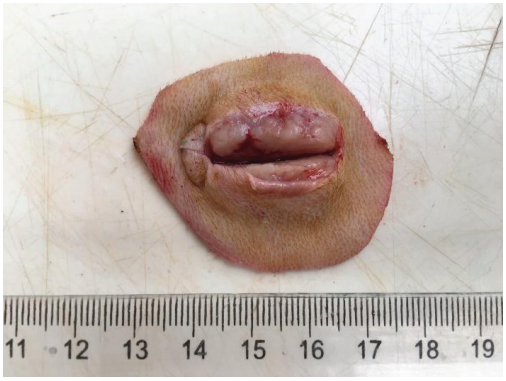

Figure 4. Kisha's mammary tumor

For the incision, a scalpel blade with a gold SERS accessory was used (Figure 5).

Figure 5. Gold SERS accessory attached to blade

The mammary tissue along with the skin were then removed using the electrocautery.
The cutaneous plane was closed by separate sutures in "U" with 2/0 Nylon.
After the mastectomy, the patients received postoperative care and medication. They were given antibiotics and anti-inflammatories. Their bodies were strapped with a piece of cotton sheet for 10 days to prevent the collection of serous fluid. After two weeks their stitches were removed.
The tumors were divided into two parts, one of which was sent for histopathological analysis and the second one was sampled for direct ex vivo (no preparation) Raman exploration.
For histopathology analysis, collected fragments from each mammary tumor were fixed in buffered 10% formalin solution for 24 h. Processing of the samples and paraffin embedding were made automatically by the tissue processor 120-3 Thermo Scientific STP. Onward, blocks were sectioned at 3 µm using Leica microtome RM 125RTS. All slides were stained with hematoxylin eosin using Thermo Scientific Microm HMS 70. The examination of the sections was made with an Olympus BX 41 microscope coupled to an Olympus DP25 video camera.
The SERS spectra were collected using a LABRAM HR 800 Horiba Jobin-Yvon spectrometer with 632nm excitation wavelength.

RESULTS AND DISCUSSION

As other research teams have found using Raman Spectroscopy in humans (Lyng et al., 2007; Surmacki et al., 2015) giving a diagnostic should need some markers (certain peaks from the Raman spectrum). Another recent study shows that a comparative analysis between humans and dogs can be useful (Birtoiu et al., 2016). Using Raman bands of carotenoids and of interfacial water as markers for benign respectively malignant tissues leads to a fast (~60 sec) and low cost diagnostic means (Birtoiu et al., 2015; 2016).
Carotenoids are exclusively present in normal tissue since they are antioxidants (substances that fight against oxidant factors like cancer cells). Their corresponding Raman bands are found in the 1000-1600 cm^{-1} spectral window (e.g. Figure 7 and Figure 9). The Raman band of interfacial water (O-H stretching) is solely found in malignant tumours. This probably shows the trend to fast multiplying of cancer cells with energy waste possibly because they need oxygen to maintain their specific redox status. Cancer cells are very rich in proteins that are hydrophilic.
The full Raman spectra of the two cases presented in this work are shown in Figure 6. Figures 7 and 8 provide a comparison of the two cases in the two regions of interest (carotenoids and interfacial water). In Figure 9 the carotenoid peak is visible, and in Figures 10 and 11, the ~3311 cm^{-1} peak corresponding to interfacial water does not appear at Cora, whereas for Kisha, the carotenoids peaks are missing and the interfacial water peak is present (Figures 12, 13, 14).

Based on this analysis we can say that Cora's tumour is benign (the carotenoid marker being observed), and Kisha's tumour is malignant (presence of interfacial water).
Histopathology analysis confirmed the results from the Raman examination.
The histopathological aspect of Cora's tumour was benign and of Kisha's tumor was malignant (Figure 15, 16).

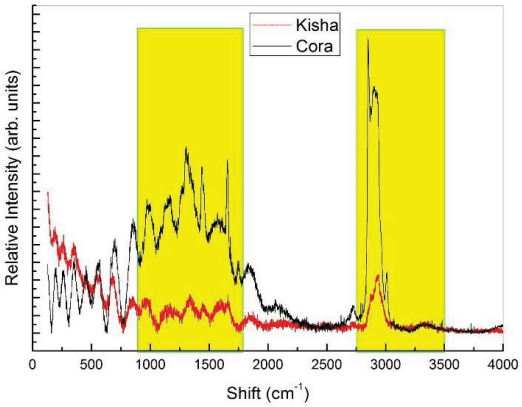

Figure 6. Full Raman Spectra for Cora and Kisha. The window 1000-1800 cm^{-1} corresponds to vibrations of carotenoids and the window 2500-3500 cm^{-1} contains vibrations of lipids and O-H.

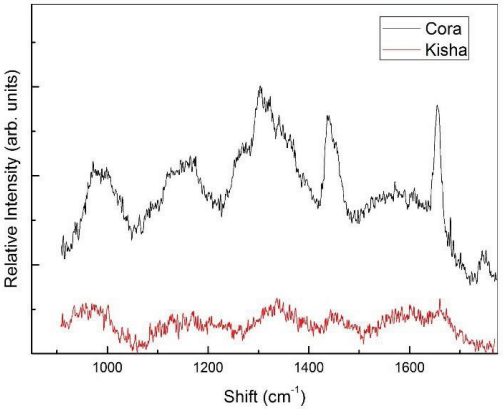

Figure 7. Raman spectra in the 1000-1600 range of both patients

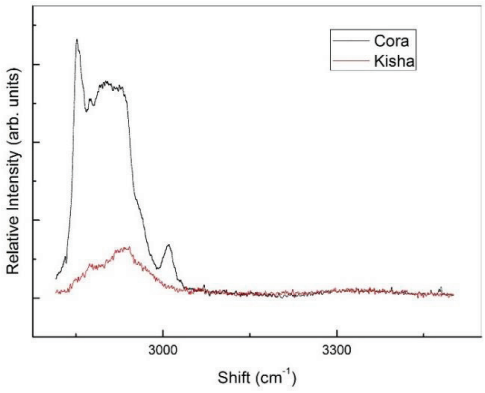

Figure 8. Raman spectra of the 3000-3500cm^{-1} region of both patients

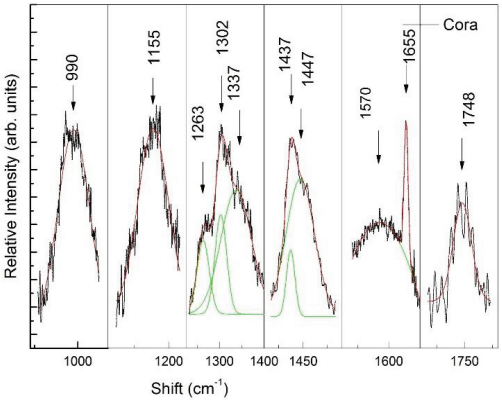

Figure 9. Deconvoluted Raman spectrum of Cora showing carotenoids at 1155cm^{-1} and 1570 cm^{-1}

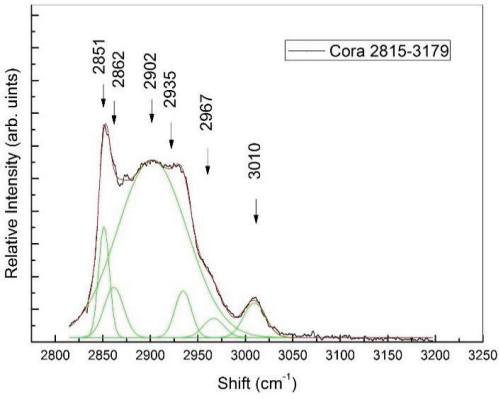

Figure 10. Deconvoluted Raman spectrum of Cora in the region 2815-3179 (lipids in normal adipose tissue)

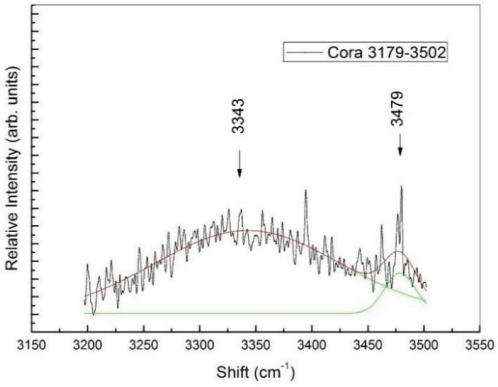

Figure 11. Deconvoluted Raman spectrum of Cora in the region 3179-3502. Benign tissue

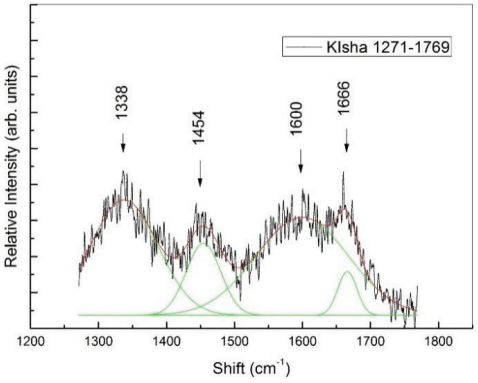

Figure 13. Raman bands of Kisha in the region1271-1769 cm^{-1}. No carotenoids. Sign of malignancy

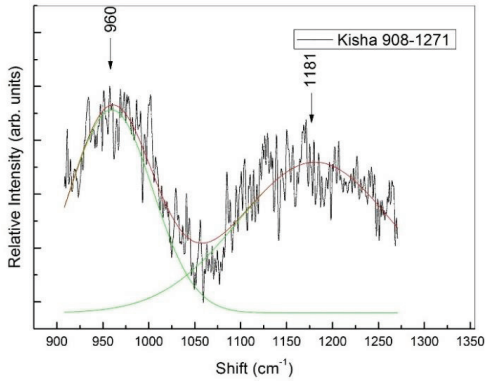

Figure 12. Raman bands of Kisha in the region 908-1271cm^{-1}. Carotenoids are absent. Sign of malignancy

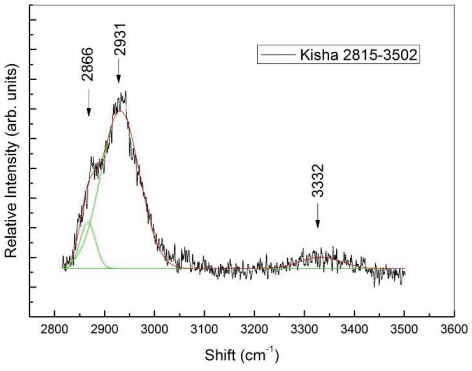

Figure 14. Deconvoluted Raman spectrum of Kisha from 2815 to 3502. 3332cm^{-1}~ interfacial water – malignancy

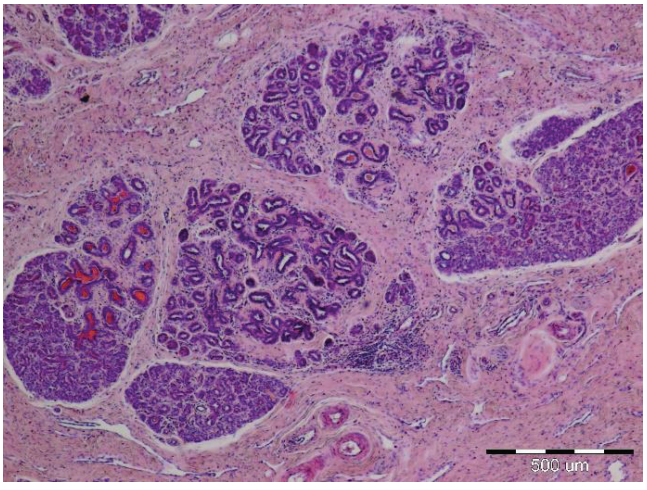

Figure 15. Histopathological aspect of Cora's tumor. Lobular hyperplasia with fibrosis, mammary gland. Increased numbers of ducts/ductules and acini per lobule. The epithelial cells exhibit no atypical changes. Note the extensive interlobular fibrosis; hematoxylin and eosin stain; objective 4x

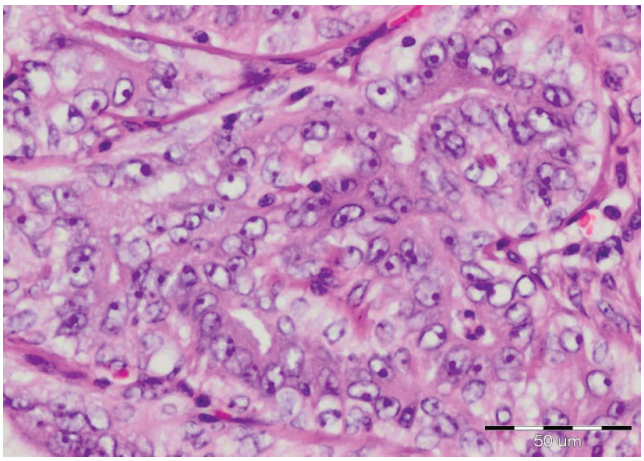

Figure 16. Histopathological aspect of Kisha's tumor. Mammary carcinoma, solid type. The neoplastic cells are arranged in solid sheets, cords or masses supported by a fine fibrovascular stroma. Neoplastic cells are polygonal to oval, with poorly demarcated cell margins and scant eosinophilic cytoplasm, moderate anisokaryosis and anisocytosis; and multiple prominent nucleoli; hematoxylin and eosin stain; objective 40x

CONCLUSION

This study on two clinical cases show a good agreement between histopathology and SERS analysis.

We set up a fast and affordable diagnostic method using the marker-like Raman bands of carotenoids and respectively interfacial water.

Visible Raman excitation wavelength (632 nm) in SERS for direct ex vivo diagnostic in dog mammary tumours is a premiere.

Owing to its non-invasiveness, high sensitivity and high specificity in fast results SERS is a useful technique to accompany histopathology.

Since SERS can give results in real time for the margin, possible recurrences may be prevented.

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