SHORT COMMUNICATION

PATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDIES ON HEMANGIOSARCOMA IN TIGERS *PANTHERA TIGRIS* AND LIONS *PANTHERA LEO*

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26 June 2018 | Vol. 10 | No. 7 | Pages: 11920–11924
10.11609/jott.2959.10.7.11920-11924
OBSERVED OPEN ACCESS

Hemangiosarcoma is a malignant tumour arising from the vascular endothelial cells and it has been reported in canines and domestic cats (Vercammen et al. 2015). But reports on hemangiosarcomas in wild felids are very few. The present paper describes the incidental findings of hemangiosarcoma in five captive wild felids. Information about the applicability of immuno-histochemical staining techniques to the tumors of captive wild felids is also limited. Keeping all these points in view, we aim to explain the pathological

features and immuno-histo-chemical expression of hemangiosarcomas in captive wild tigers Panthera tigris and lions Panthera leo.

MATERIALS AND METHODS

Three tiger and two lion carcasses were submitted by the S.V. Zoological Park, Tirupati between 2007–2014 for post mortem examination to the Department of Veterinary Pathology, College of Veterinary Science, Tirupati. At necropsy, representative tissue pieces were collected and fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5μm, and stained with haematoxylin and eosin for histopathological examination. Immunohistochemistry was performed on 4-μ sections using VEGF (Vascular endothelial growth factor), ki67 and p53 markers.

Immunohistochemistry protocol: The 4-μ sections were mounted on APES (Amino propyl ethoxy sialine) coated slides and incubated overnight at 37°C. The tissue sections were then deparaffinised by passing through xylene, two changes 15 minutes each, and dipped in absolute alcohol (2 changes) to remove xylene. The tissue was rinsed in running tap water for 10 minutes and in distilled water for 5 minutes. The

DOi: http://doi.org/10.11609/jott.2959.10.7.11920-11924

Editor: Aniruddha Belsare, University of Idaho, Moscow, USA

Date of publication: 26 June 2018 (online & print)

Manuscript details: Ms # 2959 | Received 17 January 2017 | Final received 01 May 2018 | Finally accepted 05 June 2018


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Funding: Sri Venkateswara Veterinary University, College of Veterinary Science, Tirupati.

Competing interests: The authors declare no competing interests.

Acknowledgements: We are thankful to the Dean, SVVU, College of Veterinary Science, Tirupati for institutional support.
tissue sections were then kept in Tris EDTA buffer (pH 9.0) and microwave treatment was given for two cycles 15 minutes each at 95°C to retrieve the antigenic sites. After cooling to room temperature, the tissue sections were rinsed in distilled water for 5 minutes and in Tris EDTA for 5 minutes. The slides were transferred to the humid chamber and kept in peroxidase block solution (3% hydrogen peroxide-freshly prepared) for 10 minutes to block the endogenous peroxidase. Afterwards, the slides were washed in Tris EDTA buffer for five minutes, repeated three times. The power block solution (supplied with the kit) was poured on tissue section and kept for 15 minutes. Primary antibodies ki67, p53 and VEGF (supplied with the kit) was added and kept for 20 minutes and the slides were washed in Tris EDTA buffer for five minutes each in three changes. Secondary antibody with poly HRP (supplied with the kit) was added and kept for 20 minutes and the slides were washed in Tris EDTA buffer for five minutes, then dipped in lithium carbonate twice, washed in tap water, dried and mounted in DPX.

**Results**

Five cases of hemangiosarcomas were documented; three in tigers (two females and one male) and two in female lions aged above 24 years. The cases were presented with a history of anorexia, emaciation and distended abdomen. Necropsy findings are described in detail in the following Table 1.

In all the cases, immature plump endothelial cells were noticed which were characterized as poorly demarcated and non-encapsulated proliferation of atypical ovoid to spindlyoid cells. The cells were markedly invaded into adjacent parenchyma. The neoplastic endothelial cells

<table>
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<tr>
<th>Species</th>
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<tr>
<td>1. Lion Panthera leo</td>
<td>Female</td>
<td>25 years</td>
<td>Senility, anoxemia and distended abdomen</td>
<td>· ~5 litres of sero-sanguinous fluid in the abdominal cavity · Yellowish discoloration of the liver along with multiple reddish elevated nodules of variable sizes were noticed on the dorsal surface of the liver (Image 1). Few fluid filled cysts were also observed on the liver · Reddish-brown hemorrhagic nodules were found on the spleen (Image 2)</td>
<td>· Histopathological examination of the reddish elevated areas showed necrosis and numerous blood filled spaces in liver and spleen. The anaplastic endothelial cells proliferated as solid sheets and formed tortuous vascular channels in liver and spleen (Images 7 &amp; 8). In spleen along with endothelial cell proliferation, hemosiderosis (Image 9) was also noticed. These irregular vascular spaces filled with blood.</td>
</tr>
<tr>
<td>2. Lion</td>
<td>Female</td>
<td>26 years</td>
<td>Senility, off feed for several days before death and dehydrated</td>
<td>· ~6 litres of sero-sanguinous fluid in the abdominal cavity · Liver: Multiple greyish-white and reddish-black elevated nodules of varying size were noticed throughout the liver (Image 3)</td>
<td>· Microscopic examination of liver revealed proliferation of malignant endothelial cells (Image 10)</td>
</tr>
<tr>
<td>3. Tiger Panthera tigris</td>
<td>Female</td>
<td>25–26 years</td>
<td>Anorexia and the animal was dull and depressed</td>
<td>· Abdominal cavity: ~5 litres of sero-sanguinous fluid was found. · Liver: Enlarged, reddish-brown discoloration of the ruptured liver along with attached blood clots on the dorsal surface and a big lemon sized (~2cm diameter) mass with roughened surface and focal areas of necrosis on the ventral surface were observed (Image 4)</td>
<td>· Histopathological examination of the liver revealed numerous capillary spaces disturbing the architecture of the normal hepatic parenchyma.</td>
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<td>4. Tiger</td>
<td>Female</td>
<td>25–26 years</td>
<td>Progressive emaciation</td>
<td>· Liver: Enlarged and cut section revealed oozing of several litres of blood (Image 5)</td>
<td>· The abdominal mass in the white tiger also revealed the immature endothelial cells forming vascular spaces. In addition, necrosis of the muscle fibers (Image 11) were noticed</td>
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<tr>
<td>5. White Tiger</td>
<td>Male</td>
<td>25 years</td>
<td>Respiratory distress and abdominal breathing was noticed in last 3 days. In addition to this, emaciation and anoxemia was also noticed</td>
<td>· A black coloured mass attached near the abdomen was noticed (Image 6)</td>
<td></td>
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</table>
were anaplastic, pleomorphic and were grouped into masses. Individual cells were characterized by scant to moderate eosinophilic cytoplasm and moderately pleomorphic, hyperchromatic, round to ovoid nuclei with medium-sized nucleoli.

**Immunohistochemistry result**

DAB reagent was used to show visible reaction between the primary and secondary antibodies as brown colour in the neoplastic sites of tissue sections. The development of blue colour is due to the counter stain (hematoxylin) used in the IHC procedure. In the present study, the VEGF marker expressed high positive immunoreactivity (expressed intense brown colour) in the cytoplasm of neoplastic endothelial cells (Image 12) but the proliferative markers ki67 and p53 did not express positive immunoreactivity (Images 13 & 14).

**DISCUSSION**

Few reports exist on hemangiosarcoma in captive wild felines (Ervin et al. 1988, Kang et al. 1996; Vercammen et al. 2015). The present study described the different peculiar gross features of hemangiosarcoma in five captive wild felids. Four showed elevated nodular growths on the liver (Ervin et al. 1988; Amaravathi et al. 2012). One case, without elevated nodular growths on liver, had severe oozing of blood on cut section of liver. Based on the present investigation, even though elevated growths were not observed, continuous oozing of blood from cut surface gives suspicion to hemangiosarcoma. It can be confirmed by histopathological examination and further confirmed by using tumour specific markers. The expression of VEGF in all the five cases was observed and it might be due to tumour angiogenesis and its expression correlate with tumour malignancy. This was in accordance to earlier study (Farkkila et al. 2011). Metastasis to spleen might be because of its access to vascular channels (Stannard & Pulley 1978).

**CONCLUSION**

Based on the histopathological features and immunohistochemical expression of VEGF, the present cases were identified as hemangiosarcomas. The VEGF immunoreactivity was seen in all five cases indicating the angiogenesis and malignancy.

**REFERENCES**

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Image 4. Tiger: Reddish-brown discoloration of the liver along with an attached rounded mass

Image 5. Tiger: Cut section of liver showing severe oozing of blood

Image 6. White Tiger: Note black coloured mass in the abdomen

Image 7. Liver (Lion): Note presence of proliferated endothelial cells with blood filled vascular spaces (Arrows). Circle showing the hepatocyte H&E: x 280.

Image 8. Spleen (Lion): Section showing numerous proliferated pleomorphic endothelial cells forming irregular vascular spaces (Circle). H&E: x 280.

Image 9. Spleen (Lion): Note presence of brown coloured hemosiderin pigment material (Circle) and proliferated endothelial cells (Asterisks) with RBC filled vascular spaces (Arrow). H&E: x 70.
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Image 10. Liver (Lion): Note proliferated malignant endothelial cells

Image 11. White Tiger: Tumour mass section showing plump proliferated immature endothelial cells with blood filled vascular areas. H&E: x 280.

Image 12. Liver (Lion): VEGF: Note intense brown staining of the neoplastic endothelial cells with VEGF x 700.

Image 13. Liver (Tiger): Ki67: No reactivity (no brown colour development) was observed with Ki67 marker x 700.

Image 14. Liver (Lion): p53: No reactivity was observed with p53 marker x 1000.


