Leydig cell tumor is relatively common in dogs, accounting for approximately a quarter to half of all canine testicular tumors. While most Leydig cell tumors are benign, malignant forms have been reported. A 13-year-old Pomeranian visited a local hospital with a chief complaint of edema in the left testicle. Radiography and ultrasonography revealed a mass in the left testicle; therefore, orchidectomy was performed. Microscopic analyses revealed the growth of a neoplastic mass compressing adjacent seminiferous tubules. Immunohistochemistry confirmed that the neoplasm was derived from Leydig cells. Neoplastic cells showed marked cellular and nuclear pleomorphisms, which is one of criteria for malignant Leydig cell tumor. However, the immediate postsurgical outcome was good, and seven months after surgery, the patient recovered well without any complications or recurrence. Therefore, the present study indicates that cellular and nuclear polymorphisms cannot be alone inform the differentiation between benign and malignant Leydig cell tumor in dogs.
was performed to differentiate the Leydig cell tumor from other testicular tumors. Slides were incubated with 3% hydrogen peroxide for 20 minutes at room temperature, and washed with phosphate buffered saline (PBS) for three times. The slides were then dipped into Tris-EDTA solution (pH 9.0) and heat-induced antigen retrieval was conducted with a microwave (750W). The slides were cooled down, and incubated with 5% normal goat serum for 30 minutes to remove non-specific signals, and then with primary antibodies (Table 1), 4 °C, overnight.

Table 1: Primary antibodies used for the differentiation of Leydig cell tumor.

<table>
<thead>
<tr>
<th>Primary antibody</th>
<th>Clone</th>
<th>Antigen retrieval</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vimentin</td>
<td>V9</td>
<td>Tris-EDTA, 10 min</td>
<td>1:800</td>
</tr>
<tr>
<td>c-kit (CD117)</td>
<td>Polyclonal</td>
<td>Tris-EDTA, 15 min</td>
<td>1:300</td>
</tr>
<tr>
<td>MelanA</td>
<td>A103</td>
<td>Tris-EDTA, 15 min</td>
<td>1:100</td>
</tr>
</tbody>
</table>

The slides were washed with PBS for three times, and incubated for 40 minutes at room temperature with a peroxidase-conjugated polymer, which contains antibodies to rabbit/mouse immunoglobulins. The slides were washed for three times with PBS once more, visualized by 3’3’-diaminobenzidine (DAB), and counterstained with Gill’s hematoxylin.

Microscopically, a neoplastic mass was observed in the left testicle, which proliferated to the extent that it compressed adjacent atrophic seminiferous tubules. A neoplastic mass was encapsulated by fibrous connective tissue, and demonstrated no invasive growth to adjacent tissues. In the mass and adjacent testicular structures, there were multifocal hyperemic lesions. Neoplastic Leydig cells (interstitial cells) organized solid sheet, and had indistinct cell borders, moderate amount of eosinophilic cytoplasm with variable accumulations of lipids, marked nuclear polymorphisms, anisokaryosis, and distinct single nucleoli (Figure 3).

There was less than 1 mitotic count per 10 high-power fields, and no evidence of invasion in adjacent lymphatic and blood vessels. Immunohistochemical analysis revealed that neoplastic cells showed homogenous positivity for vimentin, c-kit (CD117), and Melan A, confirming that neoplastic cells were derived from Leydig cells (Figure 4). The splenic mass was diagnosed as lymphoid nodular hyperplasia, and evidence for metastasis of the Leydig cell was not found. The immediate postsurgical outcome was good. The patient showed stable vital signs, and the patient recovered by 7 months following surgery without recurrence or any complications.
Although several studies have described variant forms of Leydig cell tumor in rats (Qureshi et al., 1991; Rehm and Waalkes, 1988), reports showing variable histological patterns for the canine incidence of this tumor are lacking. Even though most Leydig cell tumors are reportedly benign, there is evidence for their malignant counterparts. In most cases, cellular and nuclear shapes of neoplastic cells composing Leydig cell tumors are known to be small and regular (Agnew and Maclachlan, 2017). Histologic criteria for the diagnosis of malignant Leydig cell tumor is based on the irregularity of neoplastic cells, numerous mitotic counts, and vascular invasion (Agnew and Maclachlan, 2017). However, cellular polymorphism and anisokaryosis were not prominent in previous studies reporting malignant Leydig cell tumor with metastasis (Canadas et al., 2016; Kudo et al., 2019; Togni et al., 2015), in contrast to the present study. Instead, those previous studies showed relatively high mitotic count (6-21 or common in 10 high power field), whereas mitotic count was low in the present case (<1 in 10 high power field).

The present study indicates that Leydig cell tumor with severe nuclear pleomorphism could exhibit benign behaviour, thus showing uncommon histologic variant of benign tumor. The evidence provided herein indirectly proves that nuclear and cellular polymorphisms cannot alone indicate malignant interstitial cell tumor in canine species, and suggests mitotic count could be additional criteria to differentiate malignant Leydig cell tumor from its benign counterparts. However, long-term follow-up is necessary to confirm the benign feature of the tumor in the present study.

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AUTHORS CONTRIBUTION

SHK collected clinical data, drafted and revised the manuscript. BJS, SHC, HYL interpreted the clinical and histopathological data. JHS designed the project and critically revised the manuscript.

CONFLICT OF INTEREST

The Authors declare that there is no conflict of interest.

REFERENCES


