

## Multicentric lymphoma in a Rottweiler dog with bilateral ocular involvement: A case report

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Article Info	Abstract
<p><b>Article history:</b></p> <p>Received: 12 February 2018 Accepted: 22 May 2018 Available online: 15 September 2018</p> <p><b>Key words:</b></p> <p>Hyphema Lymphoma Multicentric Rottweiler dog</p>	<p>A 10-year-old, male Rottweilerdog was presented to Small Animal Hospital of Tehran University with a history of lethargy, anorexia, weight loss, vomiting, polyuria, polydipsia and blindness. The dog showed symptoms of depression, high body temperature (39.2 °C), tachypnea (40 breaths min<sup>-1</sup>) and cachexia. In ophthalmic examination, bilateral hyphema (hemorrhage in anterior chamber of the eye) and blindness were detected. The pulse of the animal was normal (90 beats min<sup>-1</sup>) and obvious general lymphadenopathy was determined. Lymph nodes were firm, freely movable, and painless on palpation. Initial differential diagnosis of lymphoma included lymphoma, metastatic neoplasia, lymphoid hyperplasia, and lymphadenitis. In hematology and cytology tests, this case was suspected to lymphoma. Immunohistochemical staining of neoplastic lymph node revealed that nearly 20.00 – 25.00% of neoplastic cells were strongly positive for anti CD3, whereas they were negative for both CD20 and CD79a. According to the microscopic and immunohistochemical findings, a diagnosis of T cell lymphoma was made. The present report is the first case of multicentric lymphoma with ocular metastasis in a dog in Iran.</p> <p style="text-align: right;">© 2018 Urmia University. All rights reserved.</p>

### لنفومای چند کانونه در یک سگ روتوایلر با درگیری چشمی دو طرفه: گزارش موردی

#### چکیده

سگ روتوایلر نر ۱۰ ساله با تاریخچه تشنگی، بی اشتها، کاهش وزن، استفراغ، پریشانی، پر ادراری و کوری به بیمارستان حیوانات کوچک دانشگاه تهران ارجاع داده شد. این سگ علائمی از افسردگی، درجه حرارت بالا (۳۹/۲ درجه سانتیگراد)، افزایش تعداد ضربان قلب (۴۰ ضربه در دقیقه) و لاغری را داشت. در معاینه چشم خونریزی دو طرفه اتاق قدامی کره چشم و کوری دیده شد. نبض حیوان طبیعی بود (۹۰ ضربه در دقیقه) و لنفادنوپاتی عمومی کاملاً مشخص، شناسایی گردید. عقده‌های لنفاوی کاملاً سفت، براحتی جابجا می شدند و در ملامسه دردناک نبودند. تشخیص تفریقی اولیه لنفوما شامل لنفوما، نئوپلازی متاستاتیک، هایپرپلازی لنفوئیدی و لنفادنیت بود. در آزمایش خون‌شناسی و سیتولوژی، این مورد مشکوک به لنفوما بود. رنگ آمیزی ایمونوهیستوشیمی عقده‌های لنفاوی نئوپلاستیک نشان داد که نزدیک به ۲۰/۰۰ تا ۲۵/۰۰ درصد سلول‌های سرطانی بطور مشخص برای آنتی CD3 مثبت بودند، در حالیکه آنها برای CD20 و CD79a منفی بودند. بر طبق یافته‌های میکروسکوپی و ایمونوهیستوشیمی، تشخیص لنفوما سلول‌های T صورت گرفت. گزارش موجود اولین مورد از لنفوما چند کانونه همراه بامتاستاز در یک سگ در ایران می‌باشد.

واژه های کلیدی: چند کانونه، سگ روتوایلر، لنفوما، هایفیما

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## Introduction

Lymphoma or lymphosarcoma is one of the most common neoplasms of the hematopoietic system and accounts for approximately 7.00% to 24.00% of all canine neoplasia.<sup>1,2</sup> Lymphoma classification is made based on anatomic location and histological criteria. The most common anatomic forms of lymphoma include; multicentric, cranial mediastinal, gastrointestinal and cutaneous. Primary extranodal forms of lymphoma such as those occurring in the central nervous system (CNS), eyes, bone, testes and nasal cavity are less commonly observed.<sup>1,3</sup> In cats, lymphoma has been associated with the feline leukemia virus (FeLV); however, in dogs, the etiology is unknown. A genetic predisposition is suspected in certain breeds, such as the Boxer, Basset hound, Rottweiler, Cocker spaniel, Saint-Bernard, Scottish terrier, Airedale terrier, English bulldog and Golden retriever. Lymphoma is commonly occurred in middle aged dogs or older (6 to 12 years of age), as in this case, with no confirmed sex predilection.<sup>4</sup> The complete blood count analysis and serum biochemical profile abnormalities are nonspecific and rarely diagnostic for lymphoma. The hematologic manifestations of lymphoma are due to the neoplastic infiltration of bone marrow; splenic malfunction; chronic disease; or paraneoplastic, immune-mediated abnormalities. Leukocytosis may result from the production of cytokines by the tumor cells. Biochemical abnormalities may be due to the release of cytokines from tumor cells or to organ damage caused by neoplastic infiltration. Anatomic forms of lymphoma found in dogs are multicentric, mediastinal, alimentary, and extranodal. The multicentric form includes more than 80.00% of lymphomas and is characterized by generalized lymphadenopathy, with or without hepatomegaly, splenomegaly, and bone marrow involvement.<sup>4,5</sup> The lymph nodes are markedly enlarged, painless, and freely movable under the skin. The most common clinical signs are nonspecific, including weight loss, anorexia, and lethargy. The airway obstruction by enlarged lymph nodes may cause coughing.<sup>4</sup> Immunophenotyping is an important prognostic tool for diagnosis of lymphoma and has become an essential step in classification of lymphoma because of the relationship between biological behavior and response to therapy. T-cell lymphoma has a worse prognosis than B-cell lymphomas. Antibody markers to CD3 and CD79 show cross-reactivity across species lines of B cells and T cells, respectively.<sup>6-8</sup> This report describes a multicentric lymphoma with metastasis in the eye.

## Case Description

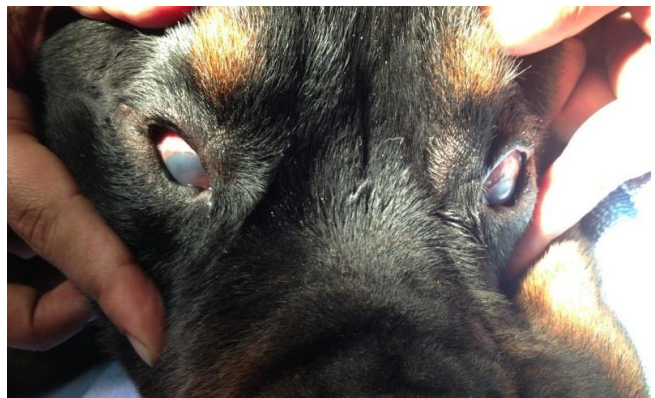
A 10-year-old, male Rottweiler dog was presented to the Small Animal Hospital of University of Tehran with a history of lethargy, anorexia, weight loss, vomiting,

polyuria, polydipsia and blindness. The dog showed symptoms of depression, fever (39.2 °C), tachypnea (40 breaths min<sup>-1</sup>) and cachexia. Bilateral hyphema and blindness were detected in ophthalmic examination (Fig. 1). The pulse rate of the animal was normal (90 beats min<sup>-1</sup>) and obvious general lymphadenopathy determined. Lymph nodes were firm, freely movable, and painless on palpation. Initial differential diagnosis of lymphoma included lymphoma, metastatic neoplasia, lymphoid hyperplasia, and lymphadenitis.

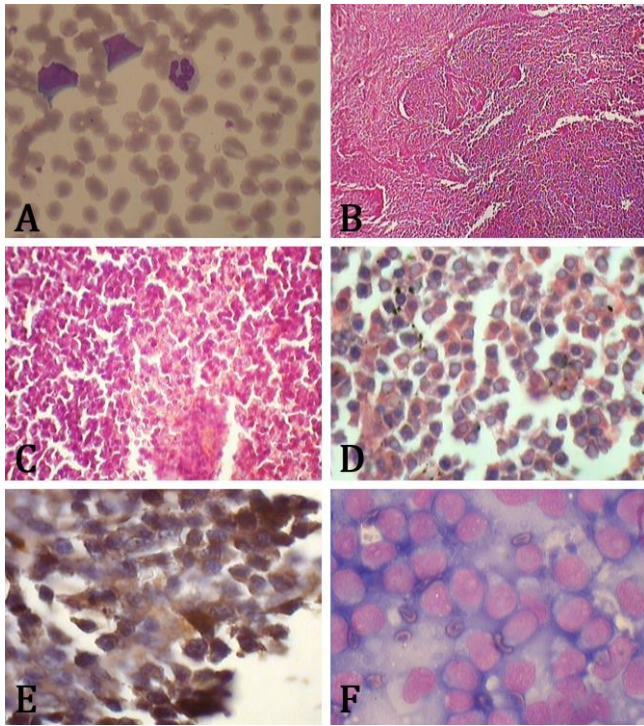
A complete blood cell counter (Nihon Kohden, Celltac; Tokyo, Japan) revealed non-regenerative anemia, with a hematocrit of 25.40% (reference range, 37.00 to 55.00%) and reticulocytes 0.08%; (reference range, 0.05 to 0.10%) hemoglobin 105 g L<sup>-1</sup> (reference range, 120 to 180 g L<sup>-1</sup>), leukocytosis of 37.00×10<sup>9</sup> cells L<sup>-1</sup> (reference range, 6.00 to 16.90×10<sup>9</sup> cells L<sup>-1</sup>), and lymphocytosis of 24.60×10<sup>9</sup> cells L<sup>-1</sup>; (reference range, 1.50 to 5.50 × 10<sup>9</sup> cells L<sup>-1</sup>). Abnormal serum biochemical results included higher alanine aminotransferase of 301.00 U L<sup>-1</sup> (reference range, 10.00 to 100.00 U L<sup>-1</sup>), alkaline phosphatase of 413.00 U L<sup>-1</sup> (reference range, 23.00 to 212.00 U L<sup>-1</sup>) aspartate aminotransferase of 170.00 U L<sup>-1</sup> (reference range, 20.00 to 66.00 U L<sup>-1</sup>).

A fine needle aspirate or biopsy of an affected lymph node was performed.<sup>4</sup> Atypical lymphocytes were observed in blood smear (Fig. 2A), and cytological examination showed an increase of more than 50.00% of the lymphoblast population. Cytologic assessment of aqueous humor from anterior chamber may help to identify infectious agents or neoplasia. Aqueous humor samples were obtained by anterior eye segment paracentesis after the dogs were euthanized. All samples were collected within 24 hr. A 22-G needle was inserted into the anterior eye segment at the limbus, above and parallel to the plane of the iris. Aqueous humor was then gently aspirated into a 2-mL syringe.

A small amount of fluid was removed from affected lymph node and evaluated immediately. Cytologically, a moderate number of lymphocytes were observed in the proteinous background of lymph node (Figs. 2B and 2C).



**Fig. 1.** Bilateral hyphema was notable.



**Fig. 2.** Pathologic evaluation of sampled lymph node. **A)** Hematologic features of blood smear; atypical lymphocytes (H & E; 1000 $\times$ ), **B)** High proliferation of neoplastic cells which obliterate the normal structure of lymph node (H & E; 40 $\times$ ), **C)** Closely packed of neoplastic cells which appear as round cell pattern embedded in loose connective tissue (IHC; 100 $\times$ ), **D)** Neoplastic cells with round vesicular nuclei and well-defined cytoplasmic borders (IHC; 1000 $\times$ ), **E)** The neoplastic cells are immuno-labeled for anti CD3 marker with brown cytoplasmic stain (IHC; 1000 $\times$ ), **F)** Cytology features of lymph node; large lymphocytes with nucleoli (lymphoblast) are predominant (H & E; 100 $\times$ ).

In the current case, biopsy was performed from lymph node under general anesthesia. A punch biopsy which was taken from the left prescapular lymph node appeared dark red and friable. The sampled lymph nodes were fixed in 10.00% neutral buffered formalin, routinely processed and embedded in paraffin, sectioned at 5  $\mu$ m, and stained with hematoxylin and eosin (H & E). For immunohistochemical analysis, the antibodies CD20, CD79a and CD3 (all from Dako, Glostrup, Denmark) were applied in appropriate dilutions on the sample sections using the labeled streptavidin-biotin method using LSAB™ kit (Dako). The slides were counterstained with Mayer's hematoxylin (Dako). The CD3 and CD20 antisera specificity was ascertained in normal thymus and lymph node of dog respectively. Complete effacement of nodal architecture was occurred by invasion of neoplastic cells within capsule and perinodal region. Moreover, the neoplastic cells obliterated the cortex of lymph nodes and infiltrated and obscured medullary cords and sinusoids. Tumor cells had large pale or vesicular nuclei which only few of them showing nucleoli in the center of nucleus.

The eosinophilic cytoplasm was fairly abundant with well-distinct cell boundaries. Mitotic figures were not numerous (Figs. 2D-2F).

Immunohistochemical staining of neoplastic lymph node revealed that nearly 20.00-25.00% of neoplastic cells were strongly positive for anti CD3 (Fig. 2E), whereas they were negative for both CD20 and CD79a. The dog died a day after hospitalization.

## Discussion

Lymphoma is one the most common hematopoietic neoplasm in dogs, occurring in 13 to 24 per 100,000 dogs.<sup>9</sup> Multicentric lymphoma is one of the most common forms of lymphoma in dogs. Ocular lymphoma occurs in both dogs and cats. As in the present case, it was commonly associated with the multicentric form. Photophobia, belfarospasm, epiphora, lymphoma, hypopyon, ocular masses, third eyelid infiltration, anterior uveitis, chorioretinal involvement and retinal detachment may be present in these animals.<sup>10</sup>

Diagnosis of lymphoma can be confirmed by cytological and histopathological tests or less frequently, using molecular techniques. In most dogs with multicentric lymphoma, a diagnosis can be easily made by cytological evaluation of fine needle aspiration of the affected lymph nodes.<sup>10</sup> Based on the microscopic and immunohistochemical findings, a diagnosis of supposed T cell lymphoma was made. The present report is the first case of multicentric lymphoma with ocular metastasis in a dog in Iran.

## Acknowledgments

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## Conflict of interest

The authors declare that there was not any conflict of interest.

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