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CASE REPORT

# Diffuse large B-cell lymphoma with conjunctival involvement in a Dobermann dog : A case report

**S. Tamizharasan, S. Vairamuthu[1], N. Pazhanivel[*], M. Chandrasekar[2], M. Tirumurugaan[3] and Ganne Venkata Sudhakar Rao**

Department of Veterinary Pathology, Madras Veterinary College, TANUVAS, Chennai-600 007, Tamil Nadu, India, [1]Centralized Clinical Laboratory, [2]Department of Veterinary Clinical Medicine, [3]Translational Research Platform for Veterinary Biologicals (TRPVB) TANUVAS, Chennai-600 051

**Address for Correspondence**

*Dr N. Pazhanivel, Professor, Department of Veterinary Pathology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 007, India, E-mail: drnpvel@gmail.com*

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## ABSTRACT

A two-year-old male Dobermann dog was brought to Madras Veterinary College Teaching Hospital with the history of a growth in the right eye. Clinical examination revealed normal vital parameters and all the peripheral lymph nodes were enlarged. Ophthalmic examination of the right eye revealed absence of menace and palpebral reflex and a growth in the junction of palpebral and bulbar conjunctiva with congestion and ulceration. Conjunctivitis, corneal opacity was also noticed. Fine needle aspiration cytology of popliteal lymph node showed large neoplastic lymphoblasts suggestive of lymphoma. Flowcytometry analysis of fine needle aspirated cells revealed 63.7% of cells reacted with CD21 markers indicative of B cell immunophenotype. Ultrasound examination revealed splenomegaly and enlarged thoracic and abdominal lymph nodes. Animal was euthanized as per owner's request. Gross examination revealed tumor mass noticed in the conjunctiva. Internal gross examination showed whitish grey miliary nodules throughout the parenchyma of spleen, liver and kidney. Histopathological examination of lymph node revealed starry sky appearance and complete evasion of architecture of lymph node by neoplastic cells. Most of the visceral organs were infiltrated with neoplastic lymphoid cells. Lymphoma may be added to the differential diagnoses when dogs show ocular involvement.

**Keywords:** B cell, canine, conjunctiva, diffuse, DLBCL, Dobermann, flow cytometry, immunophenotype, lymphoma



Lymphoma is the second most common neoplasm in dogs, with an estimated incidence in the range of 13 to 24 per 1,00,000 dogs at risk and accounts for approximately 7% to 24% of all neoplasia and 83% of all hematopoietic tumors in dogs[1]. Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma histotype affecting dogs, contributing up to 47.8% of all B-cell lymphomas[2]. Ocular manifestations in canine multicentric lymphoma are the second most common clinical signs after lymph node enlargement, have been reported to occur in 37% of cases[3]. Doberman, Rottweiler, boxer and Bernese Mountain dogs showed a significant predisposition to lymphoma and B-cell lymphoma was more common in Rottweiler, when compared with crossbred dogs[4].

Lymphoma was common in non-descript breeds in and around Chennai[5], whereas, the present case was recorded in Dobermann which was genetically predisposed to lymphoma[4]. Usually, middle to old aged dogs was affected with lymphoma, but in the present case, 2 year-old dog was affected and it was treated symptomatically for more than 4 months and finally brought to Madras Veterinary Teaching Hospital with the history of growth in the right eye region.

Physical examination revealed that the animal was dull and depressed, anorectic with ulcerated wound in the right ocular region. All the peripheral lymph nodes were characterized by painless, rubbery, and discrete generalized enlarged lymph nodes[6] (Table 1). Ocular examination of the right eye revealed absence of menace and palpebral reflexes indicated that there was loss of eye sight as earlier reported[7] and left eye was also examined and found to be normal. Corneo-conjunctival thickening might induce a friable surface and incomplete eyelid closure (lagophthalmos), leading to a painful ulcer[7] which was also noticed in the present case. Radiograph of head, thoracic and abdominal region revealed no abnormalities since it was soft tissue growth. Ultrasound examination showed splenomegaly, enlarged mediastinal, renal, inguinal lymph nodes as hypoechoic structures. Hematology revealed leukocytosis and neutrophilia, which might be due to secondary bacterial infection[1]. Serum biochemistry was found to be normal.

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Fine needle aspiration biopsy was taken from the popliteal lymph node which showed large

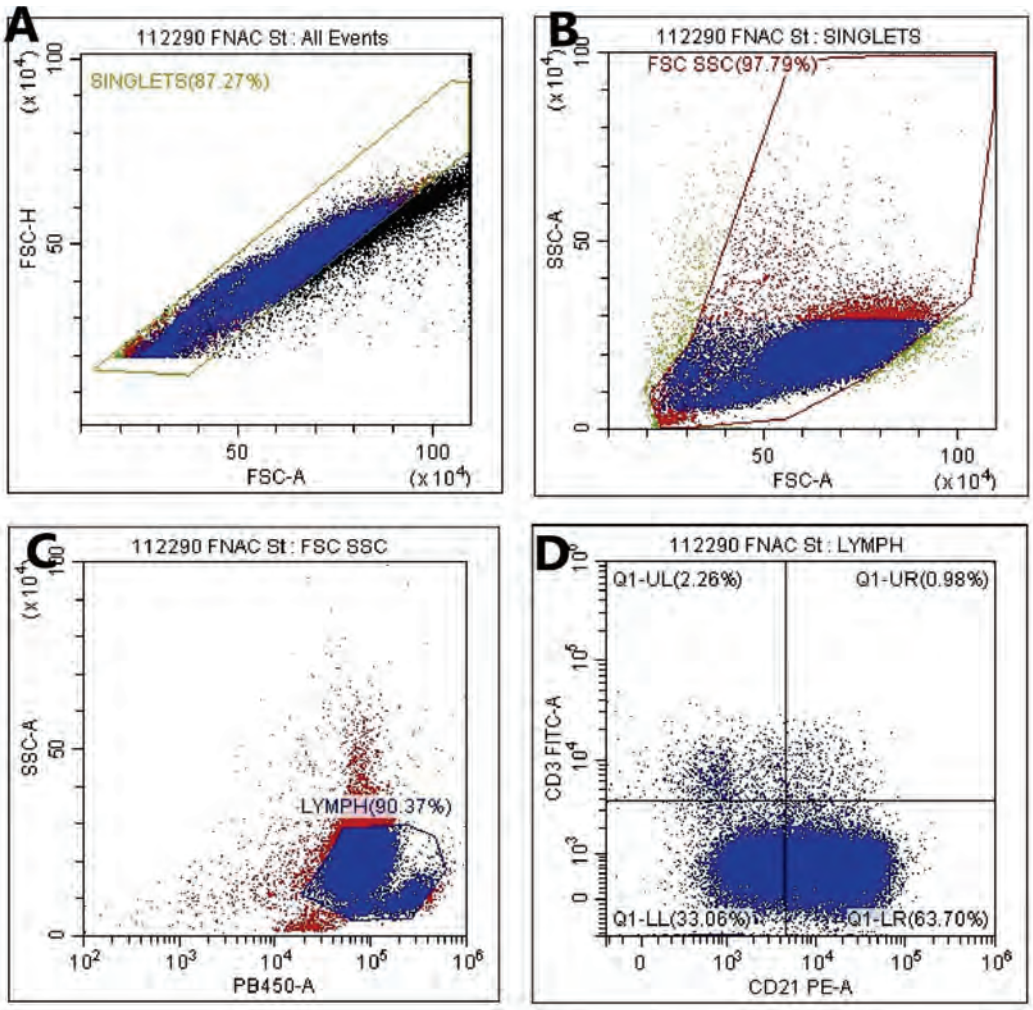


**Fig. 1.** Flow cytometry analysis of FNA cells of lymph node. **A.** Sorting out the doublets from singlets (87.27%). **B.** Sorting of agranulocytes (97.79%) from granulocytes. **C.** Sorting of lymphocytes (90.37%) under PB450-A. **D.** CD3 vs. CD21 plot of CD45-gated cells showing higher CD21 expression on large cells - 63.70% (Q1-LR) compared with CD3 expression - 2.26% (Q1-UL).



neoplastic cells with large nuclei having coarse chromatin containing multiple nucleoli. Scanty basophilic cytoplasm indicated it as centroblastic type. Mitotic index was high (≥ 5) which showed its aggressiveness and high grade. Fine needle aspirates of lymph node were collected in normal saline and subjected to flowcytometry study which showed more than 63% positivity to CD21 markers indicated that it was B immunophenotype[8] (Fig. 1A-D). But when diagnosed, it was at stage V with systemic signs (Substage b)[8]. So, Lymphoma should be considered in differential diagnosis, when the dogs were coming with ocular involvement.

**Table 1.** Dimension of the peripheral lymph nodes assessed using vernier caliper.

| Lymph Node | Left in mm | | | Right in mm | | |
|---|---|---|---|---|---|---|
| | Length | Breadth | Thickness | Length | Breadth | Thickness |
| Sub mandibular | 63 | 43 | 30 | 72 | 45 | 35 |
| Prescapular | 68 | 44 | 32 | 53 | 41 | 30 |
| Axillary | 24 | 17 | 14 | 20 | 15 | 13 |
| Inguinal | 86 | 32 | 25 | 63 | 28 | 21 |
| Popliteal | 54 | 41 | 21 | 46 | 37 | 18 |

Multidrug chemotherapy protocols are known to be more effective than single-agent chemotherapy; moreover, treatment can be tailored to the stage at presentation and the organs involved[1]. Local ophthalmic steroid therapy can be effective for alleviating ocular signs and can maintain a good quality of life by decreasing the corneal infiltration in the involved eye even without systemic chemotherapy[7]. Local ophthalmic therapy might be the only treatment option in a lymphoma patient with exacerbated systemic conditions, where systemic treatment cannot be tolerated as in the present case. To alleviate the clinical signs, dog was treated with fluids and antibiotics, but not responded. Animal was euthanized as per the request of owner. Post mortem examination showed that the

right eye totally lost its architecture due to increased pressure of tumour nodules (Fig. 2a). Left eye was not affected, but tumour nodules were present along with conjunctiva without disturbing the eye ball (Fig. 2b). All the lymph nodes were enlarged in size. Splenomegaly was seen (Fig. 2c). Miliary grayish white nodules within the parenchyma of liver, kidney (Fig. 2d) and heart were seen.

Microscopical examination of the lymph node revealed diffuse arrangement of sheets of neoplastic cells characterized by uniformly large nuclei (>2 red cells in diameter) with scanty cytoplasm. Nuclei were usually round or rarely cleaved or indented. Architectural changes were thinning of the lymph node capsule, compression of the peripheral sinus and fading germinal centers were seen in the outer cortex. There is destruction of normal nodal structures, filling of the medullary cords, and compression of medullary sinuses[2] (Fig. 3a). Neoplastic cells were medium to large sized with large nuclei containing multiple nucleoli. Discrete or solitary mass of tumour arising from the conjunctiva was noticed (Fig. 3b). Metastasis was found in heart, kidney, liver, lung, spleen, gall bladder, intestine and caecum. The tumorous nodules were present multifocally (Fig. 3c-j). Dog with metastatic lesion in the heart did not show any cardiac symptoms. Grossly, multiple white foci in the heart were noticed. Multifocal myocardial infiltration of neoplastic lymphoid cells was noticed[10] (Fig. 3c). Most neoplasms metastatic to the kidneys (Fig. 3d) also had metastases in the lungs, but in lymphoma, pulmonary tumors were rare[11], whereas in the present case metastases was seen in lungs also (Fig. 3e).

Dogs represent a genetic cluster and can be considered an excellent animal model for studying genetic predisposition to cancer[4]. When conjunctival thickening is observed in canine patients with multicentric lymphoma, conjunctival metastasis with infiltration of neoplastic lymphoid cells should be included in the differential diagnosis[7]. Lymphoma cells tend to infiltrate the tissuesof the eye rather than forming single or discrete intraocular masses[7], but in the present case discrete masses of tumour were noticed in both eyes. Iridial thickening with or without follicle formation, hypopyon, hyphema, posterior synechia formation, and glaucoma were also common[6]. Ocular examination should be used for staging and evaluation of response to therapy[3].




**Fig. 2a.** Growth in the right eye. **b.** Growth - Both left and right eye balls. **c.** Splenomegaly with multifocal cyst. **d.** Kidney - Multifocal greyish white nodules in the cortical surface.

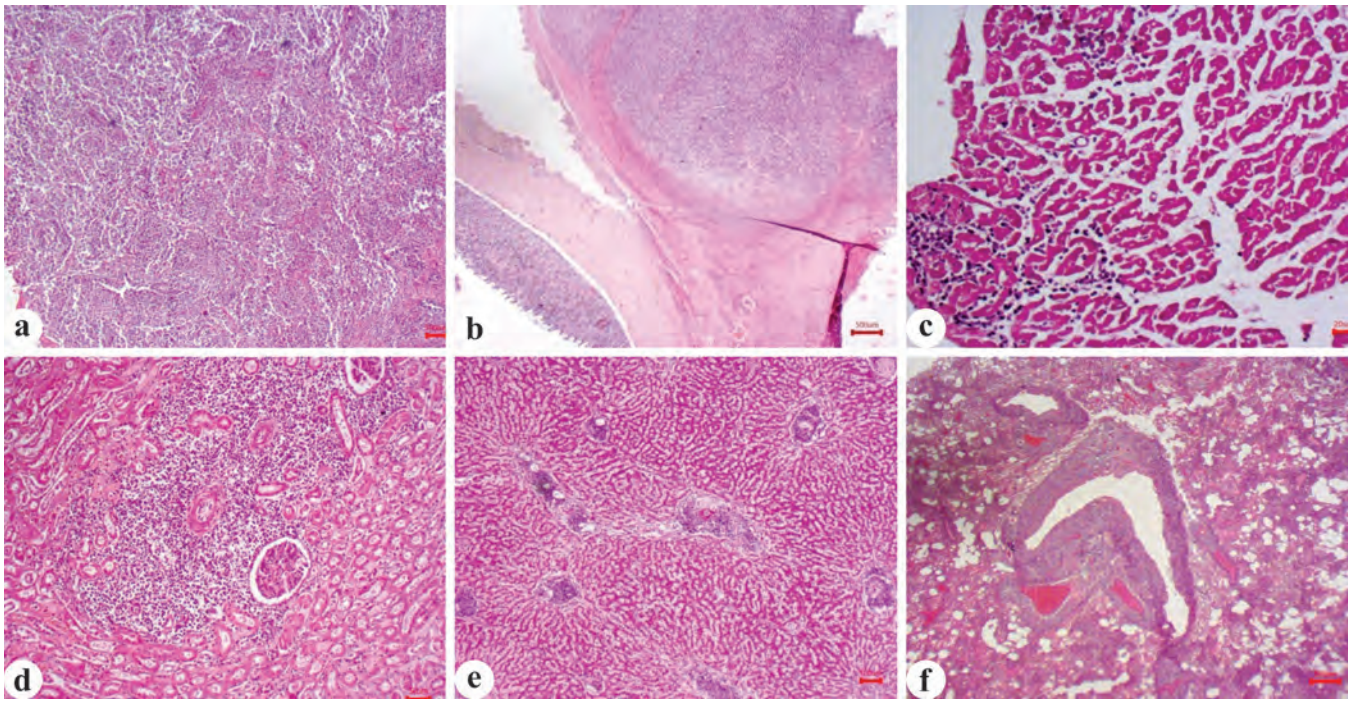


**Fig. 3a.** Lymph node - Effacement of nodular architecture by neoplastic cells (H&E x100 μm). **b.** Eye - Tumour mass originated from conjunctiva (H&E x500 μm). **c.** Heart - Infiltration of neoplastic lymphocytes in between myocardial fibres (H&E x200 μm). **d.** Kidney - Interstitial infiltration of neoplastic cell (H&E x100 μm). **e.** Liver - Neoplastic lymphocytic infiltration was seen in and around portal triad (H&E x100 μm). **f.** Lung - Neoplastic lymphocyte cell infiltration was seen in the peribronchial area and multifocally in the alveolar region (H&E x500 μm).



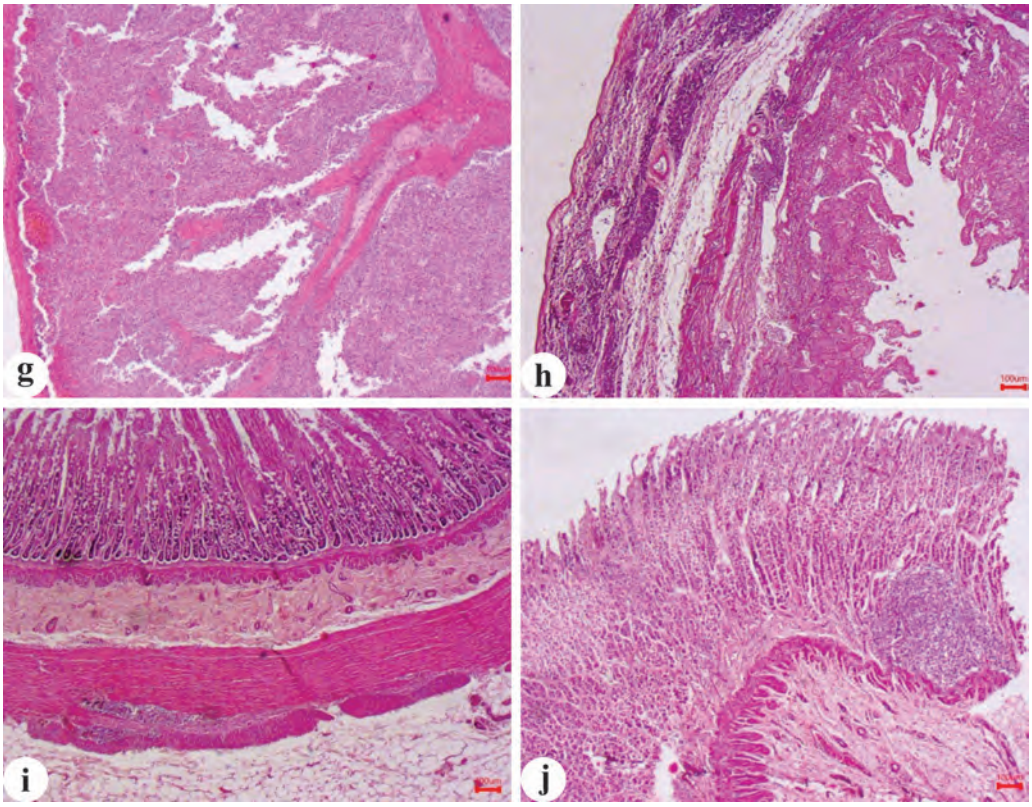


**Fig. 3g.** Spleen - Complete loss of red and white pulp and are replaced by neoplastic cells (H&E x100 μm). **h.** Gall bladder - Multifocal neoplastic infiltration in the muscular and serosal layer (H&E x100 μm). **i.** Intestine - Infiltration of neoplastic cells in the muscular layer (H&E x100 μm). **j.** Caecum - Neoplastic lymphoid cell infiltration in the mucosa (H&E x100 μm).

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