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Glioblastoma in a Pekingese

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Abstract : An 11-year-old, intact male Pekingese was brought to the Veterinary Teaching Hospital of Kangwon National University with a 10-day history of seizures. Fifteen days before coming to Kangwon National University, the dog had visited a local animal hospital for lameness, and non-steroidal anti-inflammatory drugs were prescribed to treat this symptom. However, 10 days before coming to our hospital, the dog experienced generalized seizures. Two days before his arrival, generalized ataxia and mental dullness also occurred. Our examinations revealed no remarkable findings on a routine blood test or X-ray. However, the neurological examinations confirmed mental dullness, generalized ataxia, and a lack of menace response and pupillary light reflexes. Nine hours later, dyspnea occurred, and 12 hours after that, the patient was euthanized per the client's request. A necropsy of transverse sections confirmed the presence of a prominent midline shift due to extended tumor growth. On histopathological analyses, pseudopalisading necrosis of the glial cells and microvascular proliferation were observed. In immunohistochemical analysis, glial fibrillary acidic protein, proliferating cell nuclear antigens, and ionized calcium binding adaptor molecule 1 immunoreactive cells were observed in the tumor area. Based on the results, the tumor was confirmed to be a glioblastoma. Primary intracranial tumors are rare in the veterinary field. This case report describes the clinical and histopathological findings of glioblastoma in a Pekingese.

Key words **:** brain tumor, dog, glioblastoma.

Introduction

Glioblastoma, also known as glioblastoma multiforme or grade IV astrocytoma, is the most common and most aggressive malignant primary brain tumor in humans (6). However, reports of the occurrence of glioblastomas in dogs are rare (12,13).

Nervous system tumors in dogs are mainly classified and graded according to the World Health Organization (WHO) grading system for humans (9). The WHO grading system is based on the following histological features: atypia, mitosis, endothelial proliferation, and necrosis. These characteristics reflect the malignancy of the tumors in relation to invasion and growth rates. Based on this system, grade I astrocytoma is defined as a tumor that has localized lesions without any of the listed histological features. Grade II astrocytoma is a tumor with lesions showing cellular atypia and low mitotic activity. Grade I and II astrocytomas are low-grade groups of astrocytomas, while grades III and IV are high-grade groups. Grade III tumors have two features and grade IV tumors have three or four. Glioblastoma, which is a grade IV astrocytoma, has lesions that are cytologically malignant, mitotically active, and necrotic. Glioblastoma is typically associated with rapid disease evolution and a fatal outcome. Reports have indicated that approximately 50% of the people with glioblastoma die within one year and 90% are dead within three years (6).

Glioblastoma is a highly malignant and rapidly progressive astrocytoma because tumor cells are supported by a large network of proliferated micro-vessels. This case report describes the clinical and histopathological findings of glioblastoma in a Pekingese.

Case

An 11-year-old, intact male Pekingese was brought to the Veterinary Teaching Hospital of Kangwon National University with a history of cluster seizures for the previous 10 days. Fifteen days before coming to Kangwon National University, the dog had visited a local animal hospital because of lameness; non-steroidal anti-inflammatory drugs were prescribed to treat this symptom. However, 10 days before coming to our hospital, the dog had cluster seizures. Two days before his arrival, generalized ataxia and metal dullness also occurred. The day he came to the hospital, no remarkable findings appeared on routine blood tests (complete blood counts, serum chemistry) or X-ray images. Neurological examinations confirmed mental dullness, generalized ataxia, and lack

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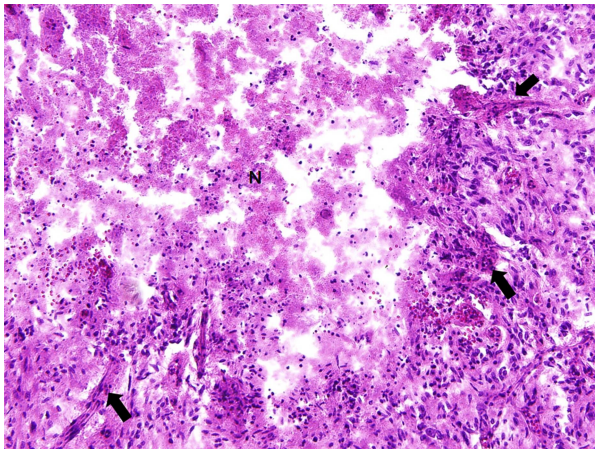

Fig 1. The extensive necrosis (N), pseudopalisading glial cells and multi-focal microvascular proliferation (arrow) on the border of necrosis were seen. H&E, × 100.

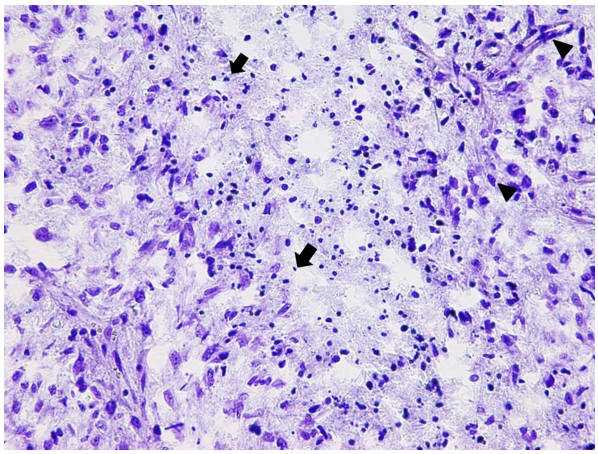

Fig 2. Apoptotic cells (arrow) having small condensed nuclei around the necrosis and abundant microvascular endothelial cells (arrow head) were observed. CV, × 100.

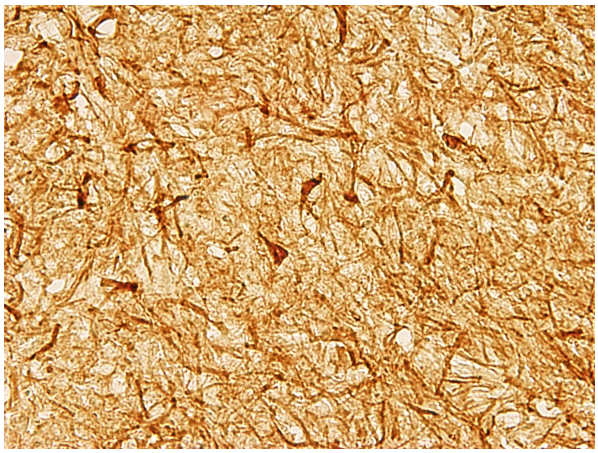

Fig 3. GFAP-immunoreactive astrocytes were observed. GFAP, × 200.

of menace response and pupillary light reflexes. Just after arriving, he suffered from cluster seizures, so phenobarbital (2.5 mg/kg, intravenously [IV], twice daily; Phenobarbital inj,

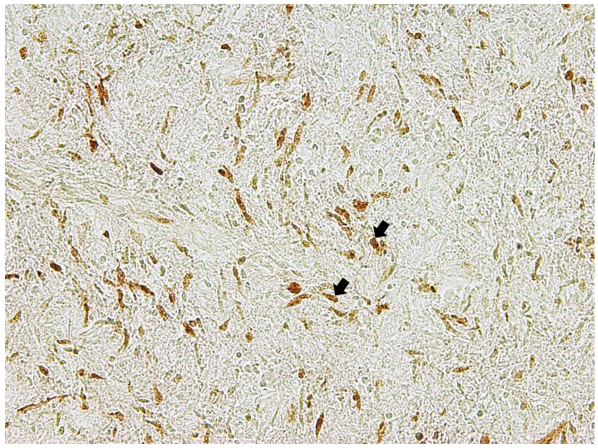

Fig 4. PCNA-immunoreactive cells having various shape of nucleus were observed (arrow). PCNA, × 200.

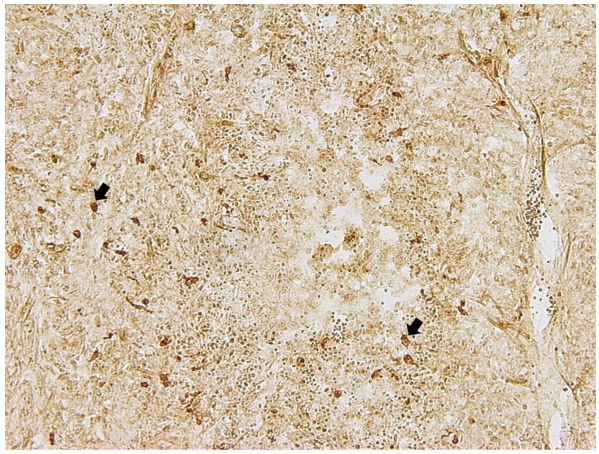

Fig 5. Iba-1 immunoreactive amoeboid type of microglia (arrow) in pseudopalisading glial cells were observed. Iba-1, × 100.

Jeil, South Korea), methylprednisolone (15 mg/kg, IV, twice daily; Methysol inj, Kunwha, South Korea), and mannitol (1.0 g/kg, IV; D-mannitol inj, Dai Han Pharm Co, South Korea) were used to alleviate the symptoms. However, nine hours later, dyspnea occurred, and 12 hours after that, the patient was euthanized, per the client's request.

Following euthanasia, a necropsy was performed. On external gross examination, no obvious lesions were seen. However, a mass was found on transverse sections of the forebrain from around the rostral horn of the lateral ventricle to the diencephalon. Microscopically, the mass was poorly circumscribed and non-encapsulated, and neoplastic cells infiltrated into the surrounding brain parenchyma. The central portion of the neoplasm contained necrotic lesions and hemorrhages. The periphery of the necrotic area was delineated by pseudopalisading of glial cells. Multifocal microvascular proliferation was confirmed near the area of necrosis. Multi-layered microvascular endothelial cells were composed of cords of vessels that formed multiple tufts (Fig 1). Hypercellularity was confirmed and most of the tumor cells were pleomorphic hyperchromatic astrocytes with prominent nuclear atypia. Cresyl violet staining confirmed normal and bizarre mitotic cells as well as apoptotic cells with small,

condensed nuclei that were observed on the border of the pseudopalisading necrosis (Fig 2). Glial fibrillary acidic protein (GFAP)-positive immunoreactivity of tumor cells was observed in the center and peripheral regions of the tumor (Fig 3). Abundant proliferating cell nuclear antigen (PNCA) immunoreactive cells were also observed in the center and peripheral regions of the mass. The nuclei of these PCNA-immunoreactive cells had various shapes (Fig 4). Some ionized calcium-binding adapter molecule 1 (Iba-1)-immunoreactive microglia were observed in the center of the tumor, where the apoptotic cells were located. These Iba-1-immunoreactive microglia had an amoeboid shape (Fig 5). On the basis of the histopathological findings, the tumor was confirmed to be a glioblastoma.

Discussion

Glioblastoma is the most common primary brain tumor in humans. The rapid progression of this neoplasm is associated with a high fatality rate (6). In veterinary medicine, the prevalence of intracranial neoplasia in dogs is 4.5%, with the prevalence of primary neoplasms at 2.3% of the total research population (7,13). According to a previous report, a glioblastoma was found in only one dog among 227 with an primary intracranial neoplasm based on postmortem examinations (13). A possible reason for the low prevalence of glioblastomas in dogs is that the rapid progression of glioblastoma results in a quick death, so there is not enough time to make a diagnosis.

The clinical signs of astrocytoma are similar to those of other brain tumors, and include behavioral changes, walking difficulties, circling, and seizures. In glioblastoma, the tumors quickly grow and spread to other parts of the brain; they can become very large before producing symptoms, which often begin abruptly with seizures (7,14,15). In the present case, the progression of clinical signs was very fast and led to an abrupt death.

There are many trials of classifying and grading astrocytomas in human medicine. The modified Ringertz grading system, the St Anne/Mayo grading system, and the WHO grading system have all been used (2,9). Although the most widely used one in both human and veterinary medicine is the WHO grading system, all three systems identify glioblastoma the same way: the presence of microvascular proliferation and necrosis is necessary, and perinecrotic palisading is common, but not essential (9). Pseudopalisading formation associated with accelerated growth in glioblastoma is considered as a result of a series of events: endothelial apoptosis, vascular occlusion, hypoxic damage, migration of glioma cells, central necrosis, angiogenic response, and enhanced outward expansion of infiltrating tumor cells toward a new vasculature (11). In our case, extensive necrosis and microvascular proliferation were confirmed. In addition, apoptotic cells were concentrated mainly in pseudopalisading cells bordering the branching zones of necrosis.

For more accurate diagnoses and grading, various immunohistochemical analyses have been tried (1,3-5,10). Immunohistochemical analyses were also attempted in this case study. GFAP is an intermediate filament protein that is expressed by numerous cell types of the central nervous system, including astrocytes, and is a long-accepted way to diagnose an astrocytic tumor (4). We confirmed GFAP-positive immunoreactivity of tumor cells in the center and peripheral regions of tumor areas. Like Ki-67, PCNA is a common biomarker used to evaluate proliferative activity in an astrocytic tumor (10). Some previous studies have suggested that the PCNA labeling index increases with the grade of a tumor, and could be used as an ancillary method to discriminate grades of astrocytomas (1,8). Abundant PCNA immunoreactive cells were also observed in the tumor areas in this study, so we can confirm the proliferative activity. Iba-1 is specifically expressed in macrophage lineage cells. The expression of Iba-1 is up-regulated in microglia following nerve injury and other brain disease (3). Any disturbance or loss of brain homeostasis leads to changes in microglial cell shape from a ramified form to an amoeboid one, which can act like other macrophages. The expression level of Iba-1 mainly increases with microglial activation (5). We also confirmed the presence of Iba-1-immunoreactive microglia that had an amoeboid shape. Although we could not conduct CD34 or Von Willebrand factor staining of endothelial cells to demonstrate proliferation, we did confirm the presence of glomeruloid tufts of the endothelium.

Unfortunately, in this case, magnetic resonance imaging could not be performed because the clinical symptoms progressed too rapidly. However, the diagnosis of glioblastoma was confirmed by histopathological studies during the autopsy. This report describes the clinical and the histopathological findings of a glioblastoma found in a Pekingese. We believe this is the first case report of glioblastoma in a dog in Korea.

Acknowledgement

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페키니즈견의 아교모세포종 증례

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요 약 : 11살 수컷 페키니즈가 10일간의 발작을 주증으로 내원하였다. 내원 15일전 파행으로 지역병원에서 소염제를 처방 받았었고 10일 전 간헐적 전신발작을 시작으로 내원 2일 전에는 실조와 정신둔감이 함께 발생하였다. 혈액검사와 영상학적 검사상 특이소견은 관찰되지 않았으나, 신경계 검사상 위협반사와 동공 빛 반사가 떨어짐을 확인할 수 있었다. 내원 9시간 후 호흡곤란이 발생하였고 그 후 12시간 후 보호자의 요청으로 안락사를 실시하였다. 부검상 가로단면에서 확장된 종양으로 인해 현저한 중심선 이동을 관찰할 수 있었다. 조직학적 분석을 통해 신경아교세포의 거짓울타리화된 괴사와 미세혈관의 증식을 확인할 수 있었다. 면역염색 결과 종양 부위에서 GFAP, PCNA, Iba-a 에 염색된 세포가 관찰되었다. 이와 같은 결과를 바탕으로 아교모세포종으로 진단되었다. 원발성 두강내 종양은 수의학에서 흔하지 않다. 이번 증례는 페키니즈견에서 아교모세포종의 임상적, 조직학적 발견에 대한 보고이다.

주요어 : 뇌종양, 개, 아교모세포종