〈Case Report〉

Even today, electron microscopy is useful for pathological diagnosis

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ABSTRACT Recently, the use of electron microscopy as a pathological diagnostic technique has become extremely rare, except in renal biopsy and neuromuscular biopsy. However, we believe it can outperform any other examination method. We present two cases wherein ultrastructural observations supported the diagnosis to highlight the importance of electron microscopy.

The first case was a patient diagnosed with progressive multifocal leukoencephalopathy, with histological examination showing atypical glial cells and immunohistochemical examination suggesting JC viral infection. Electron microscopy revealed the viral particles. The second case was a case of toxoplasmosis. Histological examination showed severe necrosis and cellular infiltration, with microorganisms suggestive of Toxoplasma, and immunohistochemistry suggesting Toxoplasma infection. Electron microscopic examination confirmed the presence of Toxoplasma.

In both cases, electron microscopy confirmed the diagnosis through direct observation of the pathogens. Although electron microscopy is a challenging diagnostic methodology, it is an important methodology that should be preserved, despite advances in medical technology.

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Key words：Electron microscopy, Pathological diagnosis, Progressive multifocal leukoencephalopathy, Toxoplasmosis

INTRODUCTION

Recently, electron microscopy has been rarely used in routine pathological diagnosis, except in renal biopsies. Immunohistochemistry has replaced electron microscopy, which has been widely used in the past. Due to advances in other new diagnostic methodologies, such as molecular pathology, electron microscopy be used less frequently.

Although these changes in the methodology of pathological diagnosis continuously reduce the opportunities for electron microscopy to play an active role in pathological diagnosis, electron microscopy can play an important role. Thus, we would like to share the usefulness and importance

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of electron microscopy by presenting a case wherein the ultrastructural observation in electron microscopy was very important and useful in supporting the diagnosis.

CASES

We present two difficult-to-diagnose cases because the patients had a history of malignancy, were immunocompromised by treatment for the primary disease, and had intracranial lesions caused by opportunistic infections. In both cases, electron microscopic observation was important and useful for the pathological diagnosis.

Case 1

We present the case of a woman in her 50s with low-grade lymphoma and a 25-year history of chemotherapy. Neurological symptoms appeared during the disease course, and brain MRI was performed, which revealed cerebellar lesions. Clinically, recurrent lymphoma was suggested, and the differential diagnoses were glioma, cerebrovascular disease, and demyelinating disease.

A brain biopsy was performed, and the specimen was submitted for intraoperative diagnosis. Frozen sections showed atypical glial cells within the lesion. Considering the possibility of progressive multifocal leukoencephalopathy (PML), the following specimen processing was performed: frozen specimens were preserved for PCR, fixed in glutaraldehyde for electron microscopy, and fixed in formalin for morphological examination in routine pathological diagnosis.

Pathological examination of the formalin-fixed, paraffin-embedded specimens showed scattered atypical glial cells on Hematoxylin-eosin (HE) staining (Fig. 1a-c). Homogeneous amphophilic materials fill the entire enlarged nucleus, suggestive of full inclusions (Fig. 1b). Immunohistochemical examination using an anti-JC viral antibody (polyclonal Rabbit Anti-JC virus, Code No. A4508, DAKO) revealed many positive cells (Fig. 1d, e). However, antibodies against the JC virus are rarely used in routine pathological diagnosis, making it difficult to evaluate staining with appropriate positive controls. These pathological findings were highly suggestive of PML, but PML was not clinically suspected, and because of the problems with immunostaining mentioned above, electron microscopy was used for diagnosis. Viral infection was proven using electron microscopy, which revealed images of nuclei filled with viral particles (Fig.1f, g). The frozen brain specimen had a high copy number (40,083 copies) of JC viral DNA. Based on these clinicopathological findings, PML (cerebellar form) was diagnosed [1, 2].

Case 2

We present another case of a woman in her 60s who had received chemotherapy postoperatively for breast cancer and bone metastases, and acute leukemia developed during the disease course. She underwent chemotherapy and cord blood transplantation for acute leukemia and was administered steroids for post-transplant cutaneous graft-versus-host disease. During the disease course, neurological symptoms appeared, including dizziness and decreased attention, and brain MRI showed an intracranial lesion with ring enhancement with severe edema. ^{123}I-IMP SPECT showed a high signal in this lesion. The differential diagnoses include metastatic recurrence of breast cancer, intracranial lesion of leukemia, or brain abscess due to any cause, and the specimen was submitted for intraoperative diagnosis to confirm the lesion and to determine the surgical strategy. The frozen section showed tissue destruction with a high degree of inflammatory cell infiltration. Clinically, brain MRI showed that the lesion had a ring enhancement after cord blood transplantation, so a specific infection, including toxoplasmosis, was also considered. The obtained specimens were processed as follows:

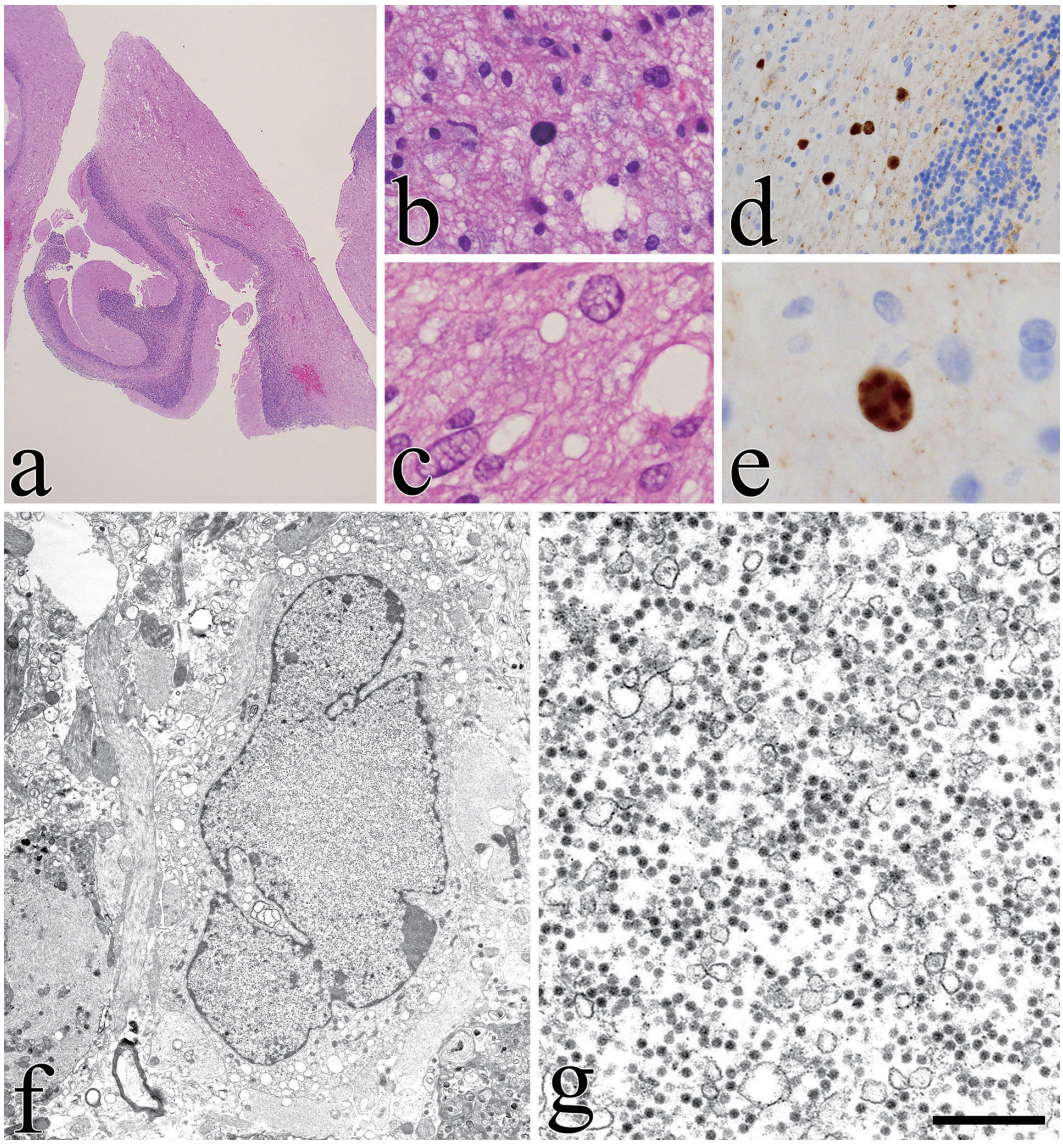

Fig. 1. Histological and electorin microspopic findings of case 1. A small number of scattered atypical glial cells are observed (a-c, HE staining). Immunohistochemistry with anti-VP-1 antibody shows many positive cells and positive nuclear findings (d, e). Viral particles are observed to fill the nucleus (f, g: electron microscopy; scale bar = 250 nm)

fixed in formalin for morphological examination in routine pathological diagnosis, cryopreserved for PCR, and fixed in glutaraldehyde for electron microscopy.

HE stained specimens prepared from formalin-fixed, paraffin-embedded specimens showed destructive lesions with extensive necrosis and a small number of cysts suggestive of Toxoplasma and structures suggestive of tachyzoite (Fig. 2a-c). Immunohistochemical examination using an anti-*Toxoplasma* antibody (anti-*Toxoplasma gondii* rabbit polyclonal antibody, Code No. PU125-UP, BioGenex) revealed tachyzoites and positive structures suggestive of cysts (Fig. 2d, e). However, antibodies against *Toxoplasma* are rarely used in routine pathological diagnosis, making the

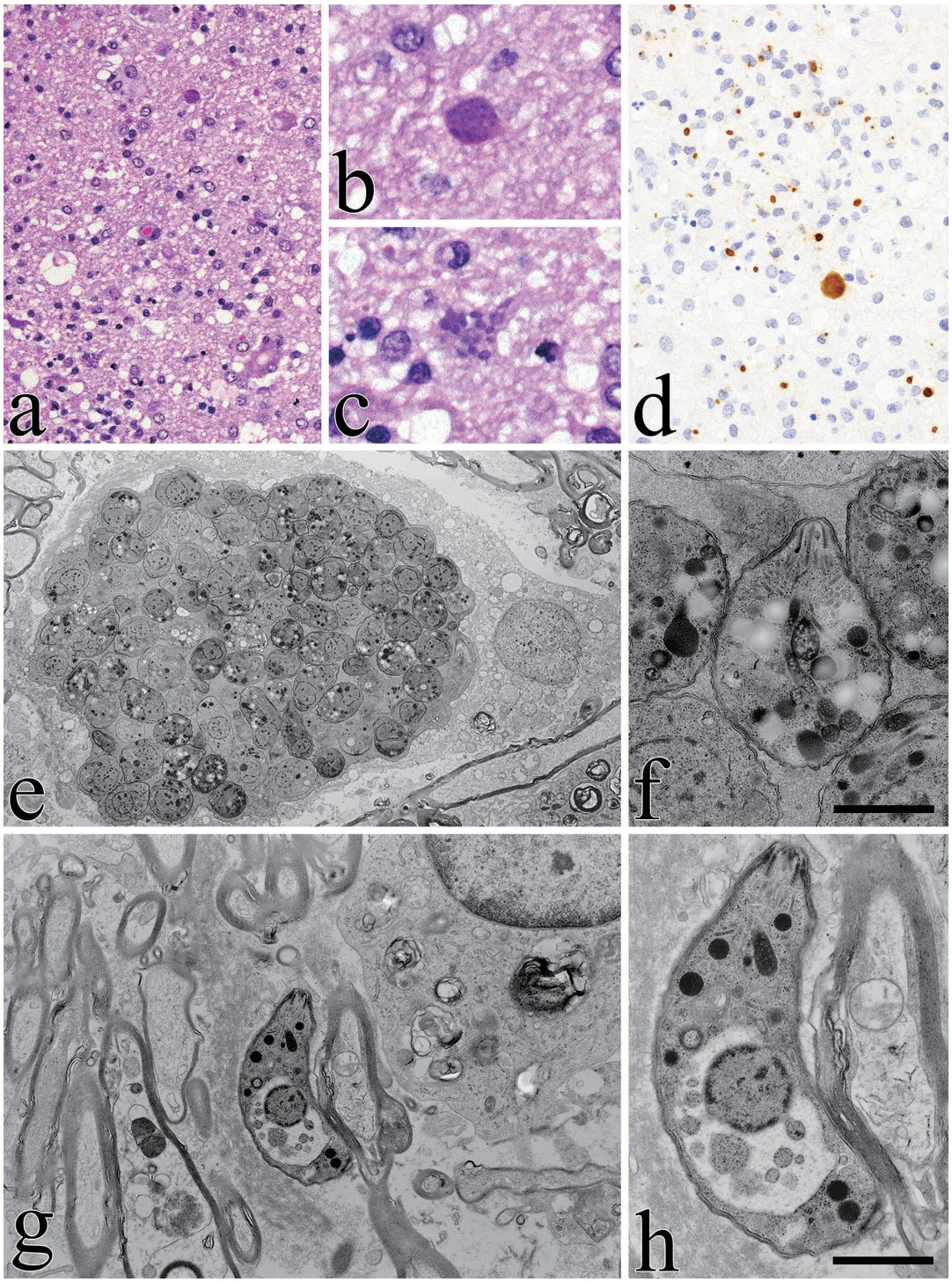

Fig. 2. Histological and electorin microspopic findings of case 2. Structures suggestive of *Toxoplasma* cysts and tachyzoites (a-c, HE staining). Immunohistochemistry using an anti-*Toxoplasma* antibody showed positive findings consistent with cysts and tachyzoites (d). Cysts filled with bradyzoites are observed (e, f: electron microscopy; scale bar = 1 μm). Tachyzoites distributed between the myelin sheaths are observed (g, h: electron microscopy; scale bar = 1 μm).

assessment of staining with appropriate positive controls difficult. Therefore, electron microscopy was performed to prove the presence of *Toxoplasma* worms, which showed cysts (bradyzoites) and tachyzoites[3, 4] (Fig. 2e-h). Clinicopathologically, she was diagnosed with toxoplasmosis, and treatment with antimicrobial agents significantly improved the brain lesions.

The pathological findings were difficult to interpret in both cases, but the direct observation of the pathogens by electron microscopy allowed a definitive diagnosis.

DISCUSSION

In the past, electron microscopy was widely used to determine cell differentiation; however, currently, most searches are performed using immunohistochemistry, and electron microscopy is rarely used for pathological diagnosis[5], except for renal biopsies. In neuropathology, electron microscopy is still useful in many cases, particularly in the search for neuromuscular diseases by nerve biopsy and skeletal muscle biopsy, and some diseases have been incorporated into the diagnostic criteria[6]. Particularly in nerve biopsy, observation of Epon-embedded semi-thin sections, which are used to prepare electron microscopy, is equivalent to HE staining of formalin-fixed paraffin sections in general pathological diagnosis, and electron microscopy is used in the pathological diagnosis of nerve biopsy[7]. Although ultrastructural observation by electron microscopy is necessary for the pathological diagnosis of nerve biopsy, a neurologist performs the pathological examination of nerve biopsy, and it is rarely performed by a pathologist as a general pathological diagnosis.

Recently, not all diseases are defined by morphology, but genetic testing results have become more important than morphology in an increasing number of diseases, such as brain tumors[8].

The role of genomic diagnosis in determining patient prognosis and treatment strategy has become increasingly important, and the role of electron microscopy in this context will become increasingly limited and may not be used in pathological diagnosis. In such a situation of pathological diagnosis, the question arises whether electron microscopy will not play an active role in the future, but we believe that some opportunities will arise for electron microscopy to play an important role in the current complex medical environment[9–11]. Electron microscopy is also important to elucidate newly emerging pathological conditions and diseases, and it is useful in the recent novel coronavirus infections[12]. In the pathological diagnosis of infectious diseases, electron microscopy serves as a crucial diagnostic tool by enabling direct observation of pathogens. Electron microscopy is not only capable of observing ultrastructural features but also capable of searching for pathological conditions for which tissue morphology is difficult to preserve with formalin fixation, such as cerebral edema, a methodology that cannot be substituted by any other examination[13]. Combined with immunostaining, it can be utilized to localize proteins in cells and tissues[14].

In both cases, the quality of the primary antibodies used in immunohistochemistry was good that the diagnosis could have been made by immunohistochemistry alone based on the combined clinical and pathological findings. However, commercially available antibodies used in immunohistochemistry are sometimes suddenly discontinued (in fact, the anti-JC viral antibody used here is not currently available), so a continuous, permanent search could not be performed.

CONCLUSION

In modern medical and pathological diagnosis, immunohistochemical and genetic tests are familiar and easy to use, whereas electron microscopic ultrastructural examination has become a high-

hurdle diagnostic methodology due to the difficulty in specimen preparation (the requirements for special fixatives, Epon embedding, specialized specimen preparation equipment, and extensive technical knowledge and experience) and the decreasing number of technicians and specialists. Despite advancements in medical science, ultrastructural examination by electron microscopy is an important methodology that should be preserved for future generations. The examination of ultrastructures using electron microscopy is the ultimate morphological diagnosis, confirming the existence and microstructure of findings visible by HE staining and immunohistochemistry, and providing absolute confidence in morphological diagnosis.

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DECLARATIONS

Conflict of interest: The authors have no conflicts of interest to declare.

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