Novel diagnostic procedure

Neurocysticercosis case with tuberculoma-like epithelioid granuloma strongly suspected by serology and confirmed by mitochondrial DNA

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Summary

An Indian woman with multiple and nodular cerebral lesions presenting epithelioid granulomas was diagnosed as suspected malignant tumours and the biopsy was carried at local hospital in Japan. The specimen was pathologically diagnosed as tuberculoma. However, due to her nationality, the authors suspected it to be neurocysticercosis (NCC) and carried out serology and applied mitochondrial DNA analysis of *Taenia solium*. These diagnostic tools revealed it as NCC radiologically and pathologically mimicking central nervous tuberculomas. Molecular confirmation of NCC cases is strongly recommended when we can get the biopsy specimens.

BACKGROUND

Neurocysticercosis (NCC), caused by the larval stage of *Taenia solium* (*T solium*), is the most common and serious parasitic disease of the central nervous system (CNS) in the majority of developing countries where people eat pork.^{1 2} Clinical manifestations of NCC are highly variable depending on the number, type, size, localisation and developmental stage of cysticerci with the host immune response.³ NCC, echinococcosis, anthrax and rabies are now recognised as re-emerging, but neglected, infectious diseases worldwide.^{2 4} If NCC is suspected through neuroimaging, either typical or atypical, and questionnaire on visiting and/or living history in endemic areas of *T solium*, it is strongly recommended to carry out serology using highly specific antigens.^{2 5-7}

NCC cases with multiple cysts are rather easily detectable by such a serology.² However, even highly specific serology is not always 100% sensitive especially in cases with solitary cyst, with calcified cysts only or with background of immunodeficiency.² Without typical neuroimaging or serological confirmation of NCC, patients are often reluctantly treated surgically with suspected diagnosis of malignant tumours, tuberculosis or any other diseases causing neurological disorders especially in non-endemic countries.² Therefore, pathological approaches on NCC cases could inevitably become feasible for crucial diagnosis to identify such NCC cases. It is not always easy that the infectious diseases including atypical NCC are confirmed with the surgical specimens of the brain in non-endemic countries. However, molecular diagnosis using PCR has been introduced and established for molecular diagnosis of many pathogens and provided satisfactory results in NCC as well.⁸⁻¹¹

CASE PRESENTATION

An Indian woman was referred to our hospital for the evaluation of multiple and nodular cerebral lesions. Two months before admission to our hospital, she noted occasional headache and paraesthesia of right forearm and MRI, examined in a local hospital, revealed Gd-well enhanced multiple nodular lesions with peripheral oedema and scattered calcifications (figure 1). Because no neoplastic lesions could be excluded on the basis of these radiological findings and because NCC is not indigenous in Japan^{1 2} and most of clinicians have no or poor knowledge on NCC without their own experiences, NCC unfortunately was not suspected as a causative disease, and a brain biopsy was performed in a local hospital. However, figure 2 strongly suggested somewhat typical image of NCC with oedema, and if the clinicians tried to get second opinions from parasitologists, chemotherapy without neurosurgery could strongly be recommended with sound serological evidence.^{1 2 6 12} The H&E-stained microsections derived from one of the cerebral nodules showed presence of epithelioid granulomas surrounded by reactive gliosis (figure 3A). These granulomas were also divided into three layers including plasma cell-rich mononuclear cell outer layer (mo), epithelioid-cell middle layer (epi) and central necrosis (nec) (figure 3B). Although these pathological findings were mimicking tuberculomas, Ziehl-Neelsen stains of the microsections as well as PCR amplification for *Mycobacterium tuberculosis* DNA were negative (data not shown) and also no microorganisms were detected by cultures.

On admission to our hospital for the evaluation as a second opinion, laboratory data showed a total white cell count of 6290/ml (with a differential of 58.0%

Figure 1 Neuroimaging figures. (A–C) T1-weighted MRI after contrast enhancement presented high signal intense lesions with gadolinium enhancement. Arrowheads show the biopsied lesion at left temporal lobe. (d) This brain CT image shows calcification scattered in the parenchyma (arrow).

neutrophils, 26.5% lymphocytes, 12.5% eosinophils), C reactive protein level of 0.04 mg/dl, and IgE level of 16.0 U/ml. NCC was highly suspected because of her nationality, multiple nodular lesions with peripheral oedema, eosinophilia and brain nodules with scattered calcifications. On the basis of those findings, serological test was performed for detection of antibodies specific to *T solium* cysticercus.^{2 6 12} The specific antibodies against *T solium* cysticercus were detected by Western blots using both native antigens purified by isoelectrofocusing from *T solium* cyst fluids⁶ and recombinant chimeric protein,¹² and by ELISA using recombinant chimeric protein (see figure 2).¹²

In order to confirm whether the epithelioid granulomas were really derived from *T solium* cysticerci or not, mitochondrial DNA (mtDNA) analysis was performed. DNA samples from formalin-fixed paraffin-embedded brain specimens were prepared according to the previously described methods.⁹ DNA sequence analysis of these PCR products was performed and revealed the causative agent to be a *T solium* Asian genotype (data not shown).¹³ Before antiparasitic treatment with albendazole,¹⁴ MRI revealed significant decrease in oedema and transformation into cystic nodules with thin wall, and neurological symptoms were completely disappeared (figure 2). The antibody titres and neuroimaging were monitored after chemotherapy.

OUTCOME AND FOLLOW-UP

After 2 years and 10 months from an attack of disease, reactivation was occurred with symptoms resolved spontaneously within several days. Inflammatory finding showed in MRI was also spontaneously improved and the antibody titres were gradually decreased from 0.121 to 0.066 (cut-off value: 0.038, figure 2).

DISCUSSION

After dissemination into CNS, cysticerci are viable as vesicular or avascular types for variable period and could elicit little inflammatory responses in the surrounding parenchyma until some cysticerci are damaged. Recent retrospective work by Yanagida *et al*¹⁵ strongly suggested that the parasite could survive at least more than 10 years. Phased degeneration into granuloma, fibrous scar and calcification without their specific structures like suckers and hooklets are followed as in our case.¹⁶ Because the foreign body granuloma of degenerating NCC could show tuberculoma-like lesions as demonstrated here or various non-characteristic morphologies, serology before treatment and monitoring of progression and mtDNA analysis using histopathological specimens are crucial tools for definitive diagnosis after surgery.^{2 9–11} Nonetheless, serology before treatment is the essential for diagnosis of NCC as it is well recognised in developed countries outside of Japan. In this case, if we applied serology before surgery (figure 2), we

Figure 2 Time course of both T1-weighted MRI after contrast enhancement and antibody responses. ELISA data were shown as the OD values using recombinant chimeric antigen (cut-off OD value: 0.038).¹² Treatment with ABZ was introduced at arrowheads.

Figure 3 Histopathologic figures. (A) The low-power view of the section with H&E staining derived from the nodules at left temporal lobe presented epithelioid granulomas. (B) High magnification of a dotted square in figure 3A with three types of distinct layers: plasma cell-rich mononuclear cell outer layer (mo), epithelioid-cell middle layer (epi) and central necrosis (nec).

could avoid the invasive and risky procedure of a brain biopsy.^{1 2}

Recent work on mtDNA of *T solium* in Bali, Indonesia has shown minor difference in the nucleotide sequences.¹⁷ Most recent similar work on another NCC case with surgery revealed that a Japanese man with travel and living history in Indonesia, Nigeria, Nepal and Malaysia, where NCC was endemic, was concluded to have been exposed to eggs of *T solium* in Nepal by haplotype network analysis.¹⁵ Another most recent NCC case of one US woman 35 years after her immigration from Lao PD also confirmed that she got infection during her repeated visits to Lao PD and enjoyed local foods through her visiting friends and relatives.¹⁸ Therefore, it may be possible for us to assess where the infection was acquired from when we can get histopathologic specimens and more polymorphic differences in mtDNA sequences in the two genotypes.^{10 13}

Learning points

- Serologic diagnosis is useful for differential diagnosis of NCC before treatment and mtDNA analysis using histopathologic specimens are crucial tools for definitive diagnosis after surgery to assess where the infection was acquired from.

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Competing interests None.

Patient consent Not obtained.

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