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Immunodiagnosis of cysticercosis using cyst fluid of *Taenia solium* metacestodes in Jeju Island

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Abstract: It is well known that immunoblot assay is valuable serological test for diagnosing cysticercosis. This study was performed to evaluate serodiagnosis of cysticercosis using immunoblotting among patients with neurologic disorders in Jeju Island. Immunoblot analysis of a total of thirty patients using the cyst fluid of *Taenia solium* metacestode as a diagnostic antigen, positive reaction was observed in six cases while active and chronic stages of the disease are not clearly known. In this regard, a combination of diagnostic methods such as radiological imaging, laboratory serological tests and clinical presentation will be necessary for further studies and for increasing diagnostic accuracy of cysticercosis.

Key words: *Taenia solium*, metacestode, cyst fluid, immunodiagnosis

Human neurocysticercosis (NCC) is a neurological disease caused by invasion of the larval worm, metacestode, of *Taenia solium*, to the central nervous system. Human become infected by ingestion food contaminated with egg of the parasite. The main clinical manifestations of the disease are frequently headache, focal and generalized seizure, hydrocephalus and nonspecific neurological deficits where the parasites invaded brain (1, 2).

The diagnoses of cysticercosis are often based on clinical, imaging, immunological and epidemiological data (3). Serological diagnoses such as enzyme-linked immunosorbent assay (ELISA) and immunoblotting are widely performed in cysticercosis. Imaging diagnosis such as computed tomography (CT) and magnetic resonance imaging (MRI) are valuable for diagnosing NCC, however, they are expensive and difficult to apply on diagnosis of all the cases of NCC in practice, therefore, many studies have been focused on the development of specific antigens of the parasite to improve specificity and sensitivity for the diagnosis of the disease (4-6). The glycoproteins of molecular weight ranging from 50 to 14 kDa of *T. solium* metacestodes had purified by lentil lectin affinity chromatography and showed that antigenic reactions of these bands to the patient's sera were specific. The glycoproteins had strongly evaluated as promising antigens for diagnosing cysticercosis (4). Another diagnostic antigen, 10 kDa, from cyst fluid (CF) of metacestode was

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highly specific for NCC and it reacted with IgG1, IgG4 subclass of NCC (7).

In the present study, sera from patients with neurologic disorder in Jeju Island were subjected to immunoblotting analysis and also, compared cysticercosis with other parasitic diseases.

Taenia solium metacestodes were collected from the naturally infected pigs. The metacestodes were homogenized with Teflon-pestle homogenizer and used as crude whole extracts. The metacestodes were punctured using sterile blades and extracted fluids were used as cyst fluid (CF) crude extracts and remaining bladder walls and tissues were designated as parenchymal extracts.

For the detection specific IgG of cysticercosis among patients with neurologic disorder, immunoblottings were performed. Briefly, the antigens prepared above, were visualized by 7.5-15% gradient SDS-PAGE and then the gels were transferred onto polyvinylidene difluoride membrane (PVDF, Amersham Pharmacia Biotech, Uppsala, Sweden). The membrane was incubated with 1:100 diluted patient's sera for overnight. The membrane was washed with phosphate buffered saline (PBS) and incubated with 1: 1,000 diluted peroxidase-conjugated anti-human IgG (Cappel, Cochranville, PA, USA) for 3 hr. The blots were developed with 4-chloro 1-naphthol and stopped with distilled water. An ELISA assay was partly performed in some experiment including comparison with other helminthic diseases. Thirty patients sera were collected in this study. They had admitted to the Cheju National University Hospital with complaint of headache, seizure and other neurologic disorder since 2001.

The crude extracts of the cysticerci were separated by 7.5-15% gradient gel of SDS-PAGE (Fig. 1). Various proteins of molecular weight ranging from 100 to 8 kDa were observed in whole, parenchymal extracts as well as CF and low molecular weight proteins (30 to 8 kDa) were especially enriched in CF. In immunoblotting of patients sera with confirmed cysticercosis, antigens of CF were more valuable than other antigens of cyticerci (data not shown) and therefore CF was used as diagnostic crude antigen.

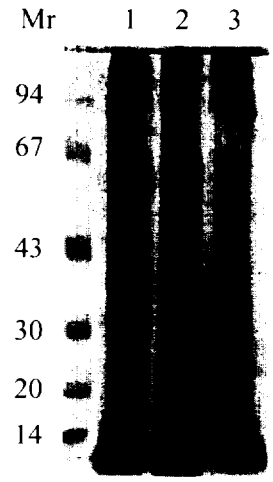

Fig. 1. SDS-PAGE findings of crude extracts of *Taenia solium* metacestode. The crude extracts were analyzed on 7.5-15% gradient gel. Mr, standard marker proteins; lane 1, whole extracts of metacestode; lane 2, parenchymal extracts of metacestode; lane 3, cyst fluid of metacestode.

Immunoblotting studies showed that the crude extracts of CF revealed major antigenic bands of molecular weight 100-200, 96, 70, 45 and 15-8 kDa (Fig. 2). Serological positive rate of cysticercosis in all the tested cases (measured by ELISA) was 30%. Of them, some cases were also reacted with antigens of *Paragonimus westermani* and *Spirometra mansoni* plerocercoid larva (sparganum). It is likely that there are present common antigenic proteins in those parasites. In addition, only six cases of the ELISA positive sera were confirmed cysticercosis by immunoblotting analysis. It seems likely that the differences between two serological methods may be arise on the presence of non-specific glycoprotein and therefore, improving diagnostic accuracy of cysticercosis may be required a set of diagnostic tools with imaging findings such as CT/MRI and with laboratory diagnosis especially immunoblot analysis.

From immunoblotting analysis, 10-8 kDa and 67 kDa protein bands were specifically reacted with patient sera (Fig. 2). In this respect, it has been reported that 10 kDa protein bands of the cyst fluid is valuable diagnostic antigen for active neurocyticercosis (5). From this experiment, it is found that all the cases sera confirmed with cysticercosis did not imply active or chronic stages of cysticercosis only in terms of positive reaction by immunoblot analysis. Taken together, a combination of methods including laboratory serological tests, radiological findings and clinical presentations will be necessary in increasing for diagnostic accuracy of cysticercosis.

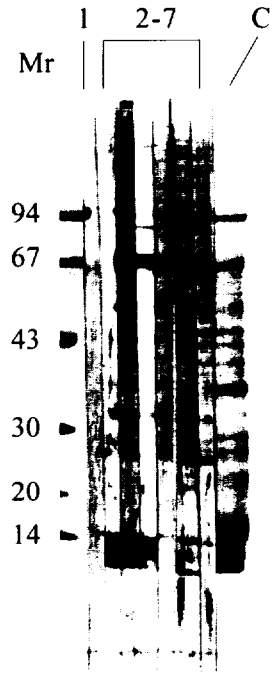

Fig. 2. Immunoblotting of patient's sera with neurologic disorder using crude CF. Mr, standard marker proteins; lane 1, healthy sea; lane 2-7, patient's sera; lane C, crude exracts of CF stained with Amido black 10B. Arrows indicate major diagnostic protein bands.

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