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☐ **Brief Communication** ☐

Antigen specificity of 36 and 31 kDa proteins of *Spirometra erinacei* plerocercoid in tissue invading nematodiasis

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Abstract: Diagnostic specificity of 36 and 31 kDa proteins of *Spirometra erinacei* plerocercoid (sparganum) was evaluated by micro-ELISA in tissue invading nematodiasis such as 25 gnathostomiasis, 33 angiostrongyliasis, 22 trichinellosis patients, and 20 normal control. All but one patient each in 3 nematodiases showed the antibody levels of negative range. The positively reacted patients were regarded as concomitant infections of sparganum because immunized or hyperinfected rabbit serum of the nematodes did not react crossly to the antigen.

Key words: *Spirometra erinacei* plerocercoid, sparganosis, gnathostomiasis, angiostrongyliasis, trichinellosis, serodiagnosis, diagnostic antigen

Antigenic proteins of 36 and 31 kDa (reported previously as 36 and 29 kDa) in crude extract of sparganum were purified by affinity chromatography using either a monoclonal antibody or a gelatin as ligand (Cho *et al.*, 1990; Kong *et al.*, 1991). The antigenic proteins revealed higher sensitivity (96.4%) and specificity (96.9%) than those of the crude extract. In Thailand, human gnathostomiasis is common (Daengsvang, 1980) and angiostrongyliasis is still prevalent in some areas (Chotimongkol, 1987). Therefore, migrating subcutaneous nodules or parasitic granulomas of the central nervous system need to be differentiated from those caused by spargana. We evaluated the diagnostic significance of 36 and 31 kDa sparganum proteins for serological differentiation of these parasitic diseases.

A total of 25 sera of gnathostomiasis were obtained from patients whose diagnosis were made either by clinical symtoms and the presence of *Gnathostoma*-specific antibodies in cerebrospinal fluid (CSF) or serum (15 patients) or confirmed parasitologically by biopsy (6 ocular, 1 subcutaneous and 3 visceral gnathostomiasis) (Morakote *et al.*, 1991a). Thirty-three sera of angiostrongyliasis were collected from 28 patients who had clinical signs of the disease and positive antibody reactions in CSF, and from 5 proven cases by biopsy (4 ocular, 1 cerebral angiostrongyliasis). Twenty-two sera were obtained from trichinellosis patients proven by biopsy (Morakote *et al.*, 1991b). Two sera of sparganosis confirmed by surgery were used. In addition, 20 sera of healthy Thai university students were employed in the study as control.

Micro-ELISA of Cho *et al.* (1990) was done with some modification. In brief, 1:100 diluted sera were reacted for 45 minutes and peroxidase conjugated anti-human IgG (heavy- and light-chain specific, Cappel, U.S.A.) diluted

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Table 1. Mean (M) and standard deviation (S.D.) of abs. for sparganum-specific (IgG) antibody by micro-ELISA in gnathostomiais, angiostrongyliasis, trichinellosis and normal control against 36 and 31 kDa proteins of sparganum

Disease category	No. of patients/ No. of positive reactors	M ± SD of Abs.
Sparganosis	2/2	0.28 ± 0.070
Gnathostomiasis	25/1	0.11 ± 0.249[a]
Angiostrongyliasis	33/1	0.08 ± 0.068
Trichinellosis	22/1	0.09 ± 0.079
Normal control	20/0	0.01 ± 0.011

[a]If M and SD are calculated in the 24 negative sera, the values were 0.06 ± 0.032.

Table 2. Antibody levels (abs.) in immunized or hyperinfected rabbit serum against each antigen

Rabbit serum	Abs. to antigen of		
	Gnathostoma	*Trichinella*	sparganum[a]
Immunized with *Gnathostoma* extract	1.63	N.D.[b]	0.01
Hyperinfected with *Gnathostoma* larvae	0.66	N.D.	0.01
Immunized with *Trichinella* extract	N.D.	1.24	0.03
Normal	0.01	0.02	0.02

[a]36 and 31 kDa proteins of *Spirometra erinacei* plerocerciod. [b]Not done.

at 1:1,000, was incubated for 30 minutes. The cut-off absorbance (abs.) was 0.22 (Cho *et al.*, 1990). As summarized in Table 1, 24, 32 and 21 sera out of 25 gnathostomiasis, 33 angiostrongyliasis and 22 trichnellosis, appeared to be very low abs. to the 36 and 31 kDa proteins of sparganum. None of control sera reacted positively. Only 1 patient serum of an each disease showed the positive reaction. The abs. were 1.30, 0.32 and 0.40, respectively.

The positive reactions in 3 nematodiasis patients may be elicited either by cross-reacting antibodies or by concomitant infections with sparganum. To explore the latter possibility, cross reactivities of rabbit sera immunized with crude extract of *Gnathostoma spinigerum* and hyperinfected with *Gnathostoma* larvae (Morakote *et al.*, 1987), and a rabbit serum immunized with crude extract of *Trichinella spiralis* muscle larvae were examined. Wells of microtiter plate were coated with 200 μl of crude extract (5 μg/ml) both of *G. spinigerum* advanced 3rd stage larvae and *T. spiralis* muscle larvae each. 200 μl of 36 and 31 kDa proteins of sparganum (2.5 μg/ml) was also coated. Rabbit serum was reacted at 1:2,000 dilution each and 1: 20,000 diluted peroxidase conjugated goat anti-rabbit IgG (whole molecule, Cappel, U.S.A.) was reacted sequentially. As seen in Table 2, no cross reaction was observed between immunized rabbit sera and the sparganum antigen. This finding supported that the patients whose sera reacted positively with the sparganum antigen have concomitant sparganum infection.

This result showed that micro-ELISA, using 36 and 31 kDa proteins of sparganum as antigen, was specific enough to differentiate the human sparganosis from gnathostomiasis, angiostrongyliasis and trichinellosis.

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