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Antigenic protein fractions of *Metagonimus yokogawai* reacting with patient sera

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Abstract: Antibody test is sometimes necessary for the diagnosis of acute human metagonimiasis because eggs may not be detected in stool. The antibody test (ELISA) was evaluated for its significance by reacting human sera from clinically diagnosed metagonimiasis, fascioliasis, clonorchiasis and paragonimiasis with 4 crude extracts of *Metagonimus yokogawai* (metacercariae), adults of *Fasciola hepatica, Clonorchis sinensis* and *Paragonimus westermani*. By ELISA, 10 of 11 metagonimiasis sera showed the highest absorbance (abs.) to the homologous antigen. Cross reactions to *M. yokogawai* antigen occurred most frequently in clonorchiasis sera. The antigenic protein fractions in *M. yokogawai* metacercarial extract were observed by SDS-PAGE/immunoblot using patients and control sera together with experimental cat sera. Out of 14 protein bands in the extract, 11 bands were reacting. Cross reacting bands to other trematodiasis sera were frequently observed. Of the reacting bands, 66 and 22 kDa proteins were recognized as specific for metagonimiasis.

Key words: *Metagonimus yokogawai*, metagonimiasis, serodiagnosis

INTRODUCTION

Metagonimus yokogawai is a minute intestinal trematode infecting fish-eating mammals and man. Endemic areas in Korea are distributed along streams drained to east and southern coasts and in adjacent islands. In unpolluted water of these streams, intermediate hosts abound. Raw sweetfish (*Plecoglossus altivelis*) is an important source of human infection. Metagonimiasis occurs more commonly in men of their 30th and 40th because raw sweetfish are eaten when drinking. They are infected commonly also with clonorchiasis (Seo *et al.*, 1981; Cho *et al.*, 1984). During the course of acute human infection, abdominal pain, fatigue, and watery diarrhea are manifested. The patients show high peripheral eosinophilia up to 40%. The symptoms regress slowly 1-2 months after the infection without any specific treatment. Chronic cases may be either asymptomatic or presented intermittently vague abdominal discomfort with diarrhea (Cho *et al.*, 1984).

The diagnosis of metagonimiasis is made by egg detection in feces. Incidental detection of adult worm sections in jejunal biopsy was described (Chi *et al.*, 1988). As for the antibody reactions or on the serological diagnosis of metagonimiasis, there are only a few results. The reasons for the lack of research may be as follows. Firstly, metagonimiasis is a locally endemic intestinal trematodiasis. The epidemiologic importance of metagonimiasis is therefore not recognized widely. Secondly, diagnosis can be, with relative easiness, made

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by egg detection in most patients. There is no urgent need of the serological diagnosis. Thirdly, preparation of the antigen is difficult because the worms are very small. Probably because of these reasons, the progress in knowledge on antibody responses in metagonimiasis is very limited. In some cases of acute metagonimiasis, however, eggs are not detected while the antibody level is in the positive range (Chai *et al.*, 1989). The specific antibody test for metagonimiasis is, therefore, necessary in clinical setting for a correct differential diagnosis of acute metagonimiasis patients.

This study was undertaken to observe the diagnostic value of antibody test by ELISA for clinical metagonimiasis and to observe the antigenic protein fractions present in crude saline extracts of metacercariae of *M. yokogawai*.

MATERIALS AND METHODS

1. Antigens

Crude extract of *M. yokogawai* metacercariae: Crude saline extract of metacercariae of *M. yokogawai* was prepared as described by Cho *et al.* (1987). Briefly, the metacercariae were harvested by peptic digestion, from na-turally infected sweetfish, which were pur-chased in Jangheung Gun, Cholla Nam Do, Korea. Purified metacercariae of 1.11 g wet weight were ground with a teflon-pestle tissue homogenizer. Cyst walls were also ground. Tissue emulsion was shaken for 2 hours, then placed for 22 hours. It was centrifuged by 10,000 *g* at 4°C for 30 minutes. Supernatant was obtained and used as a metacercarial extract. Protein content was 3.03 mg/ml when mea-sured by Lowry *et al.* (1951).

Saline extracts of other trematode parasites: Crude adult worm extracts of *Clonorchis sinensis*, *Paragonimus westermani* and *Fasciola hepatica* were prepared by the same methods as described above. These antigens were used in antibody tests to observe cross reactions with metagonimiasis.

2. Infected sera

Sera of experimental metagonimiasis of cat: Ten cats were fed orally with 10,000 metacercariae each and killed 1 week, 2 weeks, 3 weeks, 4 weeks and 8 weeks after the infection. Blood was withdrawn at the time of autopsy and 2,283-2,608 specimens of adult *M. yokogawai* were collected from each cat.

Sera of metagonimiasis patients: Sera from 11 patients of acute metagonimiasis were secured from 1987 to 1991. A case was descr-ibed elsewhere (Chai *et al.*, 1989). All the patients were ill of acute watery diarrhea with abdominal pain, fatigue, and peripheral eosinophilia. Stool examination revealed eggs of *M. yokogawai* only in a patient. Stool examinations were either not done or revealed no eggs in the remaining patients. All the patients had the history of eating raw sweetfish at a well known endemic focus of Seungju Gun, Cholla Nam Do or Hadong Gun, Kyungsang Nam Do, 1-3 weeks prior to the development of symptoms. After chemotherapy with a single dose of 25 mg/kg praziquantel, symptoms of the patients stopped within 3 days.

Sera of other trematodiases patients and normal control: Sera of serologically diagnosed clonorchiasis (5 cases), paragonimiasis (5 cases), fascioliasis (5 cases) were randomly selected from sera file of Department of Parasitology, Chung-Ang University and tested with metacercarial extracts of *M. yokogawai*, to observe the incidence of cross reactions. Normal control sera were secured from medical students of Chung-Ang University who denied any exposure to sources of the trematode infections.

3. Antibody tests by ELISA

Micro-ELISA technique of McLaren *et al.* (1979) was adopted in specific (IgG) antibody test (Cho *et al.*, 1987). Crude saline extracts of *M. yokogawai* metacercariae, *P. westermani*, and *F. hepatica* were coated to wells of microtiter plate in protein contents of 2.5 μg/ml. *C. sinensis* antigen was coated in 5 μg/ml of protein. Cat or patients sera were diluted at 1:100 and reacted for 2 hours. Peroxidase-conjugated anti-cat IgG (whole molecule, Cappel, U.S.A.) or peroxidase-conjugated anti-human IgG (heavy- and light-chain specific, Cappel, U.S.A.) were diluted at 1:1,000 and reacted. Color reaction was developed with OPD chromogen and stopped

by 8N H_2SO_4. Abs. was read at 490 nm using Bio-Rad Microplate Reader (M 3550).

4. SDS-PAGE and immunoblot

Reducing SDS-PAGE of Laemmli (1970) was carried out using crude extract of *M. yokogawai*. Stacking gel of 3% and 7.5-15% gradient separating gel were used. Constant current of 30 mA was supplied throughout the electrophresis. The banding pattern of protein bands were observed by Coomassie brilliant blue R-250 staining.

Immunoblot was done as described by Tsang *et al.* (1983). Resolved proteins bands by SDS-PAGE were transferred to nitrocellulose paper by electrophoresis at 100 V for 2 hours at 4°C. Experimental cat metagonimiasis sera and patients sera of metagonimiasis, clonorchiasis, paragonimiasis, fascioliasis and normal control were reacted in 1:100 dilution overnight. After washing, 1:1,000 diluted conjugates (peroxidase conjugated anti-cat or anti-human IgG) were reacted for additional 3 hours. Reacting bands were then colored with 4-chloro-1-naphthol chromogen solution containing 0.03% (V/V) H_2O_2.

RESULTS

1. Antibody levels as observed by micro-ELISA

Antibody levels in experimental cats: When a total of 10 infected sera were tested against crude saline extracts of *M. yokogawai*, *F. hepatica*, *C. sinensis* and *P. westermani*, mean and standard deviation of antibody levels (abs.) were 0.38 ± 0.11, 0.13 ± 0.05, 0.12 ± 0.05 and 0.06 ± 0.05, respectively. If cut off value was set at abs. 0.25, no cat sera showed positive reaction to other parasite antigens.

Antibody levels in clinical cases: As shown in Table 1, antibody levels in patients sera were the highest to homologous antigens. The number of cases who reacted in positive range of abs. was shown in Table 1. Abs. to non-homologous antigens were higher when compared with normal controls. In terms of cross-reactivity, *Paragonimus* antigen showed the lowest cross reactivity to other trematodiasis sera and *vice versa*.

Other trematodiases sera showed a considerable cross reactivity to *Metagonimus* antigens. Of them, clonorchiasis sera showed the highest abs.

2. Findings of SDS-PAGE

As exhibited in Fig. 1, crude saline extracts of the metacercariae of *M. yokogawai* showed at least 14 protein bands. Their molecular masses ranged from over 200 kDa to 8 kDa. Molecular masses of main protein bands in the crude extract were 2 bands of around 200, ca. 100, 70, 66, 62, 48, 40, 38, 28-31, 22, 16, 10 and 8 kDa, respectively.

3. SDS-PAGE/immunoblot findings

Findings in experimental cats: Antigenic protein bands in the metacercarial extract were observed by immunoblot using experimental cat sera (Fig. 2A). Out of reacting bands, 66 kDa and 22 kDa bands showed the most strong reactions. There observed at least 4 bands of antigenic proteins of which molecular mass was above 100 kDa. Eight additional antigenic protein bands between 66 and 22 kDa were also demonstrated.

Findings in metagonimiasis patients: When observed by 11 sera from acute

Table 1. Mean and standard deviation of antibody levels (abs.) as measured by micro-ELISA in patients sera of trematodiases. Protein concentrations of 2.5 μg/ml was standardized in each trematode except for *Clonorchis* which coated at 5 μg/ml of protein

Patient category	No. of cases tested	Mean ± S.D. of abs. (No. of positive cases) to antigen of			
		M. yokogawai	*F. hepatica*	*C. sinensis*	*P. westermani*
Metagonimiasis	11	**0.42 ± 0.26(10)**	0.19 ± 0.08(4)	0.15 ± 0.11(2)	0.08 ± 0.07(1)
Fascioliasis	5	0.15 ± 0.10(2)	**0.56 ± 0.18(5)**	0.14 ± 0.13(1)	0.13 ± 0.09(1)
Clonorchiasis	5	0.43 ± 0.26(4)	0.21 ± 0.08(2)	**0.55 ± 0.21(5)**	0.09 ± 0.06(0)
Paragonimiasis	5	0.21 ± 0.09(2)	0.26 ± 0.13(2)	0.35 ± 0.22(3)	**0.61 ± 0.25(5)**
Normal control	12	0.05 ± 0.02(0)	0.06 ± 0.02(0)	0.06 ± 0.02(0)	0.06 ± 0.04(0)

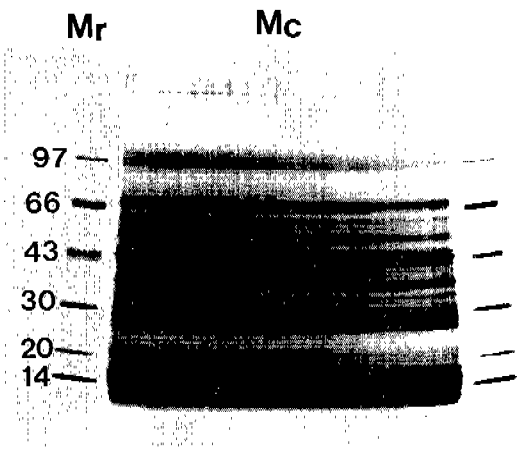

Fig. 1. SDS-PAGE findings of crude saline extract of *M. yokogawai* metacercariae. 7.5-15% resolving gel was used for protein separation. M_r: Molecular mass markers, Mc: Crude extract of metacercariae.

metagonimiasis patients (Fig. 2B), metacercarial antigen also exhibited similar patterns of reacting bands with those to experimental cat sera. Main difference from experimental cat sera was that 2 antigenic bands of molecular masses around 200 kDa were uniformly reacted to the patients sera. The protein band of 66 kDa was also recognized as antigenic. Protein bands of molecular mass, 28 and 22 kDa, were shown to be antigenic but prominent only in 4 of 11 patients.

Cross reacting bands in other trematodiasis patients: The cross reacting bands in metacercarial antigen were examined by reacting sera from 5 clonorchiasis, 5 fascioliasis and 5 paragonimiasis (Fig. 2C, 2D, 2E). Evident cross reactions were observed in all patients sera. Of them, bands of molecular masses lower than 18 kDa reacted to all of clonorchiasis sera, 1 fascioliasis serum and 4 paragonimiasis sera. Antigenic band of 22 kDa reacted crossly only to 3 clonorchiasis sera. In addition, protein band of 66 kDa showed minimal cross reactions while many bands

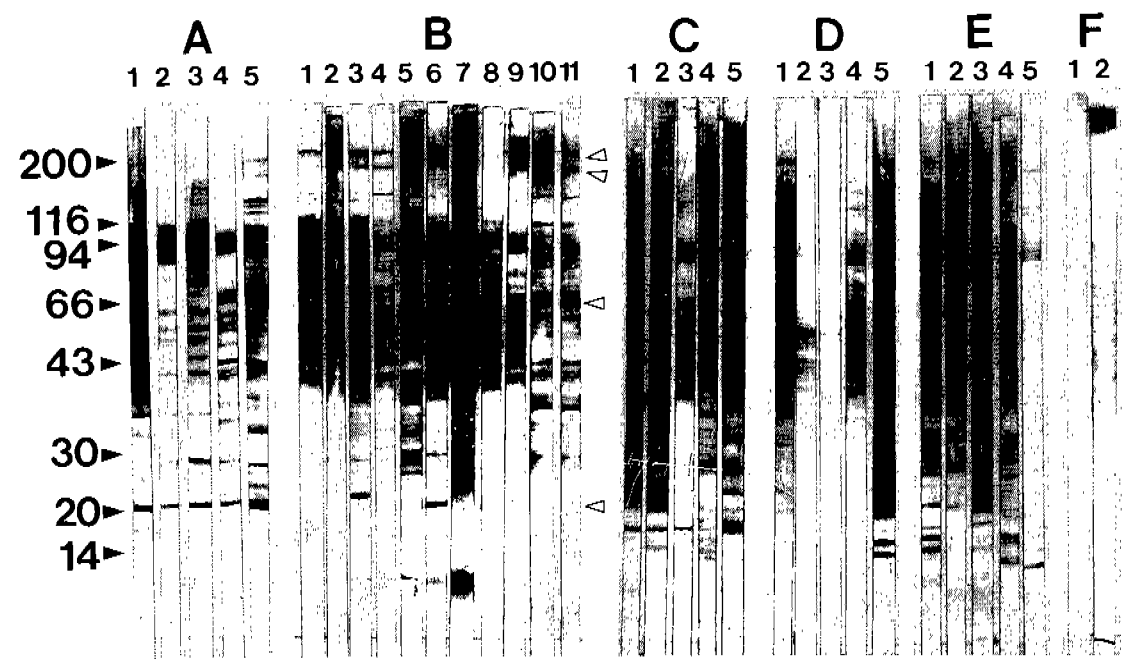

Fig. 2. SDS-PAGE/immunoblot findings of crude saline extract of *M. yokogawai* metacercariae with trematodiases sera. A: Experimental cat, B: Clinical patients of metagonimiasis, C: Clonorchiasis, D: Fascioliasis, E: Paragonimiasis, F: Normal control. Numerical at the left stand for the molecular mass (kDa). Open triangles (◁) show the specific bands for metagonimiasis. Numbers at the top of each lane indicate case number.

between 48 and 32 kDa were reacting crossly.

DISCUSSION

As observed by Cho *et al.* (1987), specific (IgG) antibody reaction was elicited in experimental cat infection, when crude saline extracts of the metacercariae or adult *M. yokogawai* were used as antigens. In their study, the adult antigen was stated to be superior to the metacercarial antigen when antibody levels (abs.) were compared in experimental cat sera. But their difference in abs. was minimal.

Metacercarial extract seems to have its value as a diagnostic antigen probably because all of the clinical cases of metagonimiasis are acute infections which manifested in a month. Unlike acute metagonimiasis, chronic infections of paragonimiasis, clonorchiasis and fascioliasis, diagnosis are made long after the infection. Therefore, adult antigens are very useful in detecting the target antibody which were formed against the adult worms. In acute metagonimiasis, however, clinical manifestations are developed only 5-7 days after the infection whereas specific (IgG) antibody responses were detected in 10 days after an experimental infection (Cho *et al.*, 1987). In such situation, specific antibodies may also be directed to the metacercarial antigen. In this respect, study on specific antibody of IgM class would be necessary.

In this study, the significance of serological diagnosis in metagonimiasis was evaluated by comparing the cross reactivities between trematodiases. By matrix tests of 4 trematodiasis sera with 4 trematode antigens, the homologous antigens exhibited the highest antibody levels than heterologous antigens. Of course there were cross reactions above cut-off abs. But homologous antigen showed the highest antibody levels. It implied that the simultaneous screening of specific antibodies to several parasite antigens are important in the serological diagnosis of acute parasitic diseases.

Identification of the specific antigenic bands for metagonimiasis was difficult. There were many antigenic bands which reacted crossly with other trematodiases sera. Of the reacting protein bands, 66 and 22 kDa in the metacercarial extract seem to be specific because they showed the highest incidence of positive reactions with both experimental cat sera and clinical metagonimiasis sera. In addition to them, 2 bands of molecular masses around 200 kDa reacted specifically to the infected human sera. They exhibited minimal reactions with other trematodiases sera.

REFERENCES

Chai JY, Yu JR, Lee SH, Jung HC, Song IS, Cho SY (1989) An egg-negative patient of acute metagonimiasis diagnosed serologically by ELISA. *Seoul J Med* **30:** 139-142.

Chi JG, Kim CW, Kim JR, Hong ST, Lee SH (1988) Intestinal pathology in human metagonimiasis with ultrastructural observations of parasites. *J Korean Med Sci* **3:** 171-177.

Cho SY, Kang SY, Lee JB (1984) Metagonimiasis in Korea. *Drug Res* **34**(II)**:** 1211-1213.

Cho SY, Kim SI, Kang SY (1987) Specific IgG antibody in experimental cat metagonimiasis. *Korean J Parasit* **25:** 149-153.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriphage T4. *Nature (London)* **227:** 681-685.

Lowry OH, Rosenbrough NJ, Lewis FA, Randall RJ (1951) Protein measurement with Folin phenol reagent. *J Biol Chem* **193:** 265-275.

McLaren M, Draper CC, Roberts JM, *et al* (1979) Studies on the enzyme-linked immunosorbent assay (ELISA) test for *Schistosoma mansoni* infections. *Ann Trop Med Parasit* **72:** 243-253.

Seo BS, Lee SH, Cho SY, *et al* (1981) An epidemiological study on clonorchiasis and metagonimiasis in riverside areas in Korea. *Korean J Parasit* **19:** 137-150.

Tsang VCW, Peralta JM, Simons AR (1983) Enzyme-linked immunoelectrotransfer blot techniques (EITB) for studying the specificities of antigens and antibodies separated by gel electrophoresis. *Methods Enzymol* **92:** 377-391.

=국문초록=

요꼬가와흡충증 항체진단의 검토 및 특이 항원 단백질 분석

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요꼬가와흡충증은 주로 은어회를 먹어 감염되는 흡충증으로서 소장염(小腸炎)을 일으켜 피로감, 복통, 설사 등 증상이 나타나며 말초혈액 호산구증가를 동반한다. 이 흡충증은 대변에서 충란을 검출하여 진단하나 급성 장염으로 발현하는 환자의 일부에서는 대변검사로 충란을 발견할 수 없어 보조적인 진단으로 특이항체검사가 필요한 경우가 있다. 이 실험은 요꼬가와흡충증의 특이항체검사의 가치를 검토하고 검사에서 항원으로 사용하는 피낭유충 생리식염수 추출액 내 특이항원 단백질을 찾기 위하여 실시하였다. 항원으로는 요꼬가와흡충 피낭유충의 추출액을 사용하였고 감염 및 대조혈청은 임상적으로 급성 요꼬가와흡충증으로 진단한 환자 11명, 간질(肝蛭) 환자 5명, 간흡충 환자 5명, 폐흡충 환자 5명과 대조군 11명의 것을 사용하였다. 항체검사로 효소면역측정법으로 특이 IgG항체를 검사한 결과 임상적으로 진단한 요꼬가와흡충증 환자 11명중 10명이 양성반응을 나타내었다. 요꼬가와흡충 항원에 대하여 다른 흡충 감염자 혈청도 교차반응을 보였으나 동종항원에 대하여 가장 높은 흡광도를 나타내었다. 따라서 특이항체검사에서는 여러가지 흡충항원에 대한 항체검사를 동시에 검사할 필요가 있다고 판단하였다. 항원 구성 단백질을 관찰하기 위하여 7.5-15% gel에서 SDS-PAGE한 결과 항원에서 주(主)단백질대 14개 이상을 구별할 수 있었다. SDS-PAGE/immunoblot을 실시하여 요꼬가와흡충증에서만 반응하는 특이항원대를 검색한 바, 항원 단백질대 대부분이 다른 흡충증 환자 혈청과 교차반응을 나타내었으나 66 및 22 kDa 단백질대는 교차반응이 제일 적어 특이항원 단백질이라고 판단하였다.

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