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SHORT COMMUNICATION

Analysis of Antibody Levels Before and After Praziquantel Treatment in Human Paragonimiasis Heterotremus

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SUMMARY **Enzyme immunoassays (ELISA) and Western blot analysis were used to determine IgG antibody levels in patients infected with** *Paragonimus heterotremus* **from Thailand before and after treatment with praziquantel. An IgG antibody ELISA showed that a substantial reduction of antibody levels occurred after one year of treatment. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis showed that** *P. heterotremus* **adult extract is highly complex, consisting of more than 9 antigenic bands with molecular size ranging from 123 kDa to < 12.3 kDa. Two prominent bands of 31.5 kDa and 18.5 kDa were found to show consistent reactions with all serum samples from the pre-treatment group. There was a marked reduction in the intensity of the reaction of the 31.5 kDa band with each serum sample from post-treatment patients but the other bands disappeared during the one year interval.**

Paragonimus heterotremus is one of several species of lung flukes which infect humans.[1] Antibody responses to lung fluke infection, caused by *P. heterotremus* have been detected in human serum by several methods, such as enzyme-linked immunosorbent assay (ELISA)[2-4] and the Western blot technique.[4,5] The aim of this study was to analyse the kinetics of antibody responses in relation to specific chemotherapy with praziquantel in sera of human paragonimiasis heterotremus.

MATERIALS AND METHODS

Adult *P. heterotremus* were obtained from experimentally-infected cats. The worms were identified as *P. heterotremus* according to criteria described previously.[6] Worms were homogenized with a tissue grinder in a small volume of phosphate-buffered saline (PBS) pH 7.2, containing 0.1 mM phenyl-methylsulfonylfluoride and 0.1 mM L-1 tosylamide-2-phenyl chloromethyl ketone. The suspension was then sonicated with the aid of an ultrasonic disintegrator (Labline Ultratrip, Melrose Park ILL, USA) set to operate at maximum amplitude at 1 minute intervals for a total of 10 minutes at 4° C. The suspension was stirred overnight at 4° C before centrifugation at 10,000 × g for 30 minutes. The supernatant fluid was used as a source of antigens. The protein content was estimated according to the criteria of Lowry *et al.*[1]

Each of the 7 paragonimiasis sera was obtained from patients in Phitsanulok Province, Thailand, whose stool and/or sputum was positive only for *P. heterotremus* eggs. Thereafter, these patient received three doses of 25 mg per kg body weight of praziquantel for two days. Two months after treatment, sputum and stool specimens were examined parasitological and were found to be negative in all patients. Ten months later, serum samples were obtained from these individuals. Stool examination was performed also at the time of blood collection and was negative for all parasites. Serum samples were kept at -70° C until analyzed for antibody activity. Control samples were obtained from

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apparently healthy adults residing in non-endemic areas. Stool examination revealed no intestinal parasites at the time of study. Mean and standard deviation of the ELISA values against *P. heterotremus* antigens derived from the healthy control sera were 0.05 and 0.04, respectively.

The solid phase enzyme-linked immunosorbent assay (ELISA) was performed in a microtitre plate as previously described.[2] Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) for analysis of *P. heterotremus* was carried out essentially as described by Laemmli,[8] with some modifications.[9] Briefly, components of the worm extract were resolved under reducing conditions on a 7-12% gradient gel. Samples containing 30 μg of protein were boiled for 5 minutes in sample buffer (10% glycerol, 5% 2-mercaptoethanol, 2% sodium dodecyl sulphate, 0.0625 M Tris-HCl pH 6.8, 0.001% bromphenol blue) before loading onto the gel. After the termination of electrophoresis, the resolved polypeptide bands were either revealed by staining with Coomassie stain or transferred to nitrocellulose membranes for Western blot analysis. Western blotting was carried out as previously described.[9,10] After protein transfer onto the nitrocellulose membrane, the membrane was immersed in blocking solution (2% skimmed milk, 0.1% Tween 20 in PBS pH 7.2) for 30 minutes. Each strip was incubated with a serum diluted 1:100 in blocking solution for 2 hours with gentle rocking. The strip was washed 5 times with fresh blocking buffer and subsequently incubated with peroxidase-conjugated goat anti-human immunoglobulin G (Cappel Laboratory, Westchester, Pennsylvania, USA). After washing, the strips were then incubated with 3, 3',4,4', tetraaminobiphenyl (Sigma Chemical Co, St Louis, MO, USA) in 50 mM Tris pH 7.6. The blot was developed at room temperature with agitation until the dark brown bands appeared. The strips were then washed with distilled water and air dried.

RESULTS AND DISCUSSION

Change in the IgG antibody levels in serum samples of patients before and 12 months after successful treatment was measured by ELISA as shown in Fig. 1. The mean ELISA values in patients before treatment were significantly higher than those after treatment (Student's *t*-test, $p < 0.05$). When pre- and post-treatment specimens from 7 patients were investigated by SDS-PAGE and Western blot analysis, it was found that *P. heterotremus* adult extract is highly complex, consisting of more than 9 antigenic bands with molecular size ranging from 123 kDa to < 12.3 kDa (Fig. 2). Only the 31.5 kDa band was found to give consistent reactions with serum samples from all of the pre-treatment group. There was a marked reduction in the intensity of the reaction of the 31.5 kDa band with samples from all of the post-treatment group.

The rationale of the present study was to evaluate the levels of IgG antibody. This may be useful

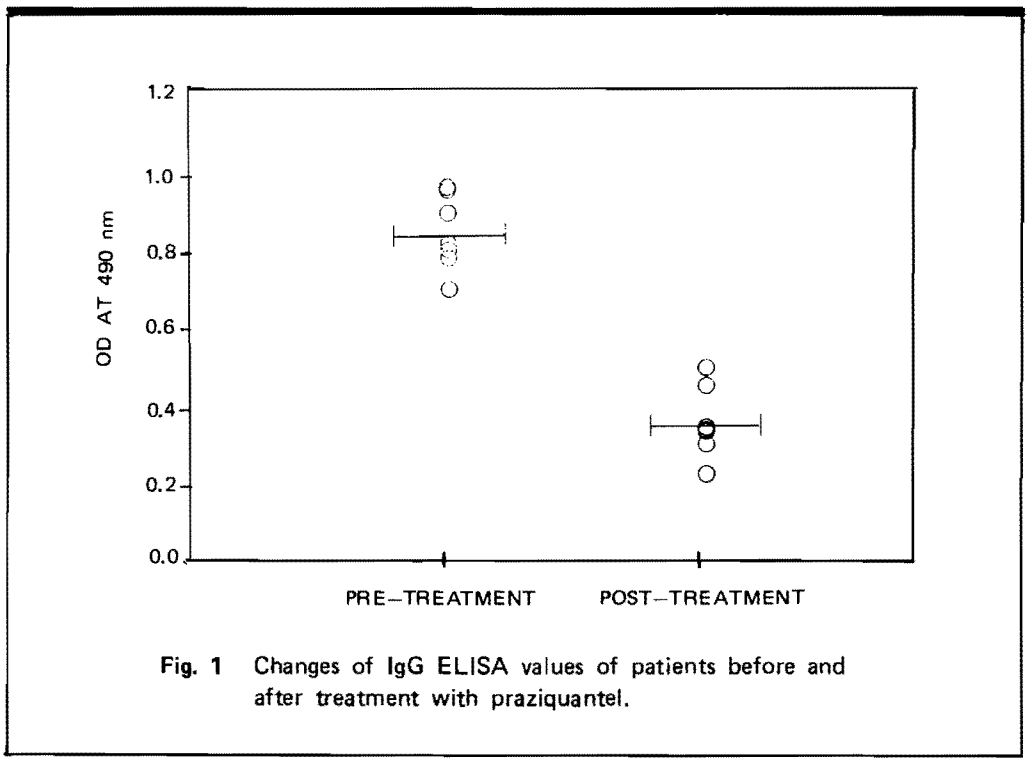

Fig. 1 Changes of IgG ELISA values of patients before and after treatment with praziquantel.

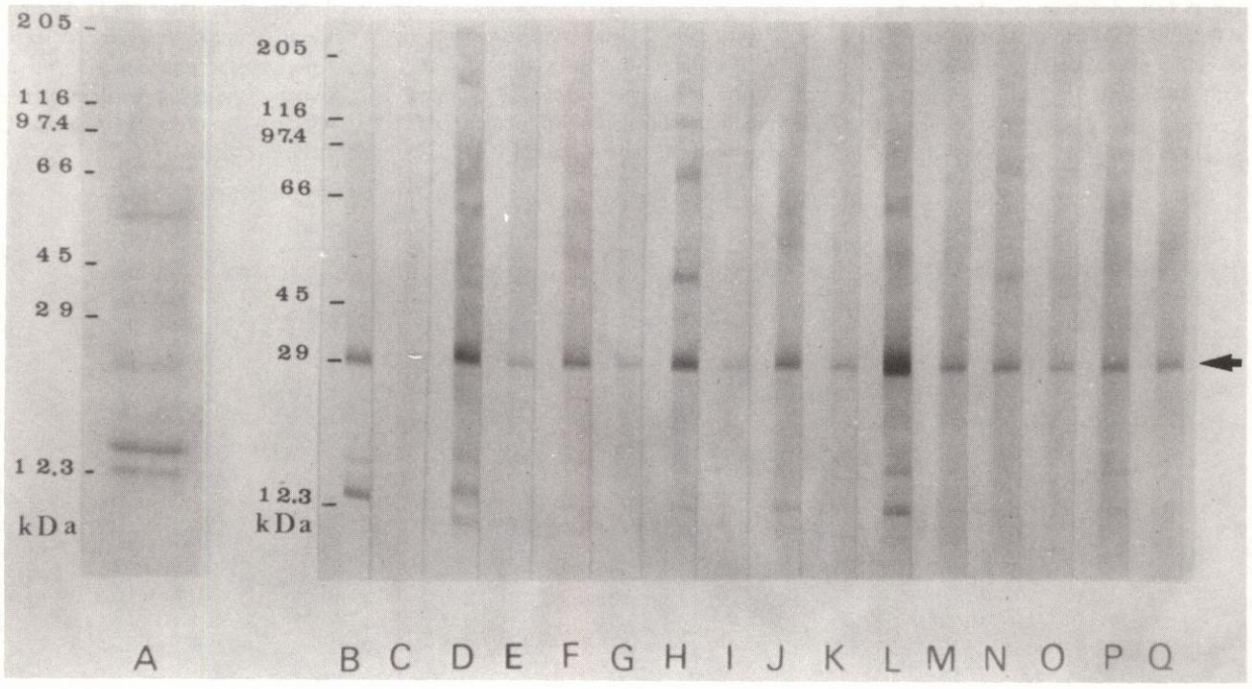

Fig. 2 SDS-PAGE and Western blot analysis of *P. heterotremus* extract recognized by sera of patients pre-and post-treatment with praziquantel. Lane A, Coomassie stain of the crude saline extract after SDS-PAGE, lane B-Q Western blot strips after reacting with pool proven paragonimiasis (B) healthy control (C), 7 pairs of pre-treatment and post-treatment (D-E, F-G, H-I, J-K, L-M, N-O, P-Q). The arrow shows the position of the 31.5 kDa antigen.

for monitoring the outcome of drug treatment and to diagnose the specific parasite infection. There was a substantial reduction of IgG antibody levels in all patients when paired serum specimens from individual patients taken at 1 year intervals were analyzed by ELISA. Consequently, the results obtained from SDS-PAGE and Western blot analysis confirmed the ELISA results. Following praziquantel treatment, there was a noticeable reduction in the intensity of the 31.5 kDa band, which has been shown previously[5] to be specific for *P. heterotremus* infection. It is possible, therefore, that the Western blot pattern of the 31.5 kDa band may also be used in the evaluation of the response of the infection to chemotherapy.

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REFERENCES

1. Vanijanontha S, Radomyos P, Bunnag D, Harinasuta T. Pulmonary paragonimiasis with expectoration of worms : A case report. Southeast Asian J Trop Med Public Health 1981; 12 : 104-6.
2. Pariyanonda S, Maleewong W, Pipitgool *V, et al.* Serodiagnosis of human paragonimiasis caused by *Paragonimus heterotremus*. Southeast Asian J Trop Med Public Health 1990; 21 : 103-7.
3. Maleewong W, Pariyanonda S, Wongkham C, Intapan P, Daenseegaew W, Morakote N. Comparison of adult somatic and excretory-secretory antigens in enzyme-linked immunosorbent assay for serodiagnosis of human infection with *Paragonimus heterotremus*. Tran R Soc Trop Med Hyg 1990; 84 : 840-1.
4. Indrawati I, Chaicumpa W, Setasuban P, Ruangkunaporn Y. Studies on immunodiagnosis of human paragonimiasis and specific antigen of *Paragonimus heterotremus*. Int J Parasitol 1991; 21 : 395-401.
5. Maleewong W, Wongkham C, Pariyanonda S, *et al.* Antigenic components of *Paragonimus heterotremus* recognized by infected human serum. Parasite Immunol 1991; 13 : 89-93.
6. Miyazaki I, Vajrasthira S. Occurrence of the lung fluke *Paragonimus heterotremus* Chen & Hsia 1964, in Thailand.

J Parasitol 1967; 53 : 207.

7. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193 : 265-75.
8. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nautre 1970; 227 : 680-5.
9. Nopparatana C, Setasuban P, Chaicumpa W, Tapchaisri P. Purification of *Gnathostoma spinigerum* specific antigen and immunodiagnosis of human gnathostomiasis. Int J Parasitol 1991; 21 : 677-87.
10. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets : procedure and some applications. Proc Natl Acad Sci USA 1979; 76 : 4350-4.