EXCRETORY-SECRETORY ANTIGENIC COMPONENTS OF ADULT *FASCIOLA GIGANTICA* RECOGNIZED BY INFECTED HUMAN SERA

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Abstract. The immunogenic components of *Fasciola gigantica* excretory-secretory (ES) products were revealed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting technic using sera from patients with *F. gigantica* infection, from patients with clinical suspected fascioliasis, from patients with other illness and from healthy adults. By SDS-PAGE, it was found that the ES products comprised more than 6 polypeptides. Immunoblotting analysis revealed 12 components which were strongly recognized by fascioliasis antisera. These antigenic components had a molecular mass ranging from less than 14.4 to 38 kDa. One antigenic band of 27 kDa was found to give a consistent reaction with fascioliasis antisera (100% sensitivity and 98% specificity). The present findings suggest that the 27 kDa components are sensitive and specific for the diagnosis of human *F. gigantica* infection.

INTRODUCTION

Fascioliasis is a disease caused by hermaphroditic trematodes of the genus *Fasciola,* of which *F. hepatica* and *F. gigantica* are the most common (WHO, 1995). It is primarily a disease of the ruminants. In Thailand and the tropical countries, the disease caused by *F. gigantica* is one of the public health problems (Kachintorn *et al,* 1988; Tesana *et al,* 1989). Humans are usually infected by the ingestion of aquatic plants that contain the infected metacercariae. Diagnosis of fascioliasis is complicated by the fact that the symptoms often occur during early infection, yet the flukes mature and lay eggs only after 8 weeks, after which parasitological diagnosis is feasible by the identification of eggs in the feces. However false fascioliasis can occur when *Fasciola* eggs are found in the stool following the ingestion of infected liver. Moreover, repeated stool examinations are usually required for the detection of eggs and in many cases, no eggs are recovered. The eggs are unembryonated and practically indistinguishable from those of the intestinal flukes, *Fasciolopsis buski* and *Echinostoma* spp. Early diagnosis is important so that treatment may be instituted before irreparable damage to the liver has occurred. For these reasons, serology plays a supplementary role to parasitological method.

Adult somatic extract and excretory-secretory (ES) products of *F. gigantica* are the effective antigens in ELISA for serodiagnosis of human fascioliasis gigantica (Maleewong *et al,* 1996). Recent studies in animal fascioliasis caused by *F. gigantica* suggest that immunoblotting technic is the useful serodiagnostic test (Sobhon *et al,* 1996). However, little is known about the antigenic profile of *F. gigantica* ES products recognized by *F. gigantica* infected human sera using immuno- blotting technic. In the present study, the ES products were analyzed to obtain this information by using the immunoblotting technic and to find their value in diagnosis of human fascioliasis.

MATERIALS AND METHODS

Serum samples

A total of 257 serum speciments were used. Ten fascioliasis sera were obtained from parasitologi-

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cally confirmed human cases of *F. gigantica* infection, all were selected on the basis of the presence of *F. gigantica* adult worms in the bile ducts during cholecystectomy and T-tube choledochostomy or bile ducts operation. Six sera were obtained from the liver abscess-patients that presented clinical findings compatible with fascioliasis. All of the clinical suspected fascioliasis sera were positive for a high level of antibodies against homologous antigens by an indirect ELISA (Maleewong *et al,* 1996). To examine the potential cross reactivity, 160 serum samples obtained from individuals with parasitic diseases other than fascioliasis were used. These samples were obtained from parasitologically cases of gnathostomiasis, angiostrongyliasis, cysticercosis, trichinosis, paragonimiasis, opisthorchiasis and capillariasis. Other parasitosis sera were from cases who were positive for parasites or their products. 15 cholangiocarcinoma sera were also included. Negative control sera were from 66 apparently healthy volunteers. Examination of their stools at the time of blood collections gave no evidence of any intestinal parasitic infection. Pool positive and negative reference sera were prepared by combining equal volumes of fascioliasis antisera or healthy control sera and used for observation of day variation in the immunoblotting analysis.

Parasite

Adults *F. gigantica* were obtained from infected bovine livers collected from the endemic area, Khon Kaen Province, Thailand. The worms were washed with sterile saline and identified as *F. gigantica* according to criteria previously described (Watanabe, 1965; Sahba *et al,* 1972).

Antigen

Adult *F. gigantica* ES antigen was prepared from spent culture medium [RPMI 1640 (Gibco, USA)] containing 0.1 mM phenyl-methylsulphonyl fluoride, 0.1 mM tosylamide-2-phenylethyl-chloromethyl ketone, 1 μM N-(N-L-3 trans carboxyoxiran-2-carbonyl-L-leucyl)-agmatine, 100 U/ml penicillin G and 100 μg/ml streptomycin in which the worms had been maintained for 6 hours at 37°C under 5% CO_2 in air. After incubation, the worms were removed and the collected spent medium was centrifuged at 10,000g for 30 minutes at 4°C. The medium was concentrated by ultrafiltration using Amicon YM3 membrane filter (Grace Co, USA) and dialyzed against distilled water containing the same proteinase inhibitors. The protein was estimated by the standard method (Lowry *et al,* 1951).

SDS-PAGE and immunoblotting technic

Component of the ES products were resolved by SDS-PAGE under reducing conditions on a 10% to 18% gradient gel prepared by the method of Laemmli (1970). Each of the ES samples containing 20 μg protein per lane (0.5 cm width) or 280 μg protein per lane (7 cm width) was loaded onto the gel. After electrophoresis, the resolved polypeptide bands were either revealed by staining with Coomassie brilliant blue stain or electrophoretically transferred to nitrocellulose membranes for immunoblotting (Towbin *et al,* 1979). After the proteins were transferred onto the nitrocellulose membrane, each membrane was immersed in blocking solution (1% skim milk and 0.1% Tween 20 in 100 mM phosphate-buffered saline, pH 7.4) for 30 minutes at room temperature and cut vertically to strips (0.5 x 5.5 cm). Each strip was then incubated with each serum sample (diluted 1 : 100 in blocking solution) for 2 hours with gentle rocking, then washed 5 times, followed by incubation for 2 hours with peroxidase conjugated goat antihuman immunoglobulin G (Cappel Laboratory, USA) in blocking solution. For visualization of the antibody reactions, hydrogen peroxide and diaminobenzidine were used as substrate and chromogen, respectively. The data analysis are presented as the diagnostic sensitivity, specificity and predictive values. These values were calculated using the method of Galen (1980).

RESULTS

SDS-PAGE and Coomassie brilliant blue staining of *F. gigantica* ES products revealed at least 6 bands with approximate molecular mass ranging from less than 14.4 to 65 kDa (Fig 1, lane A). Immunoblotting with fascioliasis antisera detected 12 major antigentic bands of less than 14.4 to 38 kDa (Fig 1, lane D to I). The frequency of reactivity against each band with the individual sera from the different patient groups and normal healthy control are summarized in Table 1. One prominent antigenic

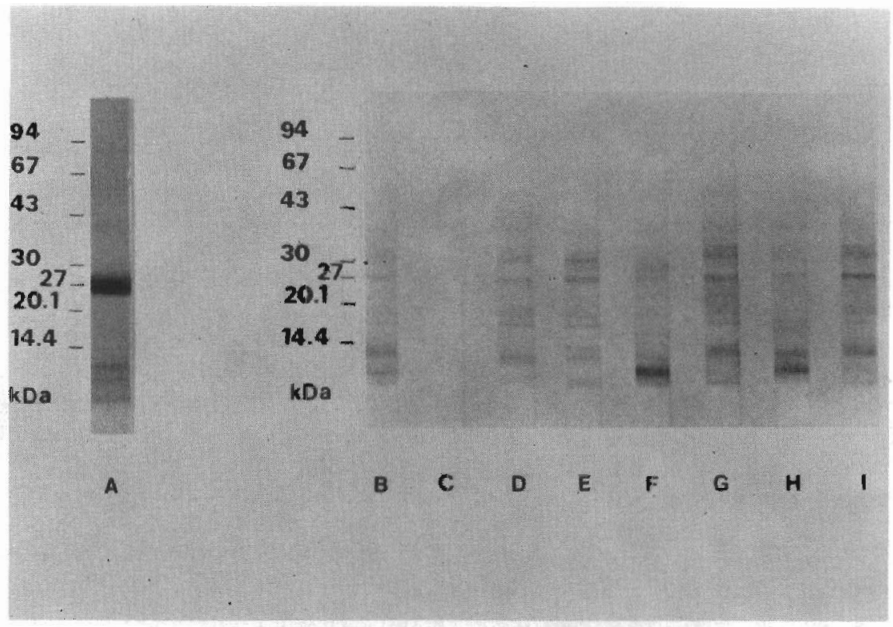

Fig 1–SDS-PAGE and immunoblot analysis of *F. gigantica* ES products. Lane A, Coomassie brilliant blue staining of ES products after SDS-PAGE; Lane B to I, immunoblot patterns after reacted with pool positive reference (B), pool negative reference (C) and individual proven fascioliasis (D to I) sera.

Table I

Number of sera which recognized each antigenic component of *F. gigantica* ES antigen demonstrated by SDS-PAGE and immunoblotting.

Serum type	No. of sera tested	No (%) reacting with component (kDa)											
		38	36	34	32	30	28	27	23	19	17	16	<14.4
Proven fascioliasis	10	9(90)	5(50)	6(60)	6(60)	6(60)	5(50)	10(100)	5(50)	7(70)	6(60)	7(70)	8(80)
Suspected fascioliasis	6	1(16.6)	4(66.6)	6(100)	2(33.3)	1(16.6)	4(66.6)	6(100)	5(83.3)	5(83.3)	1(16.6)	3(50)	5(83.3)
Opisthorchiasis	38	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Paragonimiasis	35	0(0)	0(0)	0(0)	0(0)	0(0)	1(2.9)	2(5.7)	2(5.7)	0(0)	0(0)	0(0)	6(17.1)
Trichinosis	28	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Malaria[a]	10	0(0)	0(0)	0(0)	0(0)	0(0)	1(10)	0(0)	2(20)	0(0)	0(0)	0(0)	0(0)
Angiostrongyliasis	9	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Strongyloidiasis	10	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Hook worm infection	9	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Gnathostomiasis	6	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Cysticercosis	6	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Other parasitic infection[b]	9	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Cholangiocarcinoma	15	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	3(20)	0(0)	0(0)	1(6.7)	0(0)	1(6.7)
Negative healthy control	66	1(1.5)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	2(3)	0(0)	0(0)	0(0)	1(1.5)

[a] *Plasmodium falciparum* infection.

[b] Total of 9 cases: 3 were infected with *Echinostoma* spp, 2 were infected *Capillaria philippinensis*, 1 was infected with *Schistosma japonicum*, 1 was infected with *Thelazia callipaeda*, 1 was infected with *Giardia intestinalis* and 1 was infected with *Entamoeba histolytica*.

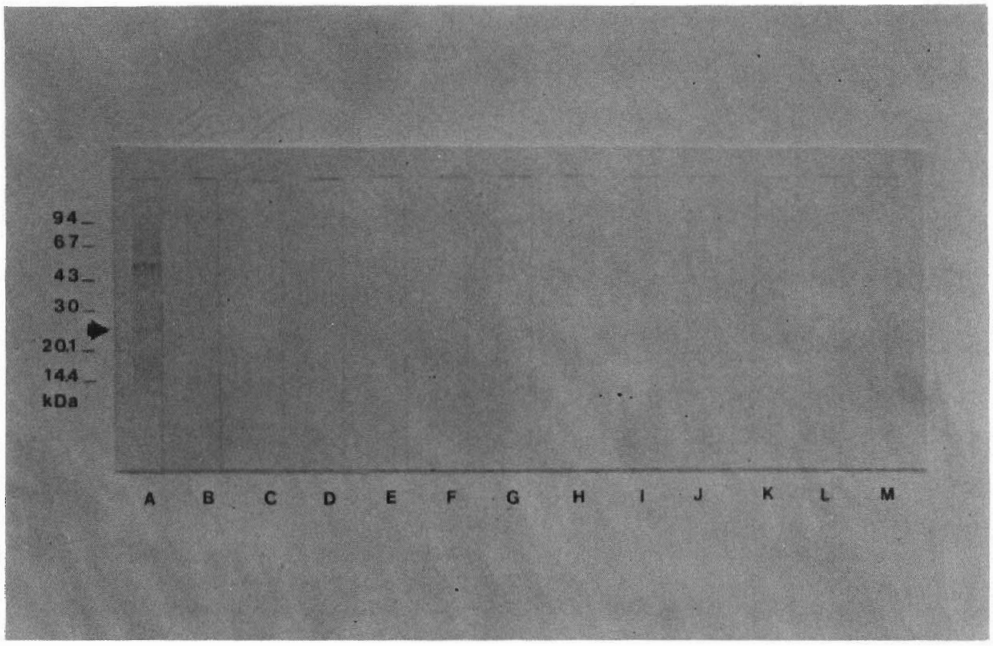

Fig 2–SDS-PAGE and immunoblot analysis of *F. gigan- tica* ES products after reaction with fascioliasis (A), opisthorchiasis (B), paragonimiasis (C), trichinosis (D) malaria (E), angiostrongyliasis (F), strongyloidiasis (G), hook worm (H), gnathosto- miasis (I), cysticercosis (J), capillariasis (K), cholangiocarcinoma (L), and healthy control sera (M).

band of 27 kDa was found to react consistently with most of the sera from patients with clinical and parasitological confirmed fascioliasis. The specificity of the 27 kDa band was defined further by comparing the serum reactivities with those of healthy controls, patients with other parasitic infections and patients with cholangiocarcinoma (Fig 2, lane B to M and Table 1). The diagnostic sensitivity and specificity of the test using the 27 kDa antigenic band were 100 and 98%, respectively. Positive and negative predictive values calculated at the prevalence of disease of 3.9% were 66.7% and 100%, respectively.

DISCUSSION

This study examined the antigenic components of the ES products of *F. gigantica*. The ES products contained a 27 kDa antigenic band which reacted mainly with sera from fascioliasis patients. The results of this study are in agreement with those of previous reports (Santiago and Hillyer, 1988; Yamasaki *et al*, 1989; Sampaio Silva *et al*, 1996). Santiago and Hillyer (1988) reported that the sera from both sheep and cows experimentally infected with *F. hepatica* recognized the *F. hepatica* polypeptides of 20-28 kDa. Yamasaki *et al* (1989) demonstrated that *Fasciola* proteinase, with approximate molecular mass of 27 kDa, was valuable as a sensitive ELISA antigen for immunodiagnosis of human fascioliasis. Sampaio Silva *et al* (1996) revealed the the 25 and 27 kDa ES antigenic components from *F. hepatica* adult worms were recognized by all 20 fascioliasis sera and may be sensitive and specific for serodiagnosis of human fascioliasis. From the present study, the antigenicity of the 27 kDa band cross-reacted with the low intensity band with sera from 2 cases of paragonimiasis and from 3 cases of cholangiocarcinoma. We consider that the cross reactivity is probably due to either cross-reaction or subclinical fascioliasis. However, cross-reactivity between *F. gigantica* antigens and some sera from patients infected with other parasitosis sera has also been demonstrated (Youssef and Mansour, 1991; Maleewong *et al*, 1996). In addition, Coles and Rubano (1988) reported that the partially purified

gut regurgitant proteinase of the *F. hepatica* were composed of two proteins with similar molecular mass of 27 kDa. These bands also reacted with serum from mice infected with *Schistosoma mansoni*.

In conclusion, the demonstration of a specific 27 kDa antigenic band of *F. gigantica* in the present study, provides a framework for further biochemical study and would lead to the development of a specific antigen for diagnosis of human fascioliasis using simple serological tests, either the ELISA or latex agglutination.

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