

Antigenic Components of Somatic Extract from Adult *Fasciola gigantica* Recognized by Infected Human Sera

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Fascioliasis is a disease caused by hermaphroditic trematodes of the genus, *Fasciola*, of which *F. hepatica* and *F. gigantica* are the most common representatives.¹ In Thailand and other tropical countries, the disease caused by *F. gigantica* is a public health problem.²⁻³ Humans are usually infected by the ingestion of aquatic plants that contain the infective metacercariae. The parasitological diagnosis of human fascioliasis is often unreliable because the parasite eggs are not found during the prepatent period.⁴ Even when the worms have matured, the diagnosis may still be difficult as eggs are intermittently secreted. Repeated stool examinations are usually required to increase the accuracy of the diagnosis. Early diagnosis of fascioliasis is necessary for institution of prompt treatment before irreparable damage to the liver has occurred. For these reasons, serology plays not only a supplementary role to parasitological method but being the most dependable means.

SUMMARY The antigenic components of *Fasciola gigantica* somatic extract were revealed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting technique using sera from patients with *F. gigantica* infection, patients with clinically diagnosed fascioliasis, patients with other infections/illness and healthy adults. By SDS-PAGE, it was found that the somatic product comprised more than 22 polypeptides. Immunoblotting analysis revealed at least 13 components which were strongly recognized by sera of patients with fascioliasis. These antigenic components had molecular weights ranging from less than 14.4 to more than 94 kDa. One antigenic component, i.e. 38 kDa was found to give a consistent reaction with sera of patients with fascioliasis (100% sensitivity and 96.7% specificity). The finding suggests that the 38 kDa components may be a potential diagnostic antigen for fascioliasis.

Numerous test systems to detect antibodies have been used for the immunodiagnosis of human fascioliasis but with emphasis on *F. hepatica* infection.⁵⁻⁸ The adult somatic extract and excretory-secretory (ES) product of *F. gigantica* are the common antigens used in an indirect enzyme-linked immunosorbent assay (indirect ELISA) for the serodiagnosis of human fascioliasis *gigantica*.⁹ However, recent studies in animal fascioliasis caused by *F. gigantica* suggested that the immunoblotting technique is a better serodiagnostic test as different antigen-antibody systems

could be simultaneously recognized and the reactions could be preserved for subsequent interpretation.¹⁰ However, little is known about the antigenic profile of *F. gigantica* somatic extract recognized by *F. gigantica* infected human sera. Thus, by using the immunoblotting technique, the present study reveals the components of the

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F. gigantica recognized by the sera of infected individuals. The aim is to find its (their) value in the diagnosis of human fascioliasis.

MATERIALS AND METHODS

Serum samples

A total of 257 serum specimens were used. Ten fascioliasis sera were obtained from parasitologically confirmed human cases of *F. gigantica* infection. *F. gigantica* adult worms could be recovered from these patients during cholecystectomy and T-tube choledochostomy or bile duct operations. Six sera were obtained from the patients with liver abscesses who presented clinical findings compatible with fascioliasis and these were treated as clinically diagnosed cases (Table 1). These six clinical suspected fascioliasis sera contained high levels of antibodies against homologous antigen by an indirect ELISA.⁹ 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 6 parasitologically confirmed cases of gnathostomiasis, 9 cases of angiostrongyliasis, 6 cases of cysticercosis, 28 cases of trichinellosis, 35 cases of paragonimiasis, 38 cases of opisthorchiasis and 2 cases of capillariasis. Other sera were from 3 cases of echinostomiasis, one each from schistosomiasis japonicum, thelaziasis, and giardiasis. Fifteen cholangiocarcinoma sera were also included. Negative control sera were from 66 apparently healthy volunteers whose stools samples around the time of the blood collections contained no intestinal parasite. Pool positive

and negative reference sera were prepared by combining equal volumes of proven fascioliasis sera and healthy control sera, respectively, and they were used for checking the day to day variation of the immunoblotting analysis.

Parasite

Adult *F. gigantica* were obtained from infected bovine livers collected from an endemic area in Khon Kaen Province, located in the north-east of Thailand. The worms were washed with sterile saline and identified as *F. gigantica* according to the previously described criteria.¹¹⁻¹²

Antigen

Adult *F. gigantica* somatic antigen was prepared by homogenization and extraction as described previously.⁹ Briefly, the adult worms were homogenized with a tissue grinder in a small volume of 0.1 M phosphate buffered saline (PBS) pH 7.4 containing 0.1 mM of phenylmethylsulfonyl fluoride, 0.1 mM of tosylamide-2-phenylethyl-chloromethyl ketone and 1 μ M of N-(N-[L-3-trans carboxyoxiran-2-carbonyl]-L-leucyl)-agmatine. The preparation was then sonicated with an ultrasonic disintegrator and centrifuged at 10,000 x g for 30 minutes at 4°C. The protein concentration of the supernatant was determined by the method of Lowry *et al.*¹³

SDS-PAGE and immunoblotting technique

The components of the somatic extract were resolved by SDS-PAGE under reducing conditions on a 10% to 18% gradient gel

prepared by the method of Laemmli.¹⁴ Antigen containing 20 μ g protein per lane of 0.5 cm width or 280 μ g protein per lane of 7 cm width was loaded on to the gel. After electrophoresis, the resolved polypeptides were either revealed by staining with Coomassie brilliant blue stain or electrophoretically transferred to nitrocellulose membranes for immunoblotting.¹⁵ The antigen blotted nitrocellulose membrane was immersed in a blocking solution (1% skimmed milk and 0.1% Tween 20 in 100 mM PBS, pH 7.4) for 30 minutes at room temperature and cut vertically into strips of 0.5 x 5.5 cm. One strip was incubated with one serum sample (diluted 1:100 in the blocking solution) for 2 hours with gentle rocking, washed 5 times, and then incubated for 2 hours with peroxidase conjugated goat anti-human immunoglobulin G (Cappel Laboratory, USA) in a blocking solution. For visualization of the antigen-antibody reactions, hydrogen peroxide and diaminobenzidine were used as substrate and chromogen, respectively. The experimental results are calculated for the diagnostic sensitivity, specificity and predictive values using the method of Galen.¹⁶

RESULTS

SDS-PAGE and Coomassie brilliant blue staining of *F. gigantica* somatic extracts revealed at least 22 bands with approximate molecular weights ranging from less than 14.4 to more than 94 (Fig. 1, lane A). Sera of patients with fascioliasis reacted to at least 13 major antigenic polypeptides with the molecular weights scattering from less than 14.4 to more than 94

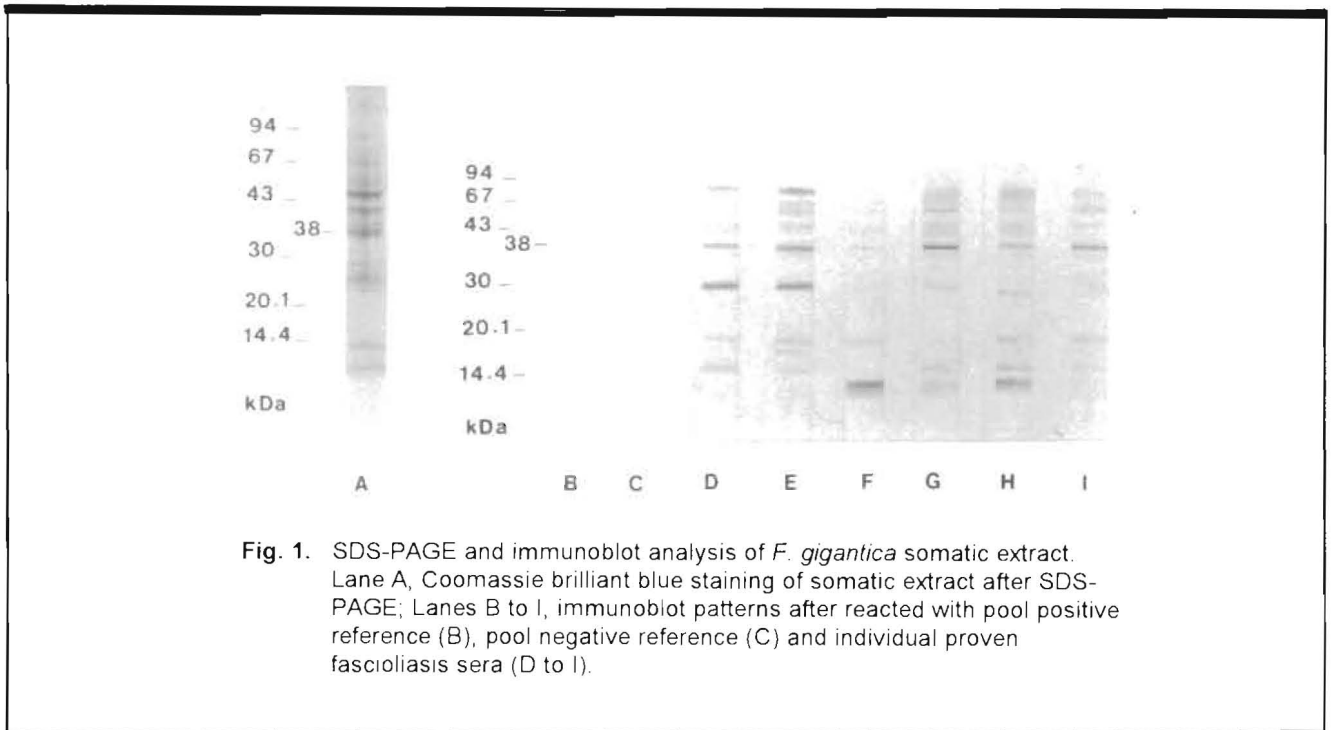


Fig. 1. SDS-PAGE and immunoblot analysis of *F. gigantica* somatic extract. Lane A, Coomassie brilliant blue staining of somatic extract after SDS-PAGE; Lanes B to I, immunoblot patterns after reacted with pool positive reference (B), pool negative reference (C) and individual proven fascioliasis sera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

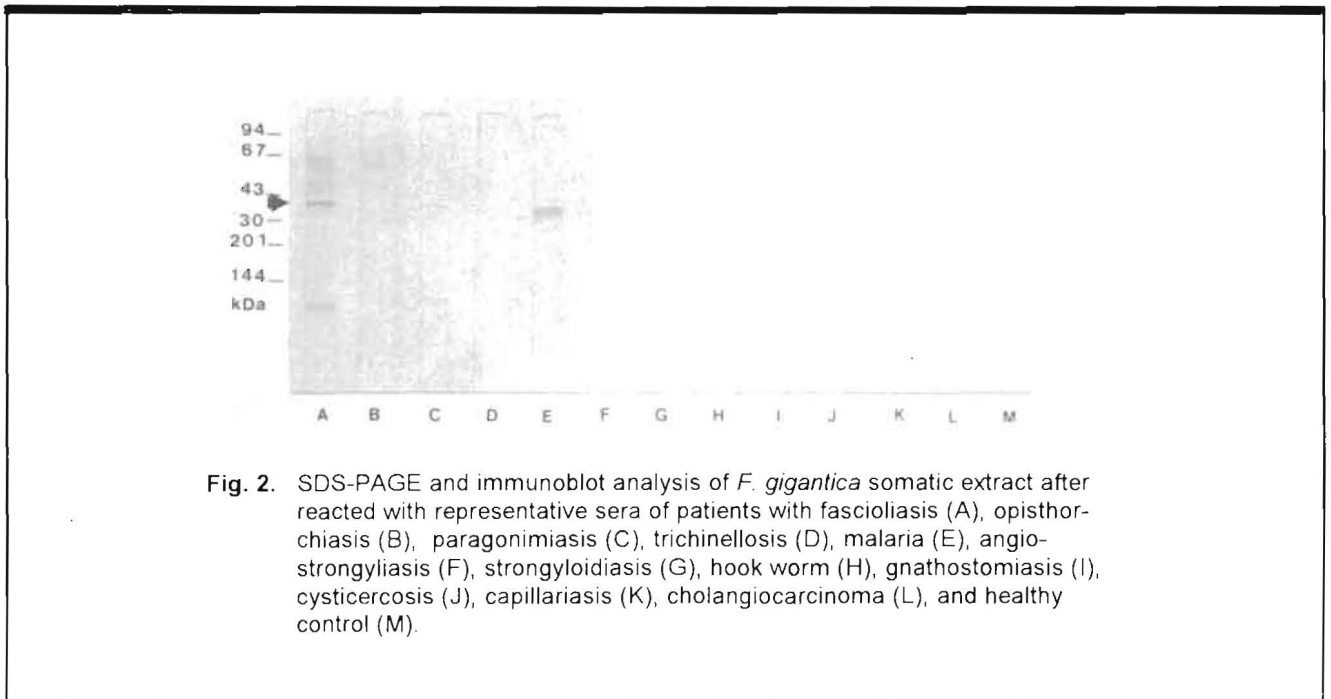


Fig. 2. SDS-PAGE and immunoblot analysis of *F. gigantica* somatic extract after reacted with representative sera of patients with fascioliasis (A), opisthorchiasis (B), paragonimiasis (C), trichinellosis (D), malaria (E), angiostrongyliasis (F), strongyloidiasis (G), hook worm (H), gnathostomiasis (I), cysticercosis (J), capillariasis (K), cholangiocarcinoma (L), and healthy control (M).

(Fig 1. lanes D to I). The frequency of reactivity against an individual component with serum samples from the different patients and normal healthy controls are summarized in Table 1. One prominent

antigenic band of 38 kDa was found to react consistently with all of the sera from patients with clinically diagnosed- and parasitologically confirmed fascioliasis. The specificity of the 38 kDa was

defined further by comparing the serum reactivities of the fascioliasis patients (both parasitologically confirmed and clinically diagnosed) with those of healthy controls, patients with other parasitic

Table 1. Number of sera which recognized individual antigenic components of *F. gigantica* somatic antigen as demonstrated by SDS-PAGE and immunoblotting

Serum type	No of sera tested	No (%) reacting with the component (kDa)												
		>94	70	65	60	54	46	38	36	30	27	24	17	<14.4
Proven fascioliasis	10	6(60)	9(90)	6(90)	8(80)	7(70)	9(90)	10(100)	8(80)	4(40)	6(60)	6(60)	6(60)	5(50)
Fascioliasis ^a	6	1(16.6)	1(16.6)	1(16.6)	2(33.3)	1(16.6)	2(33.3)	6(100)	4(66.6)	5(83.3)	4(66.6)	2(33.3)	1(16.6)	4(66.6)
Opisthorchiasis	38	0(0)	3(7.9)	4(10.5)	1(2.6)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(2.6)	0(0)	0(0)
Paragonimiasis	35	0(0)	7(20)	13(37.1)	1(2.9)	3(8.6)	7(20)	2(5.7)	0(0)	0(0)	1(2.9)	0(0)	12(34.3)	1(2.9)
Trichinellosis	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)	0(0)
Malaria ^b	10	0(0)	2(20)	0(0)	0(0)	1(10)	0(0)	2(20)	0(0)	0(0)	1(10)	1(10)	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)	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)	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)	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)	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)	0(0)
Other parasitic infections ^c	9	0(0)	0(0)	0(0)	0(0)	0(0)	1(11.1)	1(11.1)	0(0)	1(11.1)	0(0)	0(0)	0(0)	0(0)
Cholangiocarcinoma	15	1(6.6)	4(26.6)	2(13.3)	0(0)	1(6.6)	1(6.6)	3(20)	0(0)	0(0)	0(0)	1(6.6)	1(6.6)	1(6.6)
Healthy control	66	1(1.5)	2(3)	0(0)	1(1.5)	0(0)	0(0)	0(0)	1(1.5)	0(0)	0(0)	2(3)	3(4.5)	0(0)

^aClinically diagnosed

^b*Plasmodium falciparum* infection

^cTotal of 9 cases; 3 were infected with *Echinostoma* spp., 2 were infected with *Capillaria philippinensis*, 1 each was infected with *Schistosoma japonicum*, *Thelazia callipaeda*, *Giardia lamblia* and *Entamoeba histolytica*

Table 2. Sensitivity and specificity of various antigenic components for immunodiagnosis of human fascioliasis reported previously

Reacting component(s)	Antigen type	Sensitivity (%)	Specificity (%)	Reference
17 kDa	<i>F. hepatica</i> ES antigen	100	NS	Hillyer and Soler de Galanes ⁶
25 and 27 kDa	<i>F. hepatica</i> ES antigen	100	100	Sampaio Silva <i>et al.</i> ⁹
27 kDa	<i>Fasciola</i> spp. ES antigen	100	98	Yamasaki <i>et al.</i> ¹⁹
33 and 54 kDa	<i>Fasciola</i> spp. somatic antigen	100	100	Shaker <i>et al.</i> ¹⁸

ES = excretory-secretory
NS = not shown

infections and patients with cholangiocarcinoma (Fig. 2, lanes B to M and Table 1). The diagnostic sensitivity and specificity of the test using the presence of 38 kDa band as the positive marker for fascioliasis were 100% and 96.7%, respectively. Positive and negative predictive values calculated at the prevalence of the disease of 3.9% were 66.7% and 100%, respectively.

DISCUSSION

Our study examined the antigenic components of the somatic extract of *F. gigantica* adult worms. The extract contained a sensitive and specific 38 kDa antigen which reacted with sera from all fascioliasis patients. However, it also reacted with sera of few patients with other parasitic infections and patients with cholangiocarcinoma. The results of this study are similar to the previous study which reported that sera from sheep and cows experimentally infected with *F. hepatica* recognized prominent antigens located between 30 to 38 kDa of *F. hepatica* somatic antigen.¹⁷

Shaker *et al.*¹⁸ reported that the sera of patients with fascioliasis recognized the *Fasciola* somatic antigenic fractions at the 33 and 54 kDa. Yamasaki *et al.*¹⁹ demonstrated that the *Fasciola* proteinase, with an approximate molecular weight of 27 kDa, was valuable as a sensitive ELISA antigen for immunodiagnosis of human fascioliasis. Hillyer and Soler de Galanes⁶ suggested that the 17 kDa *F. hepatica* ES antigen is an excellent candidate for the immunodiagnosis of acute and chronic fascioliasis. Rivera Marrero *et al.*²⁰ have identified *F. hepatica* ES antigens with approximate molecular weights of 150-160 kDa that are recognized by early fascioliasis serum. Sampaio Silva *et al.*⁸ revealed that the 25 and 27 kDa ES antigenic components of *F. hepatica* adult worms were recognized by all 20 sera of patients with fascioliasis and the appearance of the antibodies to these components might be specific for the serodiagnosis of human fascioliasis. Comparative studies on the sensitivity and specificity of various antigenic components for immunodiagnosis of human fascio-

liasis reported previously are shown in Table 2. For animal fascioliasis, only one attempt was reported to characterize antigenic peptides from adult *F. gigantica* which could be recognized by infected animal sera.¹⁰ The tegument-associated antigens at molecular weights of 66, 58 and 54 kDa were found to be species specific and might be possible candidate antigen for serodiagnosis of fascioliasis in cattle.

In the present study, the antigenicity of the 38 kDa component from the somatic extract cross reacted, but with the low intensity of antigen-antibody band, with sera from 2 cases of malaria, 1 case of *Capillaria philippinensis* infection and 3 cases of *O. viverrini*-associated cholangiocarcinoma. We consider that such cross reactivity was probably due to either cross-reaction or the antibody sustained from undocumented self-cured fascioliasis. However, cross reactivity between *F. gigantica* antigens and some sera from patients infected with other parasitic infections has also been demonstrated previously.^{9,21}

In conclusion, the demonstration of a sensitive and specific 38 kDa antigenic component from the somatic extract of *F. gigantica* adult worms in the present study could lead to the preparation of a specific antigen for the diagnosis of human fascioliasis using simple serological tests, either the ELISA or agglutination test, provided that the specific component of the worms is prepared in adequate amount.

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