Journal of the Egyptian Society of Parasitology, Vol. 24, No. 3, December 1994

J. Egypt. Soc. Parasitol., 24 (3) 1994: 471 - 478

MOLECULAR WEIGHT DETERMINATION OF FASCIOLA ANTIGENS SPECIFIC FOR DIAGNOSIS OF ACUTE FASCIOLIASIS.

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ABSTRACT

Acute human fascioliasis depends in its diagnosis on detecting specific antibodies in the sera of patients. It was observed that both somatic fraction I and crude E/S products of adult *Fasciola gigantica* worm gave sensitive and specific results. Using IgM ELISA, the cut off O.D was 0.6 and 0.3 nm. and the accuracy was 93% and 98% for the two antigens respectively. Determination of the molecular weight of these two antigens was undertaken by SDS-PAGE. The molecular weight of the somatic fraction I ranged from 17.5 to 43.9 kd, that of the crude E/S products ranged from 12.5 to 14.7 kd. These results were compored with those of previous works performed on antigens of *F. hepatica*.

INTRODUCTION

Human fascioliasis caused by *F. hepatica* or *F. gigantica* is gaining importance (Rim et al 1994). The diagnosis of fascioliasis in the chronic stage imposes minor concern; with the use of concentration techniques for stool preparation and by repetition of examinations even cases with light infection can be discovered. Acute infection, on the other hand, depends for its diagnosis on detecting specific antibodies in the serum, besides the clinical and laboratory findings. Multiple antigens have been used for the serodiagnosis of fascioliasis and numerous attempts have been made with conventional chromatographic

techniques to isolate and characterize potential specific somatic (Hillyer & Capron, 1976, Mansour et al., 1983, Santiago & Hillyer, 1986 and Youssef & Mansour 1991) or E/S antigens (Santiago et al 1986; Espino et al., 1987; Rivera Marrero et al., 1988; Hillyer & De Galanes 1988 and Hillyer et al., 1992). Isolation and identification of parasite antigens are of fundamental importance in facilitating the preparation of purified antigens suitable for immunodiagnosis of infection and disease (Santiago et al., 1986).

The present work aimed to identify specific antigens used in diagnosis of acute fascioliasis. The molecular weight of the most specific antigens were determined.

MATERIAL AND METHODS

I. Preparation of *Fasciola* antigens: (1) Crude somatic *F. gigantica* adult worm antigens was prepared according to the method of Hillyer et al (1979). (2) Crude somatic *F. gigantica* adult worm antigen was fractionated by gel filtration chromatography using Sephadex G-75 (Hudson and Hay, 1980). A chromatogram pattern consisting of 4 peaks was obtained (Osman et al.,1992). (3) Crude E/S *Fasciola* antigen was collected from culture of *F. gigantica* adult worms according to Rivera Marrero et al (1988). (4) Fractionated E/S products of *F. gigantica* were obtained by gel filtration chromatography using Sephadex G-200 (Hudson & Hay, 1980). A chromatogram pattern consisting of 2 peaks resulted (Osman et al in press).

II. The sera tested: Sera collected from patients referred to the Medical Research Institute were stored in the deep freeze (-20°C) and used whenever needed. Three groups of sera were included in the present study.

Group I: Sera of acute fascioliasis diagnosed serologically by high IHA titres.
Group II: Schistosomiasis sera.
Group III: Sera from healthy parasite free controls.

III. Techniques for evaluation of *Fasciola* antigens: The crude somatic *F. gigantica* antigen was tested with both immunofluorescence and ELISA. The other antigens were tested by IgM ELISA.

IV: Determination of molecular weight of *Fasciola* antigens: The molecular weight of *Fasciola* antigens was determined by SDS-disc polyacrylamide gel electrophoresis (Weber & Osborn, 1969).

Preparation of antigen samples: The antigens were incubated at 37°C for 2 hrs in 0.01 M Tris- HCl buffer pH 7.8, 1% SDS and 1% B-mercapto ethanol. The protein concentration of the antigen was adjusted to 0.5 mg/ml. After incubation the protein samples were dialysed for several hours against 500 ml of 0.01 M Tris HCl pH 7.8 containing 0.1% SDS and 0.1% B mercaptoethanol.

SDS-polyacrylamide gel electrophoresis: SDS-PAGE was carried out on 10% gradient gel exactly as described by Shapiro et al (1967). All SDS-PAGE chemicals including molecular weights markers were obtained from Sigma. Gels were cast and run in glass gel tubes 10 cm long with an inner diameter of 6mm. Samples and markers [Bovine serum albumin 67,000, ova albumin 43.000, γ globulin (H-Chain) 50,000, chymotrypsinogen A 25.000, myoglobin 17.200, cytochrome C 12.132] were treated with 10% SDS and 0.1 M Tris-HCl buffer pH 7.8 solutions to a final concentration of 2.5% SDS and 5 ug/ml of protein in the final treated sample, then heated at 65°C for 15 min in water bath. Then, 20 ug of protein sample were applied per tube. Electrophoresis was performed at a contrast current of 5 mA per tube. Gels were stained with Coomassie brilliant blue RF-250.

RESULTS

I. Evaluation of *Fasciola* antigens: (Table I). Using the crude somatic *Fasciola* antigen, huge cross reactions were observed between sera of *Fasciola* and *S. mansoni* cases. So, this antigen was neglected in the present study.

The 4 peaks of the purified *F. gigantica* adult worm antigen were evaluated separately. Fraction I gave the best results (accuracy: 93%) at the cut off O.D. 0.6. Excellent results (98.1% accuracy) at O.D. 0.3 were obtained using the crude E/S *Fasciola* product in IgM ELISA. The accuracy of fraction I and II resulting from fractionation of E/S products was 72.7% and 67.2%, respectively.

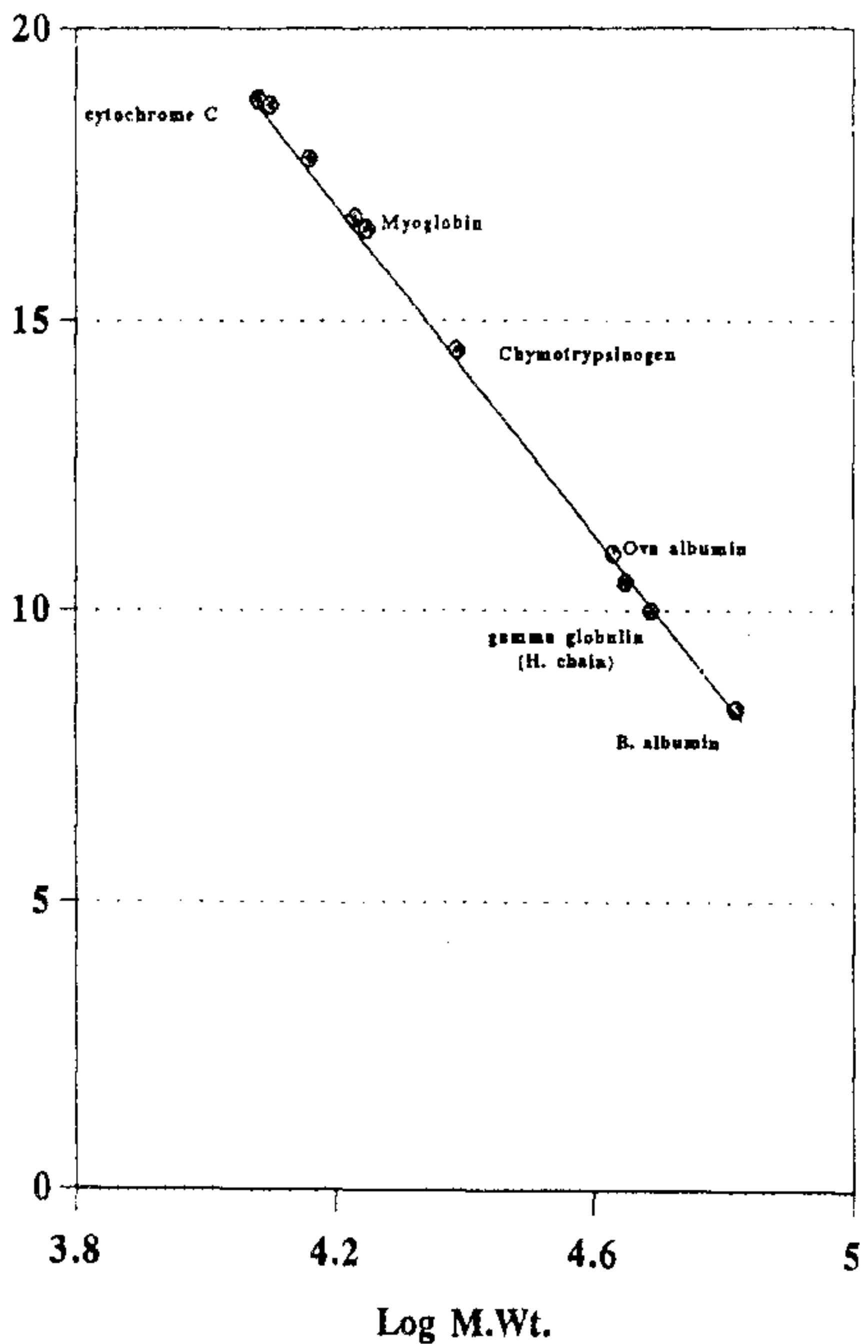

Fig.1: Molecular weight determination of Fasciola antigens by polyacrylamide gel electrophoresis.

II. Molecular weight determination of the *Fasciola* antigens giving the best results. (Somatic fraction I and crude E/S *Fasciola* product). A calibration curve was constructed by poltting the R_f value of the calibration kit proteins against the logarithms of their

corresponding molecular weights. For molecular weight determination the R_f value of the unknown was measured and its corresponding M.W was interpreted from the calibration curve (Fig. 1). The molecular weight of the somatic fraction I ranged from 17.500 to 43.960 kd, that of the crude E/S *Fasciola* antigen ranged from 12.500 to 14.700 kd.

Table I: Highest sensitivity, specificity and accuracy at the cut off O.D values by IgM ELISA in incubating fascioliasis using different *Fasciola gigantica* antigens.

Different *Fasciola* antigens	Cut off O.D	Sensitivity %	Specificity %	Accuracy %
Somatic fraction I	0.6	90.0	94.1	93.0
fraction II	0.5	60.0	70.6	66.7
fraction III	0.4	60.0	82.4	74.1
fraction IV	0.4	70.0	88.2	81.5
Crude E/S *Fasciola* Ag	0.3	100.0	94.0	98.1
E/S fraction I	0.2	89.4	35.2	72.7
E/S fraction II	0.3	71.0	58.8	67.2

DISCUSSION

Multiple *Fasciola* antigens proved to have serodiagnostic potential. In the same host with progress of infection different antigens proved more sensitive than others. In different hosts various antigens may be recognized in the same post infection period (Rivera Marrero et al., 1988). In the present study, in the early phase of infection in the human host, fraction I of *F. gigantica* somatic antigen and the crude E/S antigen were found to give the best diagnostic results by IgM ELISA. By SDS-PAGE, the molecular weight of fraction I ranged from 17.5-43.9 kd; that of the crude E/S products ranged from 12.5 - 14.7 kd.

Santiago et al (1986) reported that the crude E/S *Fasciola hepatica* product when tested on sera from rabbits with fascioliasis by ELISA, revealed high

reactivity. By EITB, the group of polypeptides of molecular weight 23-28 kd were the major antigens recognized. On the other hand, Lehner and Sewell (1980) and Rivera Marrero et al (1988) working on sheep, rats and rabbits by ELISA, reported that serum from early fascioliasis was found reactive with E/S fractions enriched in high molecular weight components 150-160 kd. Santiago & Hillyer (1986) reported that an antigen of 31-33 Kd was the most prominently recognized by sera of human and animals in the acute stage. This antigen as well as antigens in the 18-23 kd range appeared to have good specificity as they were not recognized by antibodies to *S. mansoni*. In another study, Hillyer & De Galanes (1988) reported that a 17 kd crude *Fasciola hepatica* E/S antigen was an excellent candidate for immunodiagnosis of acute fascioliasis by FAST-ELISA and EITB using sera from humans. Hillyer et al (1992) suggested that *F. hepatica* antigens 17 and 63 kd may be markers for acute infection in humans. *F. hepatica* antigens 33 and 54 kd. were reported useful for diagnosis of human fascioliasis by Shaker et al (1994).

The results of the present study provide the authors with two useful *F. gigantica* antigens which appear to be excellent candidates for the detection of acute human infection. They can also be useful in generating probes that would aid in the characterization of antigens which induce antibody production in early stage of infection.

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