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EVALUATION OF SPECIFIC FASCIOLA ANTIGEN IN THE IMMUNODIAGNOSIS OF HUMAN FASCIOLIASIS IN EGYPT.

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ABSTRACT

Enzyme-linked immunosorbent assay (ELISA) and enzyme linked immunoelectrotransfer blot technique (EITB) were employed for the detection of circulating *Fasciola* antibodies in infected human sera using a specific *Fasciola* antigen, prepared by immunoaffinity purification of homogenates of *Fasciola hepatica* adult worms. Ninty two individuals diagnosed clinically and parasitologically were classified into: Fascioliasis group (21 patients), schistosomiasis group (21 patients) and subjects harbouring other parasitic infections (50 patients). Eighteen healthy individuals served as normal controls. ELISA was 100% sensitive and 93% specific with 96.5% diagnostic efficacy, whereas EITB was 100% sensitive and specific with 100% diagnostic efficacy. Our data revealed that ELISA can be used as a good screening test while EITB can serve as a confirmatory test for immunodiagnosis of fascioliasis.

INTRODUCTION

The prevalence of human fascioliasis in Egypt is believed to be higher than reported cases (Hammond, 1974). Cases may escape diagnosis because of difficulties in detecting *Fasciola* eggs in the stools (Ragab and Farag, 1978).

However, eggs may be found in the stools of uninfected patients who have eaten raw infected liver (Bhamarapravati et al., 1983) leading to false positive diagnosis. The immunoserological diagnosis of human fascioliasis, based on the detection of antibodies, has proved to be very useful. However, cross reaction with other helminthic infections and false positivity with normal human sera constitute a major problem for specific detection of fascioliasis (Hillyer and Capron, 1976). Important serological cross-reactions are observed with sera from patients with schistosomiasis which is a prevalent disease in Egypt (Khalil et al., 1990).

This study aims at the employment of a specific affinity purified *Fasciola* antigen to raise the sensitivity and specificity of immunodiagnosis of human fascioliasis.

MATERIALS AND METHODS

Subjects: This study included 92 patients from areas of Meet Ghamr and Zagazig, Sharkiya Governorate. They were divided according to their clinical and parasitological investigations into 3 groups: 1- Fascioliasis group; including 21 patients, diagnosed by detection of *Fasciola* eggs in patients' stools. 2- Schistosomiasis group; including 21 patients, diagnosed by detection of *S. mansoni* eggs in patients' stools. 3- Other parasites group; including 50 patients infected with parasites other than *Fasciola* or *Schistosoma, as Wuchereria bancrofti, Ankylostoma duodenale, Hymenolepis nana, Ascaris lumbricoides, Entamoeba histolytica* and *Echinococcus granulosus*. Eighteen healthy individuals were also included in this study as normal negative controls.

Preparation of Specific *Fasciola* Antigen Free from *Schistosoma* Cross Reacting Fraction: Crude *Fasciola* antigen was prepared according to Hillyer et al. (1979). Crude *Fasciola* antigen was purified from cross-reacting antigenic part by immunoaffinity purification method as described by Gottstein et al. (1983), using cyanogen bromide activated sepharose 4B coupled to rabbit antiserum raised against *S. mansoni* adult worm surface antigen.

Immunological Methods Used for Assay of Circulating Fascioliasis Antibodies: Enzyme-linked immunosorbent assay

(ELISA): ELISA was performed according to Engvall and Perlmann (1971). ELISA microtiter plates were coated with 100 µl/well of 30 µg protein/ml specific *Fasciola* antigen in 0.06M carbonate buffer, pH 9.6 and incubated overnight at room temperature. Sera were diluted 1/256 in washing buffer [0.5% Tween 20 in 0.01M phosphate buffered saline (PBS), pH 7.4] and 100 µl was added per well. Plates were incubated for 30 minutes at 37°C. Hundred µl/well of goat anti-human IgG peroxidase conjugate (Miles), diluted 1/1000 in washing buffer, was incubated 30 minutes at 37°C. Following each of the above mentioned steps; plates were washed 3-6 times with washing buffer. To develop the coloured reaction, 100 µl/well of substrate solution [one tablet of 10 mg O-phenylenediamine dihydrochloride (Sigma) and one tablet of phosphate-citrate buffer with urea hydrogen peroxide (Sigma) in 100 ml distilled water] was added and kept in the dark for 30 minutes. The reaction was stopped with 50 µl/well of 8 N H_2SO_4 and read as optical density (OD) values at 492 nm (using Okidata Microline Spectrophotometer-391 plus).

Enzyme-linked immunoelectrotransfer blot technique (EITB) (Tsang et al., 1983): Purified *Fasciola* antigen resolved by SDS - polyacrylamide gel electrophoresis was transferred onto 0.45 µm nitrocellulose membrane by using Bio- Rad transblot cell and constant 50 V (Bio- Rad power supply model 200/20). The resolved *Fasciola* antigen was then probed with positive fascioliasis patients' sera as follows: Sera were diluted 1: 100 in working buffer (10% fat-free milk in 0.15 M PBS , pH 7.2). The nitrocellulose strips were exposed to the diluted serum overnight. The strips were washed 3 times (10 minutes each) with 0.15 M PBS, pH 7.2 / 1% Tween-20. The nitrocellulose strips were exposed for 1 hour to horseradish peroxidase - labelled anti-human IgG diluted 1: 2000 in working buffer. The strips of nitrocellulose were again washed as before. The processed nitrocellulose strips were then exposed for 5-10 minutes to the substrate solution (diaminobenzidine / Sigma). Positive reaction showed up as grey to blue bands within 10 minutes. The strips were then thoroughly rinsed with H_2O.

RESULTS

Enzyme-Linked Immunosorbent Assay for Detection of Anti-*Fasciola* Antibodies: The cut-off value for positivity was measured as mean

OD readings of normal controls at 492 nm + 3 standard deviation and was equal to 0.38. All normal control values were below cut off value and all 21 fascioliasis group values were above cut off value (100% sensitivity). However, 1 out of 21 of schistosomiasis group cases and 4 out of 50 cases of other parasites group were cross-reacting with the purified *Fasciola* antigen (93% specificity), as shown in table 1. The *Fasciola* antibody level in fascioliasis group was significantly higher than the other groups (P<0.001). There was no significant difference between normal controls group, schistosomiasis group and

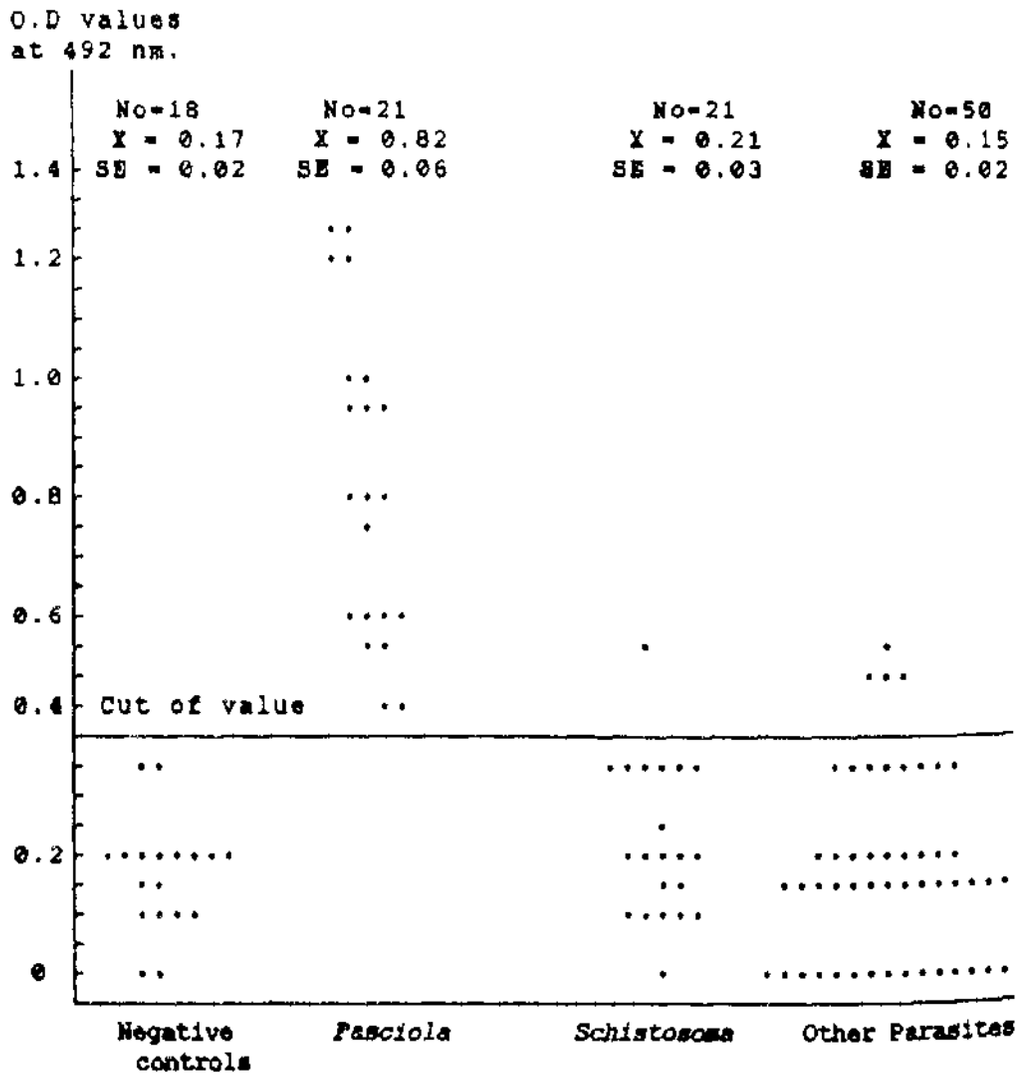

Fig.1: Incidence of positivity for *Fasciola* antibodies measured as OD values at 492 nm in various studied groups.
No = Number of cases per group
X = Mean OD values at 492 nm per group
SE = Standard error of the mean
Student's t test analysis: *Fasciola* group vs negative controls, *Schistosoma* and other parasites groups; P<0.001 (highly significant)

other parasites group (Fig. 1).

Enzyme-Linked Immunoelectrotransfer Blot Technique (EITS): Using EITB technique, sera of all fascioliasis group recognized the *Fasciola* antigen fractions at the 33 and 54 kDa regions as grey blue bands (100% sensitivity). These bands were not detected when using sera of normal controls, schistosomiasis or other parasites groups (100% specificity), as shown in figure 2 and table 2.

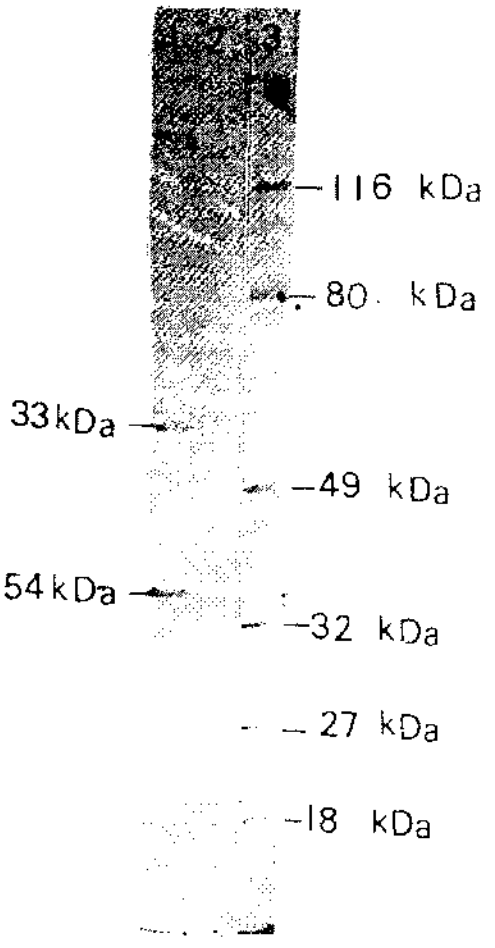

Fig.2: Enzyme linked immunoelectrotransfer blot (EITB) of affinity purified *Fasciola* antigen. Fascioliasis positive sera recongized the 33/54 kDa complex (track 1). Fascioliasis negative sera did not show any reaction (track 2). Track 3: Low molecular weight standard marker.

Table (1): Positivity percent of *Fasciola* antibodies detected by ELISA in the different studied groups.

	Negative Controls	Fascioliasis	Schistosomiasis	Other Parasites
Total Cases	18	21	21	50
No. +ve Cases*	0	21	1	4
Positivity %	0	100	5	8

* Number of fascioliasis positive cases

Table (2): Positivity percent of *Fasciola* antibodies detected by EITB in the different studied groups.

	Negative Controls	Fascioliasis	Schistosomiasis	Other Parasites
Total Cases	18	21	21	50
No. +ve Cases*	0	21	0	0
Positivity %	0	100	0	0

* Number of fascioliasis positive cases

DISCUSSION

This study aimed at getting a specific antigen for accurate diagnosis and consequently proper management of fascioliasis which would obviate many of the complications usually attributed to other causes. The affinity purified *Fasciola* antigen was employed in both ELISA and EITB techniques to evaluate its ability in improving serodiagnosis of fascioliasis. The authors found that, by coating the ELISA microplates with 3 µg/ml of purified *Fasciola* antigen and using the patients sera at 1/256 dilution, 100% sensitivity and 93% specificity

were achieved with a diagnostic efficacy of 96.5%. By using crude *Fasciola* antigen, Khalil et al. (1989) and (1990), reached 66% sensitivity and 84% specificity, while Hassan et al. (1989) found that 25% of schistosomiasis sera gave false positive reactions. Tinell et al. (1987) and Espino et al. (1987) improved both sensitivity and specificity of detection of *Fasciola* antibodies by using *Fasciola* antigen in its excretory secretory form. Using ELISA, they reached 100% sensitivity which is in agreement with the present results, whereas they reported higher specificity percentage (100%) than the present result (93%). When the authors used the EITB, all the fascioliasis group sera gave strong reaction with the 33/54 kDa complex (100% sensitivity), while none of the sera of normal controls or other parasites group, including schistosomiasis, showed any reaction (100% specificity).

In conclusion, it was demonstrated that affinity purified *Fasciola* antigen of apparent molecular weight 33/54 kDa is of diagnostic importance. Application of this purified antigen for immunodiagnosis of fascioliasis showed that ELISA is a good screening test and EITB is good confirmatory test from the sensitivity and specificity points of view.

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REFERENCES

Bhamarapravati, N.; Thamavit, W. and Limsuwan, A. (1983): Liver fluke diseases of man. In: Text Book of Medicne. Weatherall, D.J.; Ledingham, J.G.G. and Warrell, D.D.A., eds. Oxford. London.

Engvall, E. and Perlmann, P. (1971): Enzyme linked immunosorbent assay. Quantitative assay of IgG. Immunochemistry **8**: 871.

Espino, A.M.; Dumenigo, B.E.; Frenandez, R. and Finlay, C.M. (1987): Immunodiagnsis of human fascioliasis by enzyme-linked immunosorbent assay using excretory-secretory products. Am. J. Trop. Med. Hyg., **37**: 605-608.

Gottstein, B. (1983): Isolation of an antigen fraction from *Echinococcus multilocularis* with high species specificity and its identification with

the western blotting technique. In: Immunoenzymatic Techniques. S. Avrameas; P. Druet; R. Masseyeff and G. Feldman, eds. Elsevier, Amsterdam. 299-302.

Hammond, J.A. (1974): Human infection with the liver fluke *Fasciola gigantica*. Trans. Roy. Soc. Med. Hyg., **68:** 253.

Hassan, M.H.; Farghaly, A.M.; El-Gamal, R. L. R. and El-Ridi, A.M.S. (1989): Cross-reactions in immunodiagnosis of patients infected with *Schistosoma, Fasciola* and *Heterophyes* using ELISA. J. Egypt. Soc. Parasitol., **19(3):** 845-851.

Hillyer, G.V. and Capron, A. (1976): Immunodiagnosis of human fascioliasis by counterelectrophoresis. J. Parasitol., **62:** 1011-1013.

Hillyer, G.V.; Pelly, R.P. and Del-Liano De Diaz A. (1979): A solubilization of antigens of *Fasciola hepatica* which react with antibodies to *Schistosoma mansoni*. J. Parasitol., **65:** 55-60.

Khalil, H.M.; Abdel Aal, T.M.; Makled, M.Kh.; Abdalla, H.M.; Fahmy, I.A. and El-Zayyat, E.A. (1989): Sensitivity of crude and purified *Fasciola* antigens in immunological diagnosis of human fascioliasis. J. Egypt. Soc. Parasitol., **19:** 305-402.

Khalil, H.M.; Abdel Aal, T.M.; Makled,M.Kh.; Abdalla, H.M.; Fahmy, I.A. and El-Zayyat, E.A. (1990): Specificity of crude and purified *Fasciola* antigens in immunodiagnosis of human fascioliasis. J. Egypt. Soc. Parasitol. **20(1):** 87-94.

Ragab, M. and Farag, H.F. (1978): Human fascioliasis in Egypt. J. Egypt. Med. Assoc., **61:** 773-780.

Tinell, M.; Falagiani, P.; Riva, G. and Genchi, C. (1987): RAST and ELISA in three autochonus parasitic infections in Italy: Toxocariasis, hydatidosis and fascioliasis Boll. 1st. Sieroter Milan. **66:** 38-45.

Tsang, V.C.W.; Peralta, J.M. and Simons, A.R. (1983): Enzyme linked immunoelectrotransfer blot techniques (EITB) for studying the specificities of antigens and antibodies separated by gel electrophoresis. In: Methods in Enzymology Immunochemical Techniques. Langone J. J. and Vunakie H. V., eds. New York, Academic press. 377-391.