

VALUE OF ANTIGENIC SHARING BETWEEN ADULT *SCHISTOSOMA MANSONI* AND EGYPTIAN FRESHWATER SNAILS ON IMMUNO-DIAGNOSIS OF INTESTINAL SCHISTOSOMIASIS

By

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Abstract

Common antigenic molecules are found between different parasites and their intermediate hosts. This antigenic similarity may be applied for serodiagnosis or drug and vaccine development. The present study was designed to evaluate the antigenic similarity between adult *Schistosoma mansoni* and some Egyptian freshwater snails *Biomphalaria alexandrina*, *Lymnaea natalensis*, and *Physa acuta* and the antigenic value in immuno-diagnosis of intestinal schistosomiasis.

Fifteen laboratory-bred infected albino mice were used to obtain adult *S. mansoni* for adult crude antigen preparation. Forty-eight laboratory-bred rats were used to prepare hyperimmune sera by testing seven separated antigens. Crude snails' antigens were prepared from the foot and visceral parts of the tested snails. Comparing the molecular weight of the tested antigens was done using SDS-PAGE following hyperimmune rat sera preparation for western blot technique and ELISA to visualize their immunogenicity and cross-reactivity.

SDS-PAGE and immunoblotting showed the presence of strong similar bands between whole adult worm antigen and *B. alexandrina* foot antigens while poor antigenic relationship was revealed with *L. natalensis* and *P. acuta* antigens. *B. alexandrina* foot antigen was found by ELISA to be the best antigen to replace *S. mansoni* crude antigen in immuno-diagnosis of schistosomiasis in concern to their sensitivity and specificity.

Key Words: *Schistosoma mansoni*, *Biomphalaria alexandrina*, Antigen, Immuno-diagnosis.

Introduction

Schistosomiasis remains an endemic neglected waterborne parasitic disease affecting millions of people around the world with at least 236.6 million people required preventive treatment (WHO, 2018) and ranks the second prevalent neglected tropical diseases in sub-Saharan Africa among poor communities depending on surface water which is often contaminated and colonized by snails that act as the intermediate hosts for schistosomes (Parker *et al*, 2012; Toor *et al*, 2018). Egypt shows variable schistosomiasis prevalence despite the massive control and prevention efforts (El Morshedy *et al*, 2016). Elimination of such public health problem by the year 2025 is the goal of the WHO (WHO, 2017). Interest has been directed towards alternative supplementary non-drug means in adherence to treatment as

enhanced control of intermediate snail hosts in local habitats, prevention of water contamination through sanitation and vaccination strategies (King, 2017).

The antigenic sharing between adult *S. mansoni* and its specific intermediate host snail *B. alexandrina* has been demonstrated in many studies (Abd El Aal *et al*, 2016) which provide a reason to the parasite to develop in this host. This has been applied for the favour of schistosomiasis serodiagnosis and vaccine development using the snail antigen and showed promising results in those fields (WHO, 2020).

In Egypt, zoonotic trematode parasite and their intermediate hosts are reported in nearly all governorates (Al-Aboody *et al*, 2020). Some Egyptian snails showed antigenic relationships with their parasites and other parasites (Taha *et al*, 2013). The use of snail

delivered antigens in the serodiagnosis of most simple, reliable, fast and markedly available with much antigenic materials (Khat-tab *et al*, 2010; Sarhan *et al*, 2014).

The present study aimed to evaluate the antigenic materials of three Egyptian fresh-water snails in serodiagnosis of *Schistosoma mansoni* infection in albino mice.

Materials and Methods

Animals: Fifteen clean laboratory bred male albino mice aged 6-7 weeks & weighed 20-30gm was experimentally infected with *S. mansoni* cercariae to have crude adult worm antigen. Also, twenty-four male laboratory bred rats were used to produce hyperimmune rat sera (Langley and Hillyer, 1989). Rats were divided into eight groups seven groups were vaccinated by seven prepared antigens, and the 8th one was used as a control. All animals were obtained the Schistosome Biological Supply Program (SBSP), Theodor Bilharz Research Institute, Giza.

Human samples: twenty-five blood samples from *S. mansoni* patients were collected, nine from Theodor Bilharz Institute, ten from Cairo Liver Institute and six from Faculty of Medicine, Tropical Medicine Department. They were subjected to a complete history taking and faecal examination using Kato-Katz technique (Katz *et al*, 1972). They showed mild infection eggs/gram (EPG) < 100.

Blood samples from five *Ascaris lumbricoides* patients, eight *Hymenolepis nana* patients, and 12 *Blastocystis hominis* patients were collected to separate labelled sera. Also, ten sera were obtained from ten healthy volunteers as control sera.

Human consent was taken from all the donor individuals before blood sample collection.

Schistosoma mansoni cercariae were recovered from *B. alexandrina* (Theodor Bilharz Research Institute). Mice were subcutaneously injected with 100±10 cercariae (Peters and Warren, 1969).

genus trematode parasites proved to be the Mice were sacrificed by cervical dislocation eight weeks post-infection and adult worms were obtained by porto-mesenteric perfusion (Duvall and De Witt, 1967).

Preparation of *S. mansoni* whole worm antigen (WWA): *S. mansoni* adults were washed several times with 0.01M PBS, 20 minutes homogenized in a homogenizer at 6000rpm with 0.01M PBS at pH 7.4 in an ice bath and sonicated for another 20 minutes. Sonicated sample was centrifuged at 20,000rpm for an hour at 4°C (Deelder, 1973), supernatant was used as WWA and aliquot into 1 ml plastic vials, and protein concentration was measured (Lowry *et al*, 1951).

Snails & antigen preparation: *B. alexandrina* *P. acuta* and *L. natalensis* were collected from Abu-Rawash Drainage, Giza Governorate. Snails were classified and examined by light exposure for cercariae (Webbe, 1965). Snails cercaria-free were put separate in dechlorinated tap water (10 snails/L), which was changed every 2 days and supplied boiled lettuce leaves kept at room temperature (25±2°C). Preparation of snail antigens was from foot and visceral parts (Nabih *et al*, 1989) and protein concentration was measured as before.

Preparation of hyperimmune sera: Adult *S. mansoni* WWA and the snail antigens were used to produce hyperimmune rat sera (Langley and Hillyer, 1989). Hyperimmune sera were prepared for Western Blot and ELISA techniques. Freund's complete adjuvant & Freund's incomplete adjuvant (Sigma Immuno-chemicals USA) were used.

Antigens fractionation by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE): Antigens were analysed by SDS-PAGE using Laemmli buffer (Laemmli, 1970).

Immunoblotting (Western Blot): The unstained protein bands were electrophoretically transferred from SDS-PAGE to a nitrocellulose sheet (Towbin *et al*, 1979).

Immuno-detection of antigens on nitrocellulose strips: Nitrocellulose strips were washed several times with PBS then soaked in blocking buffer at room temperature for 2hrs with agitation. Strips were removed from the blocking buffer and washed twice for 5 min in PBS. Positive and negative diluted sera were added 0.5ml for each strip and incubated at room temperature for an hour with agitation. Strips were washed 4 times using PBS-T 0.05% for 5 minutes each, conjugate (1:1000) was added and incubated for an hour at room temperature and then strip was washed 4 times each by PBS-T 0.05% for 5 minutes. Nitrocellulose strips were placed in suitable container and substrate was added for 30 min. with agitation until the bands were suitably dark, and then strip was rinsed thoroughly with distilled water to stop the reaction.

ELISA using prepared antigens, hyperimmune rat sera & human sera: Sera from 25 *S. mansoni* infected patients, 25 patients infected with other parasites and ten normal controls were examined (Voller *et al*, 1976). Optimum antigen and conjugate concentrations with diluted sera were determined by checkerboard titration. Antigens were diluted in coating buffer at dilution (4µg/ml coating buffer). Each well was filled with 100µl of the corresponded antigen concentration and then plates were incubated overnight at 4°C. Plates were washed 3 times with PBS-T 0.05 % to remove excess unbound antigen and the free binding sites were blocked with Bovine serum albumin (BSA) for buffer (200µl/well) and kept for an hour. Plates were washed 3 times with PBS-T 0.05%. Sera were added to plates (100µl/well) and incubated at 37°C for 90 minutes, plates were washed 3 times with PBS-T 0.05%, and 100µl/well of conjugate (1:2000) were added to all wells and incubated at 37°C for an hour. Plate was again washed 3 times with PBS-T 0.05 %, 100µl of the substrate was added, plate was covered for 20 minutes and yellowish coloration reaction was stopped by adding 100µl/well of 1 M H₂SO₄.

Reading was done using ELISA-reader at 490nm (Titerteck multiscan-Dynatech laboratories).

Statistical analysis: Data was coded and analysed by using IBM SPSS version 24. Frequencies (number) and relative frequencies (%) were used to summarize qualitative variables while mean and standard deviations were used for quantitative variables.

Ethical approval: The experiment was carried out according to the Clinical and Laboratory Standards Institute (CLSI) guidelines by Faculty of Medicine, Cairo University.

Results

Measurement of protein concentration for the prepared snail antigens revealed that the foot parts are rich in protein if compared to the visceral parts.

Fractionation of *S. mansoni* WWA using SDS-PAGE and demonstration of different protein bands on gel using Coomassie-blue stain showed 3 clear bands in *S. mansoni* Ag at 42, 32 & 15 KDa but, in *B. alexandrina* foot Ag showed 5 bands at 90, 48, 42, 32 & 15 KDa. Visceral hump Ag showed 2 faintly stained fractions at 90 and 15 KDa. Fractionation of *L. natalensis* foot Ag showed 3 fractions at 53-68, 26 & 15 KDa but, in *P. acuta* foot Ag showed 5 bands at 180, 50-55, 35, 26 & 15 KDa, with common bands between *S. mansoni* WWA, *B. alexandrina* and *P. acuta* foot Ag at 42 & 32 KDa.

Treatment of NC strips with fractionated *S. mansoni* and other snail antigens with *S. mansoni* HIS showed 9 wide and sharp bands corresponded to MWs of 23, 25, 28, 30, 36-38, 40, 45, & 52 KDa and a wide band at level of 55-74 KDa reacted specifically with fractionated *S. mansoni* Ag. Treatment of NC strips with fractionated foot Ag of *B. alexandrina* with *S. mansoni* HIS showed 5 reacted protein fractions corresponded to MWs of 25, 36-38, 44- 48, 54-65 & 70-75 KDa. Also, 3 bands from fractionated *L. natalensis* ranged from 25 to 58 KDa and 4 fractions in *P. acuta* treated strip ranged from 25 to 58 KDa reacted with *S. mansoni* HIS. Treatment of NC strip with fractionated *S.*

mansoni Ag with negative rat sera showed 3 bands at 20, 25, & 38 KDa and a wide band ranged between 55-58 KDa. There was similarity between *S. mansoni* WWA and *B. alexandrina* foot Ag when reacted with *S. mansoni* HIS fractions corresponded to 45 & 70-75 KDa, but without similarity between *S. mansoni* antigens, *L. natalensis* foot Ag and *P. acuta* foot Ag when reacted with *S. mansoni* HIS. Bands (25 & 38KDa) were in negative sera reaction.

Treatment of NC strips with fractionated *S. mansoni* Ag and other snail antigens with *B. alexandrina* HIS showed eight reaction bands corresponded to 23, 25, 30- 32, 36, 38-43, 45, 50 & 62 KDa when reacted with fractionated *B. alexandrina* Ag. Treatment seven protein fractions corresponded to 24, 25, 30, 32-42, 45, 50 -55 & 60 -70 KDa. When NC strips with fractionated *S. mansoni* Ag treated with *B. alexandrina* HIS, seven reacted fractions corresponded to 18-23, 25, 32, 35, 45, 58 & 70 KDa were detected. When NC strips with fractionated *L. natalensis* Ag treated with *B. alexandrina* HIS, five reaction bands were detected corresponded to 15, 18- 23, 30, 32-42 & 100 KDa. Regarding *P. acuta* fractions reacted with *B. alexandrina* HIS, one band corresponded to 38-42 KDa. Treatment of NC strip with fractionated *B. alexandrina* Ag with negative rat sera showed 3 bands corresponded to 10-15, 20-30 & 75-100 KDa. There was similar protein specificity between *S. mansoni* WWA and *B. alexandrina* antigen when reacted with *B. alexandrina* HIS at fractions corresponded to 32, 45 & 70 KDa.

Treatment of NC strips with fractionated *S. mansoni* Ag and other snail antigens with *L. natalensis* HIS showed seven reaction bands corresponded to 24, 40, 45, 48, 70-80, 100 & 150 KDa when reacted with fractionated *L. natalensis* Ag. When NC strips with fractionated *S. mansoni* Ag treated with *L. natalensis* HIS, three reacted fractions corresponded to 40, 45 & 75-78 KDa was detected. Treatment of NC strips with fractionated foot Ags of *B. alexandrina* showed five re-

acted protein fractions corresponded to MWs of 26, 35-40, 52, 100 & 150 KDa. When *P. acuta* fractions reacted with *L. natalensis* HIS, six bands corresponded to 20, 24, 36, 52, 58-76 & 100 KDa. Treatment of NC strip with fractionated *L. natalensis* Ag with negative rat sera showed three bands corresponded to 15-20, 30 & 50-60 KDa. Common fractions corresponded to 40, 45 & 75-78 KDa were between *S. mansoni* WWA and *L. natalensis* Ag when reacted with *L. natalensis* HIS after exclusion of common bands reacted with negative sera.

Treatment of NC strips with fractionated *S. mansoni* Ag and other snail antigens with *P. acuta* HIS showed five reaction bands corresponded to 32-40, 45, 61- 65, 80 & 100 KDa when reacted with fractionated *P. acuta* Ag. When NC strips with fractionated *S. mansoni* Ag treated with *P. acuta* HIS, two reacted fractions corresponded to 35-40, & 45-50 KDa were detected. Treatment of NC strips with fractionated foot Ags of *B. alexandrina* showed six reacted protein fractions corresponded to MWs of 12-14, 16-18, 24-28, 35-40, 50 & 100-150 KDa. When *L. natalensis* fractions reacted with *P. acuta* HIS showed eight bands corresponded to 18, 22, 25, 38, 45, 50, 55-62 & 80 KDa. Treatment of NC strip with fractionated *P. acuta* Ag with negative rat sera showed three bands corresponded to 12-18, 22-28 & 50-60 KDa. A common fraction corresponded to MW of 35-40 KDa was between *S. mansoni* WWA and *P. acuta* foot Ag when reacted with *P. acuta* HIS.

Titration showed optimal dilution of sera was at 1:50 for all Ags and OD for conjugate was at 1:2000. Similarity between *S. mansoni* WWA & snail Ags was cut off value = double fold OD of -ve control value. Adult *S. mansoni* WWA with target Ag and other snail Ags showed highest mean OD with homologous Ag (1.042 ± 0.0551), followed by *B. alexandrina* foot Ag (1.034 ± 0.0983) respectively. Visceral hump Ags of *B. alexandrina* with *S. mansoni* rat HIS showed positive mean OD of 0.788 ± 0.1007 but lower than

that with foot Ags. *L. natalensis* & *P. acuta* crude foot Ag with *S. mansoni* rat HIS showed negative means OD of 0.396 ± 0.0085 & 0.382 ± 0.0309 respectively.

Anti *B. alexandrina* Abs from rats vaccinated by crude *B. alexandrina* Ag with target Ag and other Ags showed highest mean OD with homologous Ag (1.104 ± 0.1185), *B. alexandrina* VH Ag (0.996 ± 0.0397) and *S. mansoni* WWA (0.935 ± 0.0251). *L. natalensis* & *P. acuta* foot Ags showed negative mean OD (0.432 ± 0.0280 & 0.406 ± 0.0160 respectively) with *B. alexandrina* rat HIS.

Anti *L. natalensis* Abs from rats vaccinated by *L. natalensis* foot Ag target Ag & other Ags showed highest mean OD with homologous Ag (1.077 ± 0.0457). *B. alexandrina* foot, visceral Ags and *P. acuta* foot Ag sho

wed rare positive mean OD (0.638 ± 0.0398 , 0.641 ± 0.0376 & 0.572 ± 0.0155 respectively) compared to specific *L. natalensis* Ag. *S. mansoni* WWA showed negative mean OD with *L. natalensis* rat HIS (0.440 ± 0.0726).

Anti *P. acuta* Abs from rats vaccinated by *P. acuta* foot Ag with target antigen and other Ags showed highest mean OD against homologous Ag (1.041 ± 0.0709). *B. alexandrina* foot, visceral Ags and *L. natalensis* foot Ag showed positive mean OD with *P. acuta* HIS (0.552 ± 0.0061 , 0.581 ± 0.0346 & 0.697 ± 0.0593 respectively), but lower compared to homologous Ag, *S. mansoni* crude Ag with *P. acuta* HIS showed negative OD (0.393 ± 0.0240).

Details were given in table (1) and figures (1, 2, & 3)

Table 1: Mean ELISA OD values of all the tested Ags versus different prepared HIS

HIS Tested Ags	Adult <i>S. mansoni</i> HIS	<i>B. alexandrina</i> HIS	<i>L. natalensis</i> HIS	<i>P. acuta</i> HIS
<i>S. mansoni</i> WWA	1.042 ± 0.0551	0.94 ± 0.0251	0.44 ± 0.0726	0.39 ± 0.0240
<i>B. alexandrina</i> foot Ag	1.034 ± 0.0983	1.10 ± 0.1185	0.64 ± 0.0398	0.55 ± 0.0061
<i>B. alexandrina</i> VH Ag	0.79 ± 0.0311	1.00 ± 0.0397	0.55 ± 0.0261	0.55 ± 0.0267
<i>L. natalensis</i> foot Ag	0.40 ± 0.0085	0.43 ± 0.0280	1.08 ± 0.0457	0.70 ± 0.0593
<i>P. acuta</i> foot Ag	0.38 ± 0.0309	0.41 ± 0.0160	0.57 ± 0.0155	1.04 ± 0.0709
Negative Control	0.22 ± 0.0217	0.22 ± 0.0217	0.22 ± 0.0085	0.21 ± 0.0055

Discussion

Understanding the natural intermediate snails of schistosomes is a vital key to suppress disease transmission in Egypt (Taha *et al.*, 2013). Common molecules between trematodes and their intermediate host snails have been reported (Abd El Aal *et al.*, 2016). Snail-parasite interaction was complex, influenced by numerous genetic and physiological factors in both hosts (Abou El Naga *et al.*, 2010). Snail molecules can be a rich source of antigenic material for serodiagnosis and prophylactic studies of their parasites, being easy to obtain and of low cost, if compared to parasite antigens required maintenance of complex life cycle (Selim *et al.*, 2016). Eyayu *et al.* (2020) in Ethiopia recommended that vaccination strategy would be an ideal tool for a significant and sustainable reduction in the transmission and disease burden of schistosomiasis.

In the present study, the choice of *L. natalensis* and *P. acuta* was based on the previously reported where extensive cross-reactiv-

ity of snail antigens with incompatible trematodes and cross-reactivity between the trematode species (Negrão-Corrêa *et al.*, 2007). Also, Cross-reactivity of monoclonal antibody directed against *S. mansoni* tropomyosin isoform with *F. hepatica*, and *P. acuta* was reported (Weston *et al.*, 1994). Besides, Maghraby *et al.* (2009) and Boukli *et al.* (2011) reported common immuno-reactive proteins between *S. mansoni* and *F. gigantica* molecules.

In the present study, measurement of protein concentration in the Ags of all examined snails showed lower protein concentration in visceral Ags if compared to foot Ags. This agreed with Khattab *et al.* (2010) and Basyoni and Abd El-Wahab (2013) who found that snail foot Ags were more sensitive and specific in immunological evaluation. Also, Ekin *et al.* (2016) assumed that the foot provided higher percentage of protein than other snail tissues.

In the present study, the bands after fractionation of *S. mansoni* WWA agreed with

Shaheen *et al.* (1996) who identified a low molecular weight antigenic fraction (30-40 KDa) in soluble worm antigen preparation, and the most strongly recognized bands by IgG1 & IgG3 in human sera with pre-patent infection of *S. mansoni*. The present results also agreed with Soliman *et al.* (2003) who found that shared polypeptides of the three stages of *S. mansoni* (soluble worm antigen, cercarial antigen & soluble egg antigen) corresponded to 32 KDa under reducing condition. Moreover, Basyoni and Abd El-Wahab (2013) and Abd El Aal *et al.* (2016) reported that the prominent protein bands corresponded to the MWs at the level of 44, 38-40 and 30 KDa.

In the present study, fractionation of *B. alexandrina* foot Ag showed five clear bands while the visceral hump Ag showed only two faintly stained fractions. This more or less agreed with Shalaby *et al.* (2010) and Basyoni and Abd El-Wahab (2013) who reported prominent protein bands corresponded to MWs 97, 94, 32 & 30 KDa.

Also, in the present study, the common fractions observed between *S. mansoni*, and *B. alexandrina* foot Ag agreed with both Basyoni and Abd El-Wahab (2013) and Abd El Aal *et al.* (2016) who found that *B. alexandrina* foot Ag showed the best similarity to *S. mansoni* Ag by SDS-PAGE. However, in the present study, neither common fraction was between *S. mansoni* WWA and *L. natalensis* nor *P. acuta* Ags by SDS-PAGE.

In the present study, reaction of *S. mansoni* WWA with its homologous HIS, agreed with Noya *et al.* (1995) who reported similar bands at 74, 71, 45, 36 & 30 KDa by total IgG. Also, Shaheen *et al.* (1996) identified a similar strongly recognized antigenic fraction (30-40 KDa) in soluble worm antigen by IgG1 & IgG3 in human sera with *S. mansoni* infection. Again, Attallah *et al.* (1998) detected a similar polypeptide antigen of 74 KDa in the antigenic extracts of three developmental stages of *S. mansoni* by WB. Abd El Aal *et al.* (2016) showed similar antigenic active bands in *S. mansoni* antigen on reac-

tion with its homologous HIS at MWs of 52, 40-42 & 35 KDa.

In the present study, the similarity between *S. mansoni* WWA and *B. alexandrina* foot Ag when reacted with *S. mansoni* HIS agreed with Sulahian *et al.* (2005) who identified the band 70KDa among the diagnostic bands for schistosomiasis, and used WB for diagnosis of schistosomiasis in human sera using crude extract of *S. mansoni*. Previously, Tarabazadi and Schechtman (1998) reported that 45-kDa subunit in *B. alexandrina* Ag that induced 40% to 50% protection in mice challenged with *S. mansoni* infection. Moreover, Abd El Aal *et al.* (2016) showed that antigenic active band in *B. alexandrina* foot Ag on reaction with *S. mansoni* HIS was at 42 KDa & 70-75 KDa by WB.

In the present study, no similarity in protein specificity was between *S. mansoni* WWA and the *L. natalensis* foot Ag and *P. acuta* foot Ag when reacted with *S. mansoni* HIS, as 25KDa & 38KDa were detected with negative sera reaction. This agreed with Shalaby *et al.* (2010) who tested specificity of snail antigens of two different snail families; *L. natalensis* as an intermediate host of *Fasciola gigantica* and *B. alexandrina* as an intermediate host of *Paramphistomum microbothrium*, in detection of IgG antibodies against their trematode parasites by WB and found that *B. alexandrina* foot antigenically active polypeptides recognized by its homologous HIS showed specific reactivity toward *Paramphistomum* HIS but didn't react crossly with *F. gigantica* HIS.

In the present study, reaction of *B. alexandrina* foot Ag with its homologous HIS showed eight bands, a common band at 68 KDa between *S. mansoni* WWA and infected or non-infected snail foot antigens when reacted with HIS against foot of infected *B. alexandrina*. This agreed with Abd El Aal *et al.* (2016) they detected similar common bands at 48 & 68 KDa by WB on reacting *S. mansoni* WWA with *B. alexandrina* HIS. There was an observed similarity between *S. mansoni* Ag and *B. alexandrina* Ag when react-

ed with *B. alexandrina* HIS at the fractions corresponding to MWs of 32 and 45 KDa.

In the present study, there was some relationship between the different genera; *F. gigantica* and *S. mansoni* and their intermediate hosts; *B. alexandrina* and *L. natalensis*. Farag and Sayad (1995) in Alexandria reported *B. alexandrina* naturally infected with *F. gigantica* in Abis. Besides, Farrag *et al.* (2005) detected common antigenic bands between *B. alexandrina* Ag and *L. natalensis* Ag by electrophoresis. El-Maghraby *et al.* (2009) in Saudi Arabia found a cross-reactivity between *S. mansoni* eggs and *F. gigantica* worms and eggs.

In the present study, the ability of *L. natalensis* foot Ag to induce immune reaction in rats was observed in WB, with seven sharp bands on reaction with homologous HIS. The similarity between *S. mansoni* WWA and *L. natalensis* Ag when reacted with *L. natalensis* HIS was at 40, 45 & 75-78 KDa. This agreed with Taha *et al.* (2013) who also found that the band 45 KDa was specific for *L. natalensis* when reacted with adult *F. gigantica* HIS by WB. This may explain the cross-reactivity denoted by El-Maghraby *et al.* (2009) between *F. gigantica* and *S. mansoni*. Two common bands between *B. alexandrina* Ag and *L. natalensis* Ag when reacted with *L. natalensis* HIS were at 100 & 150 KDa. This also agreed with Farrag *et al.* (2005) who by electrophoresis reported common antigenic bands between *B. alexandrina* Ag and *L. natalensis* Ag.

In the present study, in *P. acuta* foot Ag immunogenicity, five bands were on reaction with homologous HIS. Similarity between *S. mansoni* WWA & *P. acuta* Ag when reacted with *P. acuta* HIS was at only one fraction corresponded to 35-40 KDa. This agreed with Weston *et al.* (1994) who found cross-reactivity between schistosomes and *P. acuta* by using a certain monoclonal-Ab directed against SMTM.

No doubt, apart from the vaccine development, dependable accurate diagnosis was a must. In the present study, to detect value of

Ags in diagnosis of intestinal schistosomiasis, ELISA investigated the ability of different snail antigens in trapping anti-*S. mansoni* antibodies in rat HIS and positive and negative patients' sera. ELISA proved to be cheap, reliable and widely applied in developing countries for schistosomiasis diagnosis, and more sensitive and specific than IHAT (Aboul-Hassan *et al.*, 1997) and IFAT (Sarhan *et al.*, 2014) in epidemiological surveys. The adults Ags in ELISA gave high sensitivity and specificity in diagnosing acute and chronic schistosomiasis and other parasitic diseases (Valli *et al.*, 1997; Waikagul *et al.*, 2002; Attallah *et al.*, 2013). Also, the ELISA antigenic community value between snails and other trematodes as fascioliasis (Hassan 2008), heterophyiasis (El-Seify *et al.*, 2012) and other parasitic flukes infecting farm animals (Haridy *et al.*, 2006)

In the present study, ELISA plates were coated with the antigens diluted in coating with optimal diluted buffer (4µg protein/ml coated buffer) and the optimal serum dilution was (1:50) after checkerboard titration. This went with Espino *et al.* (1987) who reported that the best result was when ELISA plate was coated with 4µg protein/ml of coated buffer, and Sulahian *et al.* (2005) and Khattab *et al.* (2010) recommended a serum dilution of 1:50 as the best to detect parasite Ags by immunoassays without differences in sensitivity or background readings when dilution 1:50, 1:100, 1:160 were used.

The present work showed that *B. alexandrina* foot Ags substituted *S. mansoni* Ag in capturing adult *S. mansoni* Abs in sera. This agreed with Shalaby *et al.* (2010) and Khattab *et al.* (2010) who found that *B. alexandrina* foot Ag was the best to replace *S. mansoni* WWA in schistosomiasis diagnosis.

In the present study, all the Ags recorded the highest mean OD values versus their homologous laboratory prepared rat HIS. This agreed with Abdel-Rahman and Abdel-Megheed (2000) and Waikagul *et al.* (2002) they reported that the best ELISA OD values in the homologous assays than in heterologous

ones. Also, this agreed with Khattab *et al.* (2010) who concluded that the ELISA visceral hump Ag of *B. alexandrina* showed lower mean ELISA OD readings with *S. mansoni* rat HIS than with their foot Ag, and that *B. alexandrina* foot Ag was more sensitive in sero-diagnosis of human schistosomiasis. Shalaby *et al.* (2010) and Basyoni and Abd El-Wahab (2013) reported also higher specificity of snail feet Ags in detection of their parasite Abs by WB.

The present of positive ELISA OD values recorded on reaction of *L. natalensis* and *P. acuta* HI sera with *Biomphalaria* Ags may explain the presence of common Ags between the cold blood invertebrates (Khattab *et al.*, 2010), where high ELISA OD values were reported between anti-*Bulinus truncatus* Abs and *B. alexandrina* Ags. Besides, *L. natalensis* & *P. acuta* foot Ags showed negative OD values on reacting with *S. mansoni* rat HIS. Thus, only *B. alexandrina* foot and visceral Ags as well as *S. mansoni* WWA were experimented with for diagnostic efficacy among low worm burden population sera.

In the present study, *S. mansoni* WWA diagnosed 92% of *Schistosoma* confirmed cases with absolute specificity, and with neither false positive ones corresponded to negative control or cross-reactivity with other helminthic parasites. This agreed with Khattab *et al.* (2010) who reported that 100% sensitivity of *S. mansoni* crude Ag in diagnosis of schistosomiasis at 1:50 serum dilution. *B. alexandrina* foot Ag came the second after *S. mansoni* WWA regarding sensitivity and specificity, as the Ag recorded positive OD values in 80% of *Schistosoma* confirmed cases, and 85.7% mean specificity without recorded false positive results in control non infected human sera. This showed an obvious similarity to Shalaby *et al.* (2010) and Khattab *et al.* (2010) who found that *B. alexandrina* foot Ag was the best to replace *S. mansoni* WWA in diagnosis of Schistosomiasis by both direct ELISA and sandwich ELISA.

In the present study, visceral hump Ag of *B. alexandrina* revealed lower sensitivity in detection of *Schistosoma* cases with 72% at 1: 50 serum dilution. They revealed also less specificity (62.8%) by recording more cross-reactivity with sera from individuals infected with other parasites. This agreed with Shalaby *et al.* (2010); Khattab *et al.* (2010), and Basyoni and Abd El-Wahab (2013), they reported the higher sensitivity and specificity of snail feet Ags in detection of their parasite Abs by ELISA and WB. Gonclaves *et al.* (2006) suggested that the screening method combining antibody detection by ELISA and repeated parasitological stool examinations could increase the chances of detecting *S. mansoni*-infected patients in low transmission areas. Attallah *et al.* (2013) also reported that immuno-diagnostic methods for the early stages of some parasitic diseases where the worm eggs are not shed in feces as well as for the chronic conditions.

Conclusion

B. alexandrina foot crude Ag is the best to replace adult *S. mansoni* crude Ag in immuno-diagnosis of intestinal schistosomiasis in patients regarding specificity and sensitivity. However, more studies are needed to categorize and test more specific epitopes within these Ags that can be vital for the development of recombinant proteins used in immuno-diagnosis and vaccine development for schistosomiasis. Also, light should be spotted on other members of family *Planorbidae* rather than *B. alexandrina* which may play a role in schistosomiasis transmission.

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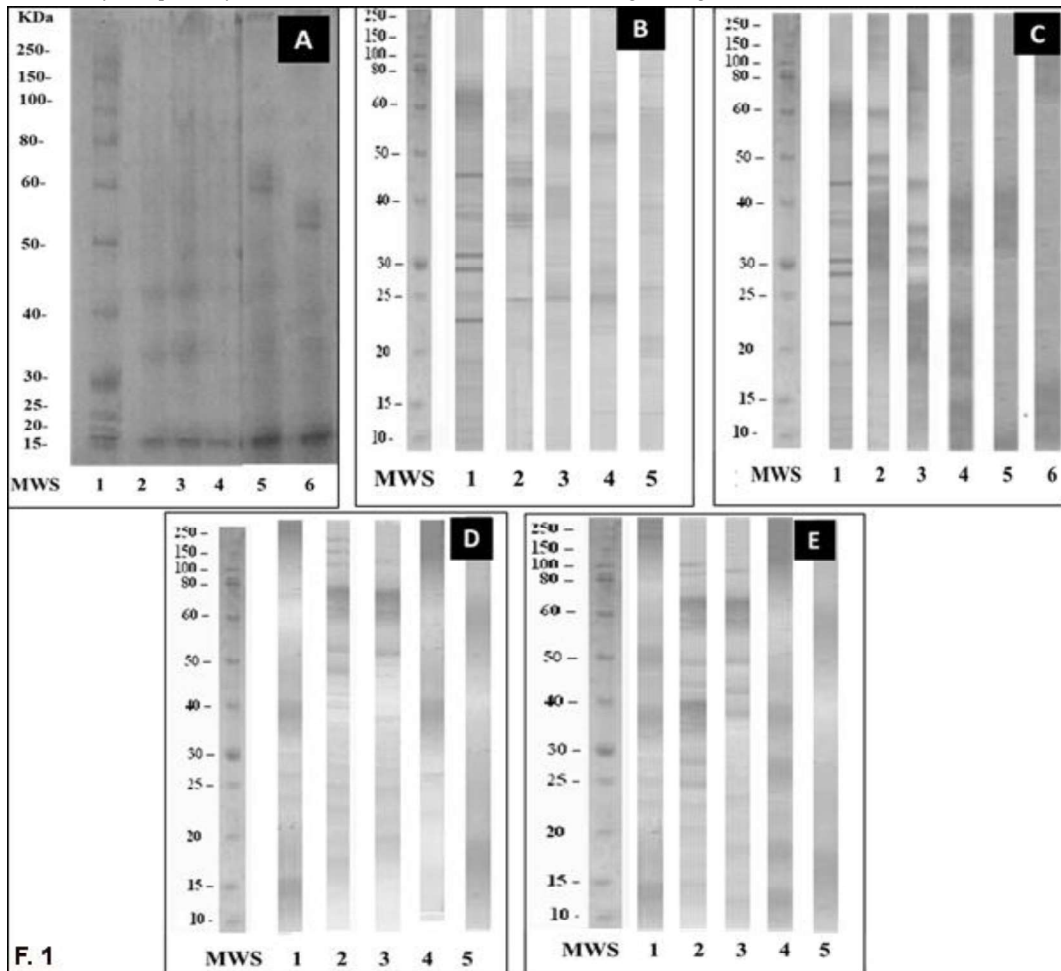
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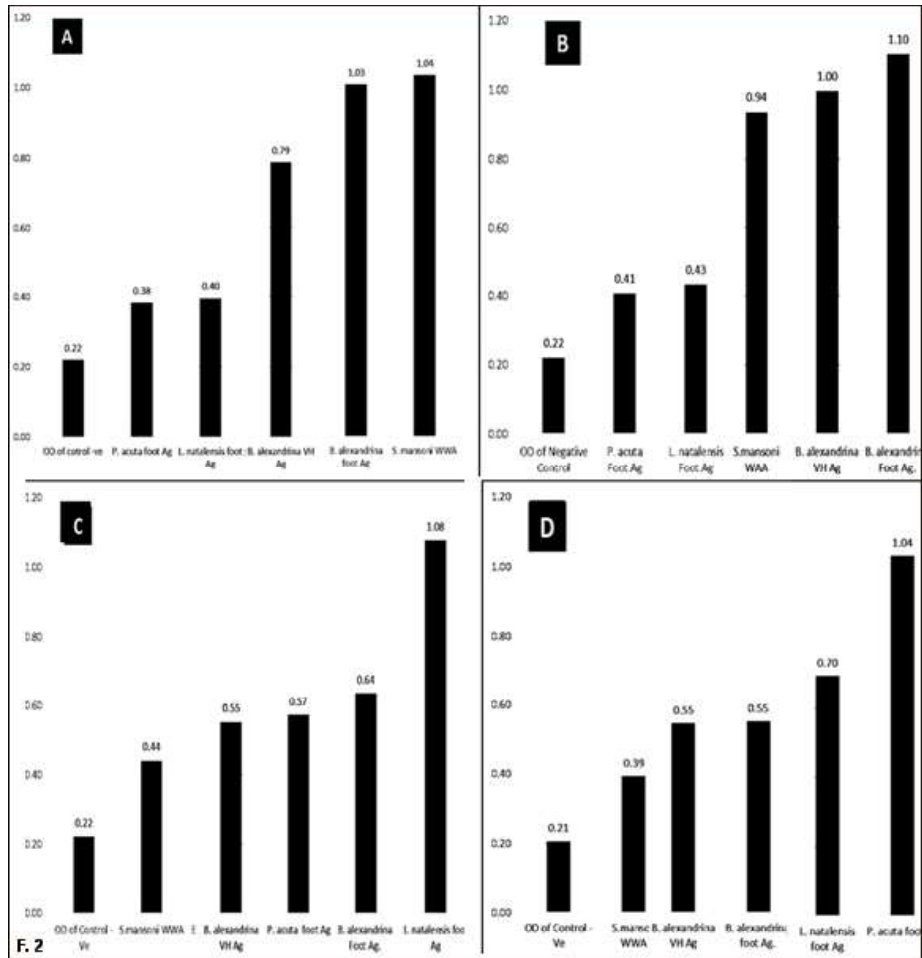
Explanation of figures

Fig. 1: A-SDS-PAGE contain fractionated *S. mansoni* and different snail antigens (Coomassie-blue stained): Lane 1: Molecular Weight protein standard marker. Lane 2: *S. mansoni* WWA. Lane 3: *B. alexandrina* foot Ag. Lane 4: *B. alexandrina* Visceral-hump Ag. Lane 5: *L. natalensis* foot Ag. Lane 6: *P. acuta* foot Ag. B- Reaction of adult *S. mansoni* HIS with fractionated *S. mansoni* Ag and different snail Ag: Lane 1: with fractionated *S. mansoni* Ag. Lane 2: with fractionated *B. alexandrina* foot Ag. Lane 3: *S. mansoni* HIS with fractionated *L. natalensis* foot Ag. Lane 4: with fractionated *P. acuta* foot Ag. Lane 5: Negative sera with fractionated *S. mansoni* Ag. C- Reaction of *B. alexandrina* HIS with fractionated *S. mansoni* and different snail Ags: Lane 1: *S. mansoni* HIS with fractionated *S. mansoni* Ag (for comparison). Lane 2: with fractionated *B. alexandrina* Ag. Lane 3: with fractionated *S. mansoni* Ag. Lane 4: with fractionated *L. natalensis* foot Ag. Lane 5: with fractionated *P. acuta* foot Ag. Lane 6: Negative sera with fractionated *B. alexandrina* Ag. D- Reaction of *L. natalensis* HIS with fractionated *S. mansoni* and different snail Ag: Lane 1: with fractionated *S. mansoni* Ag. Lane 2: with fractionated *L. natalensis* foot Ag. Lane 3: with fractionated *P. acuta* foot Ag. Lane 4: with fractionated *B. alexandrina* foot Ag. Lane 5: Negative sera with fractionated *L. natalensis* Ag. E- Reaction of *P. acuta* HIS with fractionated *S. mansoni* Ag and different snail Ag: Lane 1: with fractionated *S. mansoni* Ag. Lane 2: with fractionated *P. acuta* foot Ag. Lane 3: with fractionated *L. natalensis* foot Ag. Lane 4: with fractionated *B. alexandrina* foot Ag. Lane 5: Negative sera with fractionated *P. acuta* Ag.

Fig. 2: A- Mean ELISA OD of adult *S. mansoni* rat HIS versus target Ag and other snail Ags. B- Mean ELISA OD of *B. alexandrina* rat HIS versus its target Ag and other snail Ags. C- Mean ELISA OD of *L. natalensis* rat HIS versus its target Ag and other snail Ags. D- Mean ELISA OD of *P. acuta* rat HIS versus target Ag and other snail Ags.

Fig. 3: Sensitivity and specificity of *S. mansoni* WWA and *B. alexandrina* snail Ags in diagnosis of intestinal schistosomiasis.





F. 2

