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, Lymnea 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 genus trematode parasites proved to be the 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 freshwater 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/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).

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%, 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 (Titertec 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, one band corresponded to 38-42 KDa. Treatment of NC strip with fractionated foot Ags of B. alexandrina showed six reacted 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 mean 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 showed 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

<table>
<thead>
<tr>
<th>HIS Tested Ags</th>
<th>Adult <em>S. mansoni HIS</em></th>
<th>B. alexandrina HIS</th>
<th><em>L. natalensis</em> HIS</th>
<th><em>P. acuta</em> HIS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. mansoni</em> WWA</td>
<td>1.042±0.0551</td>
<td>0.94±0.0251</td>
<td>0.44±0.0726</td>
<td>0.39±0.0240</td>
</tr>
<tr>
<td><em>B. alexandrina</em> foot Ag</td>
<td>1.034±0.0983</td>
<td>1.10±0.1185</td>
<td>0.64±0.0398</td>
<td>0.55±0.0061</td>
</tr>
<tr>
<td><em>B. alexandrina</em> VH Ag</td>
<td>0.79±0.0311</td>
<td>1.00±0.0397</td>
<td>0.55±0.0261</td>
<td>0.55±0.0267</td>
</tr>
<tr>
<td><em>L. natalensis</em> foot Ag</td>
<td>0.40±0.0085</td>
<td>0.43±0.0280</td>
<td>1.08±0.0457</td>
<td>0.70±0.0593</td>
</tr>
<tr>
<td><em>P. acuta</em> foot Ag</td>
<td>0.38±0.0309</td>
<td>0.41±0.0160</td>
<td>0.57±0.0155</td>
<td>1.04±0.0709</td>
</tr>
<tr>
<td>Negative Control</td>
<td>0.22±0.0017</td>
<td>0.22±0.0217</td>
<td>0.22±0.0085</td>
<td>0.21±0.0055</td>
</tr>
</tbody>
</table>

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 previous reported where extensive cross-reactivity 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

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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 *B. alexandrina* foot Ag when reacted with *S. mansoni* HIS.

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, At tallah 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 reaction 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 70 KDa among the diagnostic bands for schistosomiasis, and used WB for diagnosis of schistosomiasis in human sera using crude extract of *S. mansoni*. Previously, Tarrabazadi 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 ihomologous 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* WWAg 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 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-Megeed (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 foot 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.

**References**


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Explanation of figures

Fig. 1: A- SDS-PAGE contain fractionated S. mansoni and different snail antigens (Comassie-blue stained): Lane 1: Molecular Weight protein standard marker. Lane 2: S. mansoni WWAg. 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 WWAg and B. alexandrina snail Ags in diagnosis of intestinal schistosomiasis.