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# THE ANTIGENIC RELATIONSHIP BETWEEN *SCHISTOSOMA MANSONI* AND ITS INTERMEDIATE SNAIL HOST

By
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## Abstract

Schistosomiasis is a public health problem in many developing countries including Egypt, Determination of the antigenic relationship between *S. mansoni* and its intermediate snail host (IMH) *Biomphalaria alexandrina* can open a new field for diagnosis and control of the disease. In the present study infected and non-infected *B. alexandrina* foot and visceral hump tissue as well as *S. mansion* crude Ag (SWAg) were fractionated using SDS-PAGE. It's specific and cross reacted protein fractions were determine using EITB versus experimentally prepared mice hyper immune sera (HIS) versus each antigen.

After treatment of fractionated *S.mansoni* crude worm antigens (SWAg) versus HIS produced after vaccination of mice by the same Ag, 8 kda protein fractions ranged from 35-140 kda were reacted specifically. Treatment of fractionated *B.alexandrina* infected and non-infected foot and visceral hump Ag versus previous HIS revealed presence of common polypeptides bands between SWAg and non-infected snail antigens. The fraction at 135 kda, 68 kda, were detected in all cases, while that at 40-42 kda and that at 35 kda was diagnosed in SWAg and that of infected snails only. The fraction at 68 kda was reacted specifically between SWAg and all tested fractionated snail antigens either that of foot or visceral hump when they treated separately by HIS of mice vaccinated by each snail Ag separately. The fraction at 135 kda was common between SWAg and snail (infected and non-infected) visceral hump antigen. The fraction at the level of 110 kda was diagnosed in SWAg, in non-infected foot antigen and visceral hump Ag. The fraction at the level of 46-48 kda are common between SWAg and snail foot and visceral hump Ag after treated by HIS of mice vaccinated by foot Ag,

Presence of common antigenic fractions between snail tissues and *Schistosoma* species can prefer an easily source of antigen valuable for diagnosis or vaccination as well as can be considered as new tool for determination to the snail IMH of new discovered trematode parasites.

**Key words:** *S.mansoni*- *B.alexandrina*- EITB- Common fractions.

## Introduction

Schistosomiasis is a public health problem in many developing countries including Egypt, *S. mansoni* is the most widespread species of the causative trematode parasite (WHO, 2010). The prevalence of schistosomiasis was related to the number of infected snails in the area (De Santana *et al*, 1992).

Numerous genetic and physiological factors in both the snail and the parasite are critical for determining the interaction between *S. mansoni* and *Biomphalaria.* The most important of which is the internal defense system (IDS) of the snail (Abou El Naga *et al*, 2010).The parasite can escape the IDS by two mechanisms, molecular mimicry and antigenic masking. In the molecular mimicry, the parasite expresses glycoprotein epitopes on its surface that mimics host molecules. While the antigenic masking is the absorption and incorporation of the snail agglutinins and hemolymph soluble components to the sporocyst surface (Abou EL Naga, 2011).

Common antigen fractions were demonstrated to be shared by schistosome larval stages (miracidia, sporocysts and cercariae), adult schistosomes and their intermediate hosts (Lehr *et al*, 2008). These antigenic fractions proved to be active in inducing immunity against schistosomiasis. Previous studies had shown that anti snail antibodies

had been demonstrated in sera of patients infected with *S. mansoni* and *S. haematobium* using hepatopancreas of infected and uninfected *B.glabrata* snails (Van Lieshout *et al*, 2000). *Biomphalaria*, contain circulating haemocytes participate in the protective mechanism against pathogens. These compounds can interact directly with pathogenic agents producing toxic substances or lytic peptides, or indirectly through mediator molecules for recognition of the pathogen or haemocyte activators (Martins-Souza *et al*, 2011). The existence of a cellular defense mechanism deployed by molluscs against trematode infection was initially suggested by the histological reactions around parasite sporocysts. Susceptible snail parents showed a normal development of the parasites with wide spread of cercariae in the different organs. There were neither dead parasites nor cellular reactions around the living ones (Abou EL Naga, 2011). While Resistant snail parents when exposed to miracidium showed diffuse cellular infiltration with phagocytosis, granuloma formation, haemocyte rich nodules and focal thickening of the stroma and the absence of viable parasites and the presence of remnants of dead forms (Abdel Aal, 2016). The presence of common antigenic fractions between snail host tissues and Schistosomes directed many researchers to focus their work on the vaccination of the final host of *S. mansoni* with the constituents of its intermediate host (snails); these constituents include protein, nucleoprotein, lipid and carbohydrate (Lehr *et al*., 2008). Moreover molecular similarity between Parasite and snail component especially hemolymph cells open anew filed for identification of the suitable snail intermediate host for that of unknown snail IMH

The present study investigated the antigenic relation between *S. mansoni* and *B. alexandrina* foot and visceral hump antigens using SDS PAGE and Western blot technique.

## Materials and Methods

The present study was done from February 2015 to January 2016. The study was assessed and approved by Faculty of Veterinary Medicine Cairo University Ethics Committee and have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. This study was conducted between March to June 2015.

Preparation of *B. alexandrina* crude snail antigens: *B. alexandrina* snails were collected from Abu-Rawash canals at Giza Governorate. The snails were reared in the laboratory for production of laboratory-bred snails. Experimentally infected *B. alexandrina* by *S. mansoni* miracidium were provided by Schistosome Biological Supply Program Unit, Theodor Bilharz Research Institute, and Giza (Smithers and Terry, 1965). Infected and non-infected snails medium to large sized were cleaned, dissected under the dissecting microscope in suitable Petri-dish. After removal of the shell each animal was cut into two parts, a foot and visceral hump and used for antigen preparation (Nabih *et al*, 1989) with some modification. Foot and visceral hump were separated and suspended in l0 ml of 0.01MPBS pH 7.4, homogenized in ice bath (at 4°C) for 20 minutes and sonicated for 5 minutes. The extract was centrifuged at 6000 r.p.m. for one hour at 4°C and the supernatant was collected. Its protein content was measured (Lowry *et al*, 1951) then divided into aliquots and stored at -20°C until used.

*S. mansoni* adult worm crude antigen: *S. mansoni* whole worms were obtained from Schistosome Biological Supply Program Unit (SBSP), Theodor Bilharz Research Institute, Giza, Egypt They collected the worms eight weeks post experimental infection of mice by perfusion with citrated saline and the worms were collected from liver and pre-mesenteric veins (Smithers and Terry, 1965). Adult worm antigen was prepared (Deelder *et al*, 1976). The worms were washed several times and homogenized with (0.01M) phosphate buffered saline (PBS) (pH 7.4), in a homogenizer then sonicated, and separation of the supernatant and its pro-

tein content was measured and preserved until use as before.

Preparation of hyper immune sera in mice: Hyper-immune sera (HIS) were raised versus *S. mansoni* and non-infected *B. alexandrina* foot and visceral hump prepared antigen (Langley and Hillyer, 1989). Three mice for each antigen were bled for negative control sera then injected with 1.2 mg protein for each antigen, mixed in an equal volume of mineral oil subcutaneously. After three weeks, 3 consecutives injections of 0.4 mg protein antigen in equal volume of oil were given intramuscularly at biweekly intervals. A week after the last injection, the blood was collected from the Retro orbital venous plexus behind the eye using a glass capillary tubes, this method was performed under anesthesia by inhalation of ether. Serum separation was obtained by centrifugation at 3000 rpm for 5 min. and stored at -20°C until used.

Electrophoretic fractionation of antigens: The prepared antigens were resolved using 1.5 mm thickness, Sodium dodecyl Sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli, (1970) in 12 % polyacrylamide gel slabs in Tris-glycine buffer, pH 8.3 under reducing conditions. The stacking gel consisted of 5 % acrylamide prepared in 12.5 mM Tris-HCL buffer (pH 6.7) (Sigma chemical Co.). Pre-stained low molecular weight (MW standard was employed (Sigma SDS-100B). The comb was adjusted as one small well for standard and one large for the sample.

Transfer of protein fractions onto nitrocellulose (NC) sheet: Electrophoresis transfer of fractionated proteins onto NC was performed (Towbin *et al,* 1979) using transfer buffer (25 mM tris-base, 192 mM glycine, 20% (v/v) methanol at pH 8.3). Transferring was carried out at 10V, 100 mA overnight at 4°C. The sheet was dried and stored in freezing until use in the form of 0.5 cm longitudinal strips.

Determination of specific protein fractions using EITB: A longitudinal NC strips (15 x 0.5 cm) containing the fractionated antigen were cut out and allowed to react versus known positive and negative control serum samples at 1:100 dilution, 0.5ml of sera/strip via Western-blot assay (EITB) according to Towbin et al. (1979).

Horseradish peroxidase conjugated anti-protein A (Sigma Immunochemicals) was used as conjugate at 1:1000 in 3% BSA/PBS. The used substrate is 4-chloro-1-naphthol. Fractions that react versus reference positive sera and at the same time did not react versus negative control one considered as specific protein fractions.

Statistical analysis: Data were computerized and analyzed using SPSS, version 16.0 (Kirkwood and Sterne, 2003).

## Results

Treatment of fractionated SWAg versus HI mice sera obtained after vaccination of mice by the same Ag using EITB revealed 7kda protein fractions ranged from 35-140 kda (Tab. 1; Pl. 1). Treatment of fractionated *B. alexandrina* infected and non-infected foot and visceral hump Ag versus the previous mice HI sera revealed variable kda fractions at corresponding level to that of SWAg with more bands in infected snail antigens than that in non-infected one. Data revealed common bands between SWAg and non-infected *B. alexandrina* snail antigens. The fraction at 135 kda, 68 kda, could be detected in all cases, while that at 40-42 kda and that at 35 kda was diagnosed in SWAg and that of infected snails only. No cross reacted bands could be detected on treatment of fractionated SWAg versus negative control mice sera (Tab. 1)

By the same way the fraction at 68 kda was react specifically between SWAg and all tested fractionated snail antigens either that of foot or visceral hump when they treated separately by HI mice sera prepared after vaccination of mice by snail foot and visceral hump antigen (Tab. 2; Pls. 2, 3, 4). The fraction at 135 kda was common between SWAg and snail (infected and non-infected) visceral hump antigen. The frac-

tion at the level of 110 kda was diagnosed in SWAg and in non-infected foot antigen after treatment of the NC strips by HIS of mice vaccinated by the snail visceral hump Ag. The fraction at the level of 46-48 kda are common between SWAg and snail foot and visceral hump Ag when treated by HIS of foot vaccinated mice.

Table 1: EITB reactive bands of fractionated *S. mansoni, B. alexandrina* foot and visceral hump versus *S. mansoni* HIS produced after vaccination of mice.

| | Tested fractinated of Ag | | | | | |
|---|---|---|---|---|---|---|
| | *S. mansoni* Ag versus | | *B. alexandrina* foot Ag. | | *B. alexandrina* visceral hump Ag | |
| | HIS | - ve Sera | Non-infected | infected | Non-infected | infected |
| 1 | 140 | | - | - | - | - |
| 2 | 135 | | 135 | 135 | 135 | 135 |
| 3 | 100 | | | - | | - |
| 4 | 68 | | 75-68 | 75-68 | 75-68 | 75 |
| 5 | 52 | | - | 52 | - | 63 |
| 6 | 42-40 | 28 | - | 42-40 | 45 | 48-43 |
| 7 | 35 | 20 | - | 35 | - | 35 |

Table 2: EITB reactive bands of fractionated *S. mansoni, B. alexandrina* foot and visceral hump versus specific HIS produced after vaccination of mice by each antigen

| Tested HIS | NC Strips contained fractionated of Ag | Level of Reacted KD bands | | | | | |
|---|---|---|---|---|---|---|---|
| HIS of mice vaccinated by non-infected snail foot Ag | *S. mansoni* | | | 68 | | 48 | |
| | Foot Ag of non-infected snails | | 100 | 68 | 52 | 46 | |
| | Foot Ag of infected snails | | 100 | 68 | 52 | 48 | |
| | Visceral hump Ag. of non-infected | | 100 | | 52 | 46 | |
| | Visceral hump Ag. of infected snail | | 110 | 68 | | 48 | |
| -Ve sera versus non-infected fractionated snail foot Ag | | | 82 | | | | |
| HIS of mice vaccinated by non-infected snail visceral hump Ag | *S. mansoni* | 135 | 110 | 68-72 | 60-63 | 38 | 32 |
| | Visceral hump Ag. of non-infected | 135 | | 68-72 | | | |
| | Visceral hump Ag. of infected snail | 135 | | 68-72 | | 35 | 32 |
| | Foot Ag of non-infected snails | | 110 | 68-75 | | | |
| | Foot Ag of infected snails | | | 68 | | 35 | |
| -Ve sera versus non infected fractinated visceral hump Ag | | | | | 52 | | 20 |

## Discussion

Schistosomiasis is a major health issue in the tropics and subtropics. The early diagnosis of schistosomiasis is essential for adequate treatment of the acute phase of infection, as the initial symptoms and signs are not pathognomonic and may be neglected leading to chronic disease. Accuracy of serological tests are related to the degree of specificity and sensitivity of used antigens for this reason much researches had been performed to develop sensitive and specific antigens for the diagnosis of schistosomiasis (Van Lieshout *et al*, 2000).

The presence of common antigenic fractions between snail host tissues and schistosomes directed many researchers to focus their work on the vaccination of the final host of *S. mansion* with the constituents of its intermediate host snails (Lehr *et al*, 2008).

The existence of a cellular defense mechanism deployed by molluscs against trema-

tode infection was initially suggested by the finding of histological reactions around parasite sporocysts. Susceptible snail parents showed a normal development of the parasites with wide spread of cercaria in the different organs. There were neither dead parasites nor cellular reactions around the living ones (Abou EL Naga, 2011). Resistant snail parents when exposed to miracidium showed diffuse cellular infiltration with phagocytosis, granuloma formation, hemocyte rich nodules and focal thickening of the stroma and the absence of viable parasites and the presence of remnants of dead forms.The present study demonstrated that detection of similarity in antigenic composition between parasite and its snail IMH can be considered as new tool for identification to snail IMH of new discovered trematode species.

In the present work specific treatment of fractionated SWAg versus HIS using EITB technique, revealed multiple bands in all fractionated antigens. The band number was high in infected snail than that in non-infected one. Five bands were identified in infected foot, while, two bands were only identified in non-infected snail foot. The same was true concerning infected and non-infected snail visceral hump antigens in comparison with SWAg. Presence of high number of similar bands in infected snail with that of SWAg fraction was mainly related to the present S. mansion partheniatea among the infected snail tissue. This was agreed with El-Dafrawy *et al.* (2007) who reported a positive reaction of the haemolymph and the tissue of infected intermediate hosts (*B. alexandrina* and *Bulinus Truncatus*) to *S.mansoni* and *S. haematobium* antigens that was considered an indicator for the presence of a schistosome-antigen in the snails. These results agreed with Attallah *et al*. (1998) who by EITB detected a polypeptide antigen of 74kda molecular weight in antigenic extracts of *S. mansoni* (eggs, cercariae, and adults).

The present results revealed a band of MW at 40-42 kda in fractionated infected foot antigen when reacted against SWAg HIS. A finding nearly similar to a study that reported two *S. mansoni* proteins of 43 & 39 kda (Sm43 and Sm39), which reacted with rabbit antibodies produced against *B. glabrata* proteins. Cross-reactive components were found in fresh water and land snails but not in vertebrate tissues, suggesting that the 39 K Da protein was specific for invertebrates (Dissous and Capron, 1989).

The present study revealed a common band corresponding to MW at 45 KDa between SWAg and visceral hump (both infected and non-infected) of snail when reacted with SWAg HIS. These results agreed with Tarrab-hazadi *et al*. (1998) who reported that 45-kDa subunit was capable of inducing a significant level of protection of mice (30 to 50%) challenged with *S. mansion* infection.

In the present study, there was a common band at 68kda between SWAg and (infected and non-infected) snail foot antigens when reacted with HIS against foot of infected *B. alexandrina*. These results agreed with Rupple *et al*. (1987) who reported that in chronically infected mice, antibodies against *S. mansoni* SWAg proteins of 67 kda were prominent. Also, a common band at 32 kda was detected in fractionated antigens of both SWAg and visceral hump of infected *B. alexandrina* when reacted with HIS against visceral hump of infected *B. alexandrina*. This agreed with Soliman *et al*. (2003) who used SDS-PAGE to analyze soluble worm antigens, cercarial antigen preparations and soluble egg antigens of *S. mansoni* and found that the shared polypeptide of the immature stages of *S. mansoni* was 32 kda under a reducing condition.

Also, Noya *et al.* (2003) recognized the 32 kda native proteins from the SWAg by WB using EITB technique on SWAg of *S. bovis*. Fractionated antigens of SWAg and visceral hump of (infected and non-infected) *B. alexandrina* snail showed antigenically active bands using EITB when reacted with HIS against visceral hump of non-infected *B. al-*

*exandrina*. The variations in our results and other similar studies concerning the identified bands may be due to differences in the antigen preparations, different technique, use of different concentrations of the resolving gel and protein concentrations in antigens of *S. mansoni* and snails.

In the presence study of antigenic relationship between *S. mansoni* and *B. alexandrina* agreed with Theron and Coustau (2005) they found that interactions between *B. glabrata* and *S. mansoni* was characterized by a compatibility polymorphism with a specific snail strain resistant to a specific *S. mansion* strain but susceptible to another strain. This was responsible for the antigenic cross-reactivity (Schmitt *et al*, 2002). Hamed (2010) reported that the nucleoprotein of susceptible snails showed reduction in worm and ova counts by 70.96% & 51.31%, res- pectively, whereas the nucleoprotein of resistant snails showed reductions of 9.67% & 16.77%, respectively. This agreed with the present result that as fractionated infective snail was more reactive than fractionated non infective snail with HIS against SWAg.

Numerous genetic and physiological factors in snail and parasite were critical for determining interaction between *S. mansoni* and *B. alexandria* due to snail' internal defense system (Abou El Naga *et al*, 2010).

Generally speaking, schistosomiasis remains the truly neglected tropical disease caused by blood flukes of the genus *Schistosoma*, with the three species *S. mansoni*, *S. haematobium*, and *S. japonicum* responsible for the majority of human infections (Colley and Secor, 2014). Recent reports of WHO (2015) suggested that more than 249 million people have been infected in 78 countries where the disease is endemic, located in sub-Saharan Africa, the Middle East, the Caribbean, and South America resulting in approximately 200,000 deaths annually.

In Egypt, both *S. mansoni* and *S. haematobium* species are endemic. The number of infections due to *S. mansoni* exceeds that of *S. haematobium* due to ecological changes influenced by the shift in irrigation system from basin to perennial following the construction of the Aswan High Dam (Elmorshedy *et al,* 2016). In the Egyptian hyperendemic foci of *S. mansoni* infection, the prevalence even approaches 70% and the percentage of heavily infected individuals, i.e. those excreting more than 400 eggs per gram (EPG) of feces accounts for 20% of those infected (Barakat, 2013).

## Conclusion

The outcome data support the hypnosis that the antigenic community between *S. mansoni* and *B. alexandrina*, through exhibition of common antigenic epitopes with its intermediate host. SWAg against HIS was more reactive to infected snail than non-infected snail by using WB.

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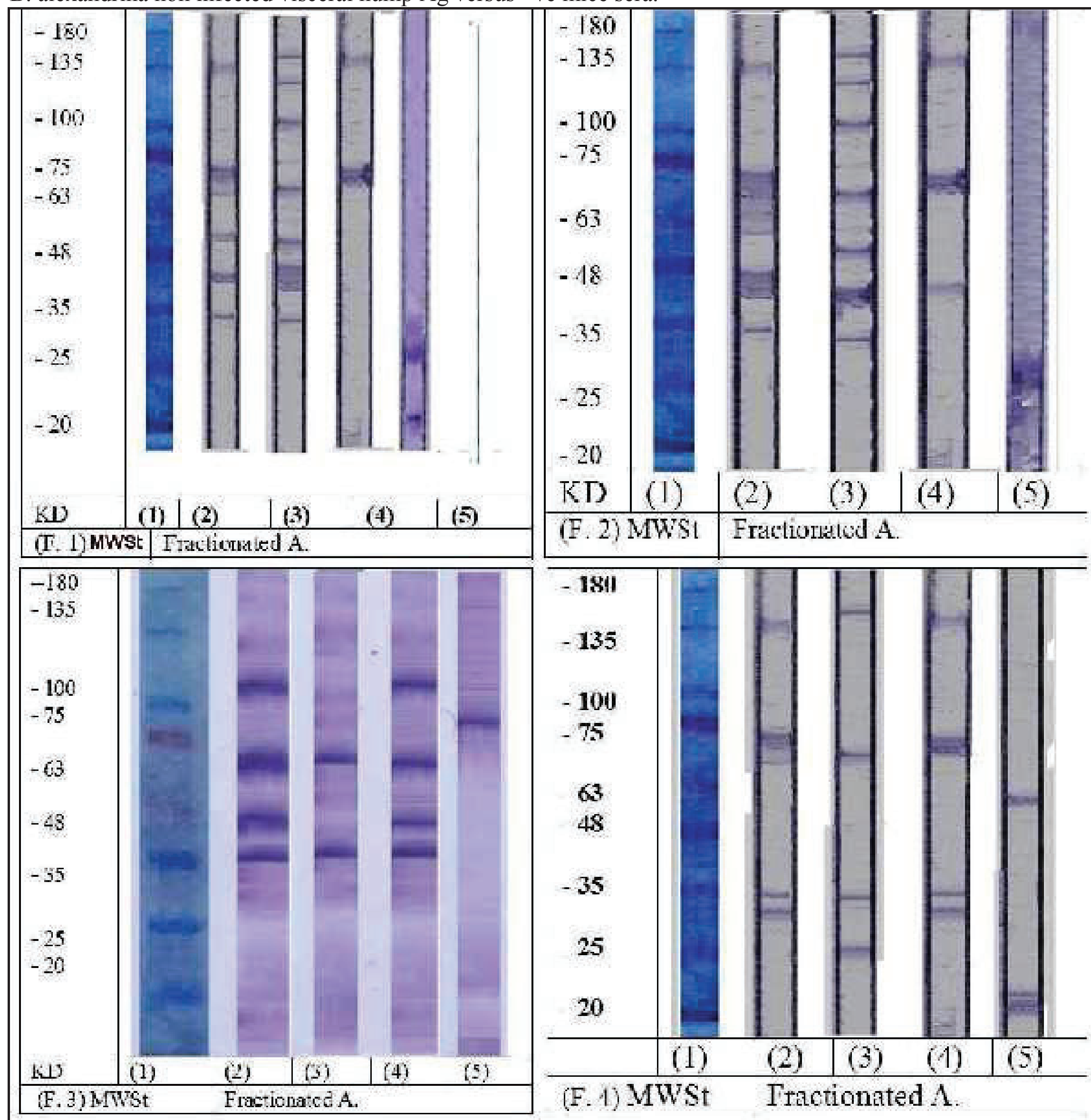
## Explanation of figures

Fig.1: Specific protein fractions recorded after treatment versus *S. mansoni* HIS using EITB technique: Lane (1) MWS, Lane (2) *B. alexandrina* infected foot antigens. Lane (3) *S.mansoni* fractionated antigens, Lane (4) *B. alexandrina* non-infected foot antigens, Lane (5) *S. mansoni* Ag versus –ve mice sera.

Fig. 2: Specific protein fractions recorded after treatment of different fractionated snail visceral hump antigens versus *S. mansoni* HIS using EITB technique: Lane (1) MWS, Lane (2) *B. alexandrina* infected Visceral hump antigen, Lane (3) *S.mansoni* fractionate antigen, Lane (4) *B. alexandrina* non-infected Visceral hump antigens, Lane (5) *S. mansoni* Ag versus –ve mice sera.

Fig. 3: Specific protein fractions recorded after treatment of different fractionated antigens versus *B. alexandrina* foot HIS using EITB technique: Lane (1) MWS, Lane (2) *B. alexandrina* infected foot antigen, Lane (3) *S.mansoni* fractionate antigen, Lane (4) *B. alexandrina* non-infected foot antigens, Lane (5*) B.alexandrina* infected foot Ag versus –ve mice sera.

Fig. 4: Specific fractions recorded after treatment of different antigens versus non-infected *B. alexandrina* Visceral hump HIS using EITB technique: Lane (1) MWS, Lane (2) *B. alexandrina* infected visceral hump antigen, Lane (3) *S.mansoni* fractionate antigen, Lane (4) *B. alexandrina* non-infected visceral hump antigens, Lane (5) B. alexandrina non infected visceral hump Ag versus –ve mice sera.



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# CHARACTERIZATION OF THE HEMOCYTES OF SUSCEPTIBLE AND RESISTANT *BIOMPHALARIA ALEXANDRINA* SNAIL

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## Abstract

The internal defense system consists of soluble components of hemolymph and circulating cells known as hemocytes. The circulating hemocytes play a central role in innate immunity. This work aimed to study the hemocytes of both susceptible and resistant *B. alexandrina* snails exposed to *S. mansoni* infection using light and electron microscopes. Two tested groups were included in the study; 60 susceptible and 60 resistant *B. alexandrina* snails. Both tested groups were studied as regad the hemocyte count (before and after infection) and the morphological characteristics of both circulating and tissue hemocytes by light and electron microscopes. Before infection, there was no significant difference between the two groups as regard the hemocyte count, however after infection, there is a significant decrease in the circulating hemocytes of the resistant group. Light microscopy revealed five morphological types of circulating cells of both susceptible and resistant snails. Regarding scannig electron microscopy, hemocytes of susceptible snails appeared rounded with smooth or slightly rough surface. However, that of the resistant snails appeared irregular in shaped with corrugated surface. Furthermore, Light microscopy and the transmission electron microscopy revealed signs of cell activation in the hemocytes of the resistant group. The circulating hemocytes consist of five cell types in both susceptible and resistant *B. alexandarina* and morphologies of these cells are quite similar, but with more signs of cell activations in the resistant group. More specific studies on the functional activities of the hemocytes and mechanisms that may affect or influence the susceptibility and/or non-susceptibility of molluscs to invade microorganisms is essential and how they can act in the immune response.

**Keywords:** Egypt, Hemocytes, Susceptibility, Immune response, Scanning electron microscopy, Transmission electron microscopy.

## Introduction

*Schistosoma mansoni* has a complicated life cycle involving freshwater snail intermediate hosts. Fresh water pulmonate snails of the genus *Biomphalaria* act as intermediate hosts of the widely distributed parasite, *S. mansoni* (Mossalem and Mossa, 2014). *Schistosoma* miracidia that penetrate *Biomphalaria* may be destroyed within hours or produce infections that yield human-infective cercariae several weeks later (Abou-El-Naga and Radwan, 2012). The internal defense system (IDS) is one of the factors that influence the susceptibility pattern of the snails. It consists of both cellular and humoral components and circulating hemocytes are the principle defense line of cellular defense. The susceptibility of *Biomphalaria* to infection by *S. mansoni* is regulated by the hemocytes present in the hemolymph, while the humoral component includes lysosomal enzymes helps in recognition of penetrating pathogens (Helal *et al*, 2014). The host-parasite interactions represent a major challenge in biology and control methods against *Schistosoma* in intermediate snail hosts (Abou-El-Naga and Radwan, 2012). The snail-parasite interaction is very important to understand mechanisms by which snails resist parasite infection (El-Ansary and Al-Daihan, 2006).

This work was designed to study the circulating and tissue hemocytes of both susceptible and resistant *B. alexandrina* snails exposed to *S. mansoni* infection as regard their count and morphological features using light and electron microscopes owing to find an opportunity to break the life cycle of man