Interspecific variations of *Biomphalaria alexandrina* and *Biomphalaria glabrata* snails in the presence and absence of *Schistosoma mansoni* by using of protein profiles.

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ABSTRACT

Schistosomiasis, the most important parasitic disease in Egypt, has plagued its people since ancient times. Two species of *Biomphalaria* are reported from Egypt, the indigenous *Biomphalaria alexandrina* and *Biomphalaria glabrata*, the latter is believed to be introduced during the past few decades. Both are known to be excellent hosts of *Schistosoma mansoni*, in Egypt. SDS-PAGE was used to separate tissue proteins of control and *Schistosoma*-infected *Biomphalaria alexandrina* and *B.glabrata* snails. Also total protein of these groups was measured using Bradford assay method. The present data showed that there is a variation in the protein profiles under the effect of infection, and the days of infections can also affect total and protein profiles pattern. There was a significant decrease in *B.alexandrina* total protein, in contrary, total protein of *B.glabrata* groups exhibited significant and insignificant increase under the effect of infection. The electrophoretic pattern showed that there is an interspecific variation between *B.alexandrina* and *B. glabrata* control (non-infected) and infected ones. Protein profiles showed 13 bands ranged between 15-300 KDa, with a unique bands to some groups. The similarity indices showed the high value (0.8) between the two Biomphalarian species.

This study is an attempt to specifiy characterization of similar species of animals using modern simple technique rather than morphological old methods, and further detects the variable protein bands due to infection with *S.mansoni*.

Keywords: Interspecific; *Biomphalaria alexandrina* ;*Biomphalaria glabrata*; protein profile.

INTRODUCTION

Parasitic platyhelminthic infections such as schistosomiasis affect humans in many countries of the western hemisphere, the pacific region, the Middle East, Asia and Africa. The reduction of transmission levels has been attempted in numerous ways, including mass chemotherapy and the application of molluscicides to restrict the spread of infection. It has long been recognized that control strategies aimed at the long term reduction of schistosomiasis cannot ignore the role of snails in its transmission. Preventing the spread of this disease in the long term has been difficult to sustain because of the tendency of snails for recolonization, the lack of surveillance of treated sites, extreme poverty, civil strife and the expansion of irrigation projects in high-risk regions of Sub-Saharan Africa and the Pacific Rim (Sturrock, 2001).

There are 34 described species of *Biomphalaria* snails, of which about 18 were known to be or presumed to be capable of supporting the complete larval development of ***Schistosoma mansoni*** (Malek, 1985; Brown, 1994). Two species of *Biomphalaria* were reported from Egypt, the indigenous ***Biomphalaria alexandrina*** and ***Biomphalaria glabrata***, the latter is believed to be introduced during the past few decades, which is an ecologically successful and highly adapted member of the class Gastropoda (Hickman, 2000). Both snails are known to be excellent hosts of ***S.mansoni***, the human infecting blood fluke common in Egypt.

In order to differentiate between ***B.glabrata*** and ***B.alexandrina*** in Egypt, Yousif *et al*. (1996) used shell morphology and morphometry, presence or absence of the renal ridges, and certain anatomical features of the pallial cavity and radular teeth. While, Lotfy *et al*. (2005) and Bakry (2009) differentiate between ***B.glabrata*** and ***B.alexandrina*** using PCR assays based on nuclear sequences.

Snails like other invertebrates have efficient innate immune systems which is one of the potential approaches for controlling shistosomiasis development and transmission. This depends on an in-depth understanding of the molecular interaction between schistosomes and snails (Zhang *et al.*, 2008). Snails have potent internal defence systems and penetrating parasites are frequently eliminated (Van der Knaap & Loker, 1990; Yoshino & Vasta, 1990). To survive within their hosts, parasites have developed means to evade or interfere with snail internal defence responses. Digeneans can be used as tools to study snail immunobiology because of their diverse abilities to influence snail host internal defences. *Biomphalaria glabrata* responds to infection with *Echinostoma paraensei* by producing increased quantities of plasma polypeptides with the properties of lectins. Molluscan lectins are likely to mediate non-self recognition by binding and opsonizing foreign particles (Fryer *et al.*, 1989; Richards&Renwrantz, 1991).

So this study, aimed to **firstly**; differentiate between the two species of *Biomphalaria* using simple, cheap and modern tool of protein profile, rather than traditional and difficult morphology method or expensive PCR method. **Secondly**; comparative study to the response of both *B.alexandrina* and *B.glabrata* to *Schistosma mansoni* infection 7 and 14 days post exposure using total protein and protein profiles by SDS-PAGE.

2. Materials and methods

1. Biological Materials:

Control and infected Biomphalaria alexandrina and glabrata snails were purchased from Theodor Bilharz Reseach Institute (TBRI), Egypt. Six identical groups (5-7mm) of Biomphalaria spp (20 snails/ group) .The first and the second one are control snails of both Biomphalaria alexandrina and glabrata respectively. The third and fourth groups, are B.alexandrina and glabrata 7- days post exposure(7 PE.) to Schistosoma mansoni miracidia, while, the fifth and sixth groups are B.alexandrina and glabrata 14-days post exposure to S.mansoni miracidia(14 PE.) respectively. Snails from each group were transferred to clean aquaria with declorinated tap water. They were daily fed boi+led lettuce leaves. Dead snails were daily removed from the aquaria. The control and infected snail groups were collected after seven and fourteen days post exposure and kept at 4◦C until use.

2.2. Protein assay:

Protein contents measured by Bradford protein assay. Total protein concentration in tissue snail groups compared to a protein standard using spectrophotometer at 595 nm (Bradford, 1976).

2.3.Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE):

SDS-PAGE (12% gel) analysis of control and infected snails tissue and their protein bands were carried out according to Laemmli (1970). Proteins were stained with 0.1% Coomassie Blue R-250 Silver. Broad range molecular weights marker (Promega, V8491) was run in parallel in order to calculate the molecular weights of proteins. Then, gel was photographed and the molecular weights were calculated using Molecular Imaging Software (MIS, Kodak).

2.3. Similarity matrix:

A similarity matrix was constructed on the basis of the presence / absence of bands from Dice's similarity coefficient (Dice, 1945) using the formula:

S= 2a/2a+b+c

Where a = number of bands shared between samples 1 and 2, b= the number of bands present in 1 but not in 2 and c = number of bands present in 2 but not in one.

2.4. Statistical methods:

The independent t-test was used to distinguish the significant difference between snail groups on the means of total protein concentrations in uninfected (control) and infected snails.

Results

Total protein contents:

Figure (1) and Table (1) showed the total protein concentration of the tissue of control and infected B.alexandrina and B.glabrata snail groups. The total protein of B.alexandrina control group was 10 mg/ml. While, total protein of B.glabrata control group was 3 mg/ml. There was a significant decrease (P=0.003 & 0.001) in total protein contents (6 & 4 mg/ml) respectively in B.alexandrina after 7 days & 14 days of exposure to S.mansoni miracidia (7 PE B.alexandrina. and 14 PE B.alexandrina) relative to control one (10 mg/ml). While, there is a significant increase (P= 0.002) of total protein (7.7 mg/ml) in B.glabrata 7 days after exposure to S.mansoni (7 PE. B.glabrata) and insignificant increase (P=0.065) of total protein (4.7 mg/ml) in B.glabrata 7 days after exposure to S.mansoni (14 PE. B.glabrata) relative to control one (3 mg/ml).

Table (1): Total protein contents (mg/ml) of different groups of control and infected Biomphalaria snails. * Significant at P≤ 0.05:

Snail groups	Total protein concentration (mg/ml)
Biomphalaria alexandrina control (B.a.clt)	10 mg/ml ± 0.2
Biomphalaria glabrata control (B.g.clt)	3 mg/ml ± 0.4*
Biomphalaria alexandrina 7 days post exposure (7 PE.)	6 mg/ml ± 0.1*
Biomphalaria glabrata 7 days post exposure (7 PE.)	7.7 mg/ml ±0.14
Biomphalaria alexandrina 14 days post exposure (14 PE.)	4 mg/ml ±0.05*
Biomphalaria glabrata 14 days post exposure (14 PE.)	4.7 mg/ml ± 0.43

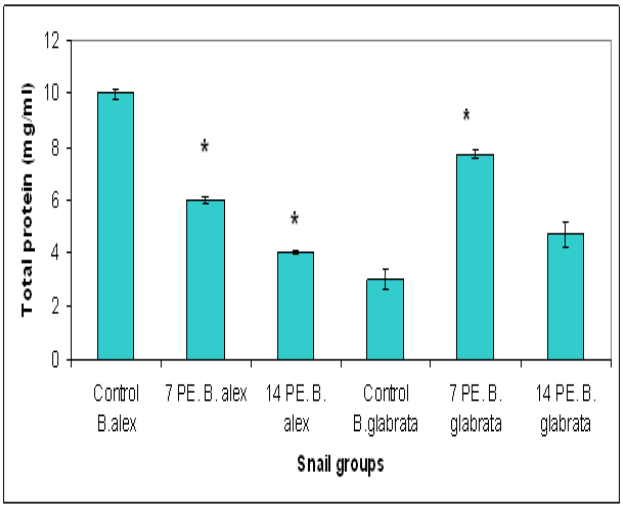


Figure (1); protein content (mg/ml) in *Biomphalaria alexandrina* control (control B.alex) and infected groups, control *B.glabrata* and infected groups (7 &14 days post exposure to *S.mansoni. Significant P≤ 0.05.**

Protein profiles by SDS-PAGE:

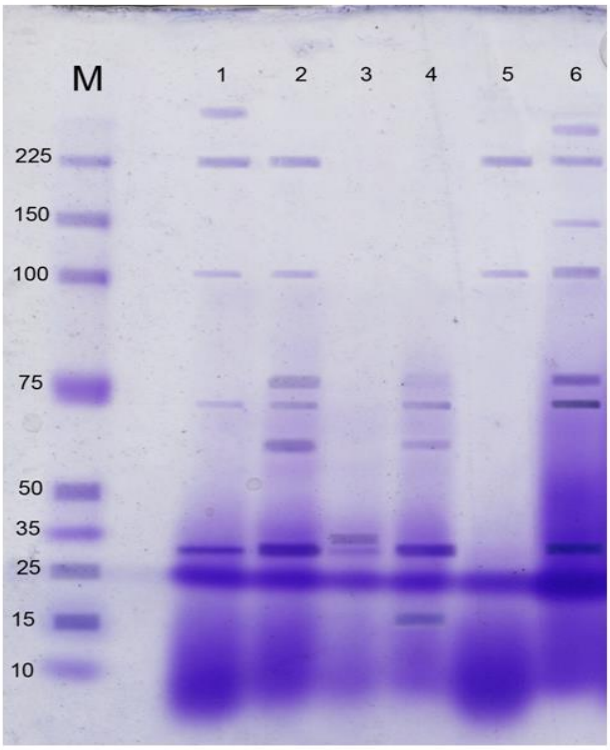
The protein profile pattern identified by SDS-PAGE electrophoresis for the infected and non-infected groups is shown in Table 2 and Figure 2. The results indicated that the protein profiles of different groups exhibited a complex pattern of polypeptides with a total number of 13 bands have molecular weights ranged from 15 to 300 KDa. The results also pointed out that the band with molecular weight of 25 KDa is the most common in all groups. 300 KDa protein band is the specific band to *B.alexandrina* control (non-infected) snail. While 75 and 65 KDa protein bands are specific bands to *B.glabrata* control (non-infected) snails. 275 and 150 KDa protein bands are occasionally appeared in *B.glabrata* 14 days

post-exposure (PE.) to *S.mansoni* miracidia. Whereas, low molecular weight protein bands of 35 and 15 KDa appeared in both *B.alexandrina* and *B.glabrata* only at 7 days post-exposure (7 PE.) to *S.mansoni* miracidia respectively.

Table (2): Protein profile of *Biomphalaria alexandrina* and *B.glabrata* control and infected snails.

Band no.	Control *B.alexandrina*	Control *B.glabrata*	*B.alexandrina* 7days PE.	*B.glabrata* 7days PE.	*B.alexandrina* 14days PE.	*B.glabrata* 14days PE.
300	+++					
275						+++
225	+++	+++			+++	+++
180	+++	+++	+++	+++		
150						+++
100	+++	+++			+++	+++
75		+++		+++		+++
70	+++	+++		+++		+++
65		+++		+++		
35			+++			
30	+++	+++	+++	+++		+++
25	+++	+++	+++	+++	+++	+++
15				+++		
Total protein bands	7	8	4	7	3	8

Figure (2): protein profile of snail groups. M: protein marker, lane 1: control *B.alexandrina*, lane 2: control B.glabrata, lane 3: 7 days *Biomphalaria alexandrina* post exposure to S.mansoni, lane 4: 14 days *Biomphalaria alexandrina* post exposure to *S.mansoni*, lane 5: 7 days *Biomphalaria glabrata* post exposure to *S.mansoni*, lane 6: 14 days *Biomphalaria glabrata* post exposure to *S.mansoni.*



Similarity matrix:

The similarity coefficient "S" based on the number of protein profiles separated by SDS-PAGE, shown in Table (3), revealed that there is a high similarity between the two non-infected snail species of which exhibited '0.8'similarity. The similarities between control *B. alexandrina* group and its two exposed ones at 7 & 14 days PE are 0.5 & o.6 respectively. The similarities between control *B.glabrata* group and its two exposed ones exhibited higher value (S= 0.8 & 0.75) than those of *B.alexandrina*.

Table (3): Similarity matrix of different electropherograms of protein subunits of different *Biomphalaria* groups:

Snail groups	1	2	3	4	5	6
1	1					
2	0.8	1				
3	0.5	0.5	1			
4	0.57	0.8	0.5	1		
5	0.6	0.5	0.4	0.2	1	
6	0.6	0.75	0.36	0.5	0.5	1

Discussion

Preliminary observations on tissue polypeptide pattern indicated the differences between infected and control snails by both qualitative and quantitative techniques. The total protein contents of *B.alexandrina* generally decreased significantly as a result of infection. While, the total protein contents of *B.glabrata* increased due to infection compared with control group. Loker and Hertel (1987), revealed a significant increase in total protein in the plasma of *B.glabrata* infected for 4 and 8 days with *Echinostoma paraensei*. Previous studies on *B.glabrata* infected with *Schistosoma mansoni* indicated that after 11-14 days of infection, the plasma protein content of infected snails is significantly reduced (Lee and Cheng, 1972; Gress and Cheng, 1973; Michelson and Dubois, 1975; Stanislawski and Becker, 1979). So, those previous studies are tolerant with the present study of infected *B.alexandrina*. While, total protein of infected *B.glabrata* in the present study is coordinated with the results of Loker and Hertel (1987). *Schistosoma mansoni* therefore seems to evoke different response in different hosts as *B.alexandrina* or *B.glabrata*.

The electrophoretic pattern by SDS-PAGE revealed that, there were 13 different protein bands with molecular weights ranged from 15-300 KDa. Each group has a specific band, *B.alexandrina* has a specific band with 300 KDa, whereas, *B.glabrata* has a specific bands with molecular weights of 75 and 65 KDa. A band with molecular weight of 180 KDa in the present study considered to be hemoglobin similar to that described previously by Loker and Hertel (1987). In contrary, Granath et al. (1987) described this band with molecular weight of 160 KDa. This band with 180 KDa diffused completely in 14 days postexposure to parasite in both *Biomphalaria* species, this may be due to consumption of hemoglobin by parasite after prolonged exposure to

S.mansoni. Uchikawa and Loker (1992), designed a group of molecules G1M (200 KDa) and G2M (80-120 KDa) as a response to infection with Echinostoma paraensei. But in the present study there were only two bands (275 and 150 KDa) appeared in B.glabrata after prolonged exposure (14 days) to parasite. A band with molecular weight of 65 KDa appeared in both control and 7days PE. B.glabrata and disappeared in 14 days PE.

Adema et al. (1997 a & b) demonstrated that 65 KDa band appeared in infected B.glabrata, and this band was strongly bound by anti-fibrinogenes antibodies, comprised of at least two members of the fibrinogene related protein (FREPs) and responsible for the internal defence of the snail. Also FREPs have been identified by Stout et al. (2008). These apparent bands indicate the presence of immune polypeptides that bind to protein of S.mansoni miracidia and sporocyst and ensured by the study of previous authors who demonstrated that FREPs, G1M, and G2M may agglutinate different types of erthrocytes and other particulate materials or bind protein of echinostome miracidia and sporocyst both in vivo and in vitro (Hertel et al., 1994 and Locker et al., 1994). The 35 and 15 KDa protein bands of the present study appeared in B.alexandrina and B.glabrata only 7 days PE respectively.

According to similarity matrix in the present study, there is an obvious high similarity index (S=0.8) between the two species of Biomphalaria, despite of their morphological and molecular differences. Also higher values of similarity indices (S= 0.8 & 0.75) obtained between all the groups of B.glabrata compared to control. However, moderate values of similarity (S= 0.5 & o.6) obtained between B.alexandrina groups compared to control. El- Dafrawy et al. (2006) demonstrated the highest similarity index 0.667, in 2 weeks and 5 weeks post exposure to S.mansoni miracidia groups of B. alexandrina and the lowest one was in 3-days post exposure (0.5) compared to control group.

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الملخص العربي

"ألاختلافات النوعيه بين قوقع بيومفلاريا الكسندرينا و بيومفلاريا جلابراتا في وجود و غياب البلهارسيا المعويه باستخدام تحليل البروتين."

تعتبر البلهارسيا من أكثر الامراض الشائعه في مصر منذ قديم الاذل. و قد تم التعرف علي نوعين من القواقع هما بيومفلاريا الكسندرينا و بيومفلاريا جلابراتا و التي تعتبر من انجح العوائل الوسيطه في مصر. في هذة الدراسه تم قياس كلا من البروتين الكلي بأستخدام طريقة برادفورد، و قياس الحمل الكهربي لفصل البروتينات بأستخدام صوديوم دودوسيل سلفات بولي أكريلاميد جل في أنسجة المجموعات الغير مصابه و المصابه بالبلهارسيا المعويه لكل من قوقع بيومفلاريا الكسنرينا و جلابراتا. و قد أظهرت النتائج ان العدوي و ايام الاصابه يمكن ان تؤثر علي التحليل الكمي و النوعي للبروتين. حيث وجد انخفاض معنوى ملحوظ في نسبة البروتين الكلي في قوقع بيومفلاريا الكسندرينا نتيجة الاصابه بالبلهارسيا المعويه، علي عكس ما حدث في قوقع بيومفلاريا جلا براتا حيث ظهر زياده في نسبة البروتين الكلي تحت تأثير العدوي. أوضح قياس الجهد الكهربي للبروتين ان هناك اختلافات بين النوعين من القواقع سواء المصابه او الغير مصابه بالبلهارسيا المعويه. حيث وجد عدد ١٣ شريط من البروتينات لها اوزان تتراوح بين ١٥-٣٠٠ كيلو دالتون، مع وجود أشرطه مميزه لكل مجموعه. و قد اوضحت مؤشرات التشابه ان اعلي نسبة تشابه٠,٨ هي بين مجموعات بيومفلاريا الكسندرينا و بيومفلاريا جلابراتا الغير مصابه.

تعتبر هذه الدراسه هي محاوله لتعريف الانواع المتشابهه من الحيوانات باستخدام تقنيات حديثه و بسيطه بدلا من الطرق القديمه التي تعتمد علي الشكل الظاهري فقط. و ايضا اكتشفت الدراسه تنوع في اشرطة البروتين نتيجة الاصابه بالبلهارسيا المعويه.