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Assessment of *Bulinus truncatus* Immune Response *Against Schistosoma haematobium* Infection by Tissue Reaction and Hemocytes Count.

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ABSTRACT

Bulinus truncatus is the main intermediate host for *Schistosoma haematobium* in Egypt. The fates of *Schistosoma* miracidia in the snails varies between different populations of *B.truncatus*. The internal defense system is one of the factors that influence the susceptibility pattern of the snails. The interaction between *Bulinus* snail and *S. haematobium* need to be identified for each population, and even between the members of the same population with different degrees of susceptibility. In the present study, the first generation of *B. truncatus* collected from Giza and Damietta in addition to Schistosome Biological Supply Center (SBSC) was examined histologically at the 5week post exposure. The study includes the characterization of the immune response, as expressed by tissue reactions and hemocytes count of *B. truncatus* snail against *S.haematobium*. The results showed that the experimental snail groups were classified as follows: moderate susceptibility [SBSC (42.1%) and Damietta (39.4%)] and low susceptibility [Giza (17.85%)]. These results characterized the immune response of *B. truncatus* snail against *Schistosoma* infection which was found to occur by two different mechanisms. The results showed that granulocytes were present in significant greater number in all samples as compared to hyalinocytes ($p<0.001$). Exposure of *B. truncatus* to *S. haematobium* caused gradual increase in the number of circulating hemocytes in the three experimental groups. The variations in the rates of infection and immune response of different *B. truncatus* groups with *S. haematobium*, are dependent on collection site of the snails. Introduction of this variability into endemic areas may reduce the ability of the parasite to infect local hosts and consequently reduce schistosomiasis epidemiology.

Keywords: *Bulinus truncatus*, *Schistosoma haematobium*, susceptibility, immune response, hemocyte, histopathology.

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INTRODUCTION

Schistosomiasis, is the most important parasitic disease in Egypt, has plagued its people since ancient times [1-4]. Geographical distribution of intestinal schistosomiasis is directly associated with the presence of susceptible freshwater snails of genus *Biomphalaria* with etiological agent, *S. mansoni* and genus *Bulinus* with the etiological agent, *S. haematobium*. These trematodes are stenoxenic parasites, i.e., it uses specific intermediate host species. However, not all *Bulinus* species are susceptible to *S. haematobium,* it varies among snails according to different ages, genetic variation, immune system status and geographic areas in which both snails and the trematode parasite were occurred [5-7].*B. truncatus* is the main intermediate host of *S. haematobium* in Egypt [8].

As typical invertebrates, molluscs possess a well-developed internal immunodefense system [9], in which phagocytosis is the main innate process by which the organism can protect itself against invading microorganism and discriminate between self and non-self. The principal line of cellular defense against both externally and internally generated injury is phagocytosis or encapsulation involving phagocytic cells [10]. The internal defense system (IDS) of snails is composed of soluble components of hemolymph and circulating cells, termed hemocytes, which work in association during the snail responses against infectious agents [11].Circulating hemocytes in molluscs represent the primary effector component involved in the destruction and elimination of metazoan parasites [12-14]. However, the parasite can escape the IDS.

The distribution of parasites among hosts is the result of interaction between numerous factors including genetic, biological, behavioral, and ecological processes [15]. During the life cycle of trematodes, parasites need to penetrate into this host, develop, multiply asexually and finally leave the host to continue their life cycle [16].

The circulatory system of gastropod molluscs are opened, and the hemolymph bathes the organs and supplies tissues by means of network of sinsoids. Consequently, the intramollus can stages of trematode parasites develop in the tissues an intimate association with the hemolymph of their host snails [17]. Understanding the host-parasite interactions represents a major challenge in biology and may help in finding a novel control method against *Schistosoma* in different snail species. Differences in the immune responses were represented by different species of *Biomphalaria* against *Schistosoma* infection and even between members of the same species with different degrees of susceptibility [18-25]. So, the mechanisms involved in these interactions need to be specified for each species.

In *Bulinus truncatus rohlfsi* previous studies have generally shown two types of hemocytes: granuolocytes and hyalinocytes [26].Granulocytes spread rapidly on glass, constituting 90% of the hemocytes population and are directly involved in phagocytosis, having different cell organelles with numerous lysosome-like structures containing digestive enzymes. The other types of hemocytes, hyalinocytes, attach to and not spread on glass. The present work was undertaken to study susceptibility and characterization of the immune response of *B. truncatus* snails from different Egyptian localities to *S. haematobium* infection.

MATERIALS AND METHODS

Collection of snails: *Schistosoma haematobium* snails were found in large number in small dishes and several minor snails at small depth and were scanty in big canals. On the other hand, *Bulinus* snails were found in main stream and canals. Populations of *Bulinus* snail were collected from two Egyptian Governorates (Giza and Damietta) [27] and the 3rd group lab bred strain was obtained from Schistosome Biological Supply Center at Theodor Bilharz Research Institute (SBSC-TBRI).

Snails breeding: Snails were allowed to lay egg masses on small foam pieces placing on the water surface of aquaria in dechlorinated tap water under laboratory conditions [28]. The egg masses of adult *Bulinus* snails were collected, relocated and transferred to smaller plastic aquaria of one liter capacity containing dechlorinated tap water at room temperature. Hatching snails were transferred with a fine brush to another aquarium where they were feeding on blue green algae, mainly *Nostoc muscorm* and aseptic soil until they reach to 5 mm shell height then feeding on lettuce leaves and Tetramin (Fish food). Water in aquaria was continuously changed, a photoperiodicity of 12hr. light/12 hr. dark.

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Parasites: The Egyptian strain of *Schistosoma haematobium*, applied by the SBSC (TBRI, Giza) was used in this study. Eggs and freshly hatched miracidia were collected from intestine of ten Syrian golden hamsters *Mesocricetus auratus* (18-21 g) infected 6-8 weeks earlier with 300 *S. haematobium* cercariae[29]. About 200 ml of 0.85 % saline-solution were added to the minced tissue and the suspension was homogenized for 5-10 sec at very low speed using a warring blender. Homogenate was sieved using a tiered column of sieves arranged in descending order of mesh opening (420 μm, 177 μm, 105 μm and 45 μm). The eggs were washed through bottom sieve with 100 ml of 0.85 % saline solution and rinsed with 100 ml of aerated tap water. The eggs were pipette into a small 1.5 x 6 cm petri dish (Nunc) and kept under ceiling illumination for about 5 min for hatching.

Snail exposure and infection: The snails from each group were reared in the laboratory and their first generations (F1) were used throughout the infection experiment.50 lab-bred *Bulinus* snails (4-6mm in shell length) from each Governorate offspring as well as snail group from SBSC were exposed individually to 10 newly hatched *S. haematobium* miracidia. After exposure, groups of snails were maintained communally in plastic tray (20 x 30 cm) containing 1.5 liters of dechlorinated tap water, supplied with lettuce leaves and *Nostoc* algae. Starting from 4 week post-exposure (WEP), the snails were examined individually once a week for cercarial shedding. They put in multi wells containing 2 ml of dechlorinated tape water/ snail under artificial light for two hours (stimulated period). After initial shedding was observed, snails were screened individually once weekly till the death of snails. Survival rate, infection rate and cercarial production per snail per week were recorded. The classification of snails susceptibility to infection was dependent on the infection rate of the snail in which snails were considered refractory below 10%, low susceptible 10-25 %, moderate susceptible 25-50% and high susceptible at infection rate over 50%[30].

Light microscopic study: Ten infected snails from each group, together with the three non-infected snails were subjected to histological examination. They were relaxed with menthol crystals (approximately 2 x 10^{-4} M). Each snail was carefully crushed between two microscope slides, and the broken shell was pulled away from the body. The columellar muscle was separated from the shell, and the snail was extracted intact. Snails were fixed in Bouin's fixative for at least 24 hr and then placed in ascending concentrations of ethanol. Hematoxylin-eosin-stained 5-µm sections were examined microscopically for histological condition of larval trematodes [19].

Hemocytes count: The hemolymph was collected from control and experimental groups at 1, 2, 3 and 4 WPE to *S. haematobium* miracidia[31]. Each snail shell was cleaned with 70% alcohol, dried with absorbent tissue paper, and the hemolymph was collected by a cardiac puncture using a 21-gauge needle. It collected from randomly selected 7snails, in each specified group. Total hemocyte count were performed using 10 µL of whole hemolymph diluted (1/10) in CBSS buffer containing 0.5% neutral red solution. A 10% solution of lymph in neutral red was placed in a Naubauer chamber and observed under an optical microscope at 400 magnification. Hemocytes present in 4 randomly selected fields were counted. Hemocytes that stained red were considered granulocytes and those did not stain considered hyalinocytes[32].

RESULTS

Table 1: Classification of snail's susceptibility according to their infection rates

Snail groups	SBSC (No. exposed=50)	Damietta (No. exposed=50)	Giza (No. exposed=50)
Survival rate of snails (%)	76	66	56
Infection rate of snails (%)(	42.1	39.4	17.85
Number of cercariae/ snail/ week (Mean ±S.D)	110 ± 33.16	47.5 ± 20.2	29.66 ± 13.65

The results of experimental infection of the *B. truncatus* snail groups with *S. haematobium* are presented in Table 1. The experimental snail groups were classified according to their infection rate: moderate susceptibility SBSC (42.1%) and Damietta (39.4%) snails with mean number of cercariae/ snail/ week being 110 ± 33.16and 47.5 ± 20.2, respectively for two snail groups. Giza snails showed low susceptibility 17.85% and the mean number of cercariae/ snail/week was 29.66 ± 13.65.

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The infected snails from SBSC group showed a moderate degree of susceptibility. At 5 WPE, live sporocysts were present in a large numbers with normal development (Fig.1A). The sporocysts were widely spread in different organs especially the digestive glands and ovotestis. No cellular reaction was present around the sporocyst(Fig. 1B). Mild generalized diffuse cellular infiltration was present in the tissues and in between the organs.

The moderate susceptible snails from Damietta group showed sporocysts in different organs at 5 WPE. A large number of them were able to complete their development, although some dead ones were present surrounded by cellular aggregations (Fig.1C). Moderate generalized diffuse cellular infiltration was present in the tissues and between the organs. Sometimes granulomata were formed around the remnant of sporocyst. These granulomata were spherical to oval in sections and appear in two forms. The first form is consisted of hemocytes and fibers encircling the dead sporocyst (Fig. 1D),while the second form is consisted of layers of flattened hemocytes and fibers encircling the dead sporocyst and surrounded by layers of un-flattened hemocytes(Fig. 1E).

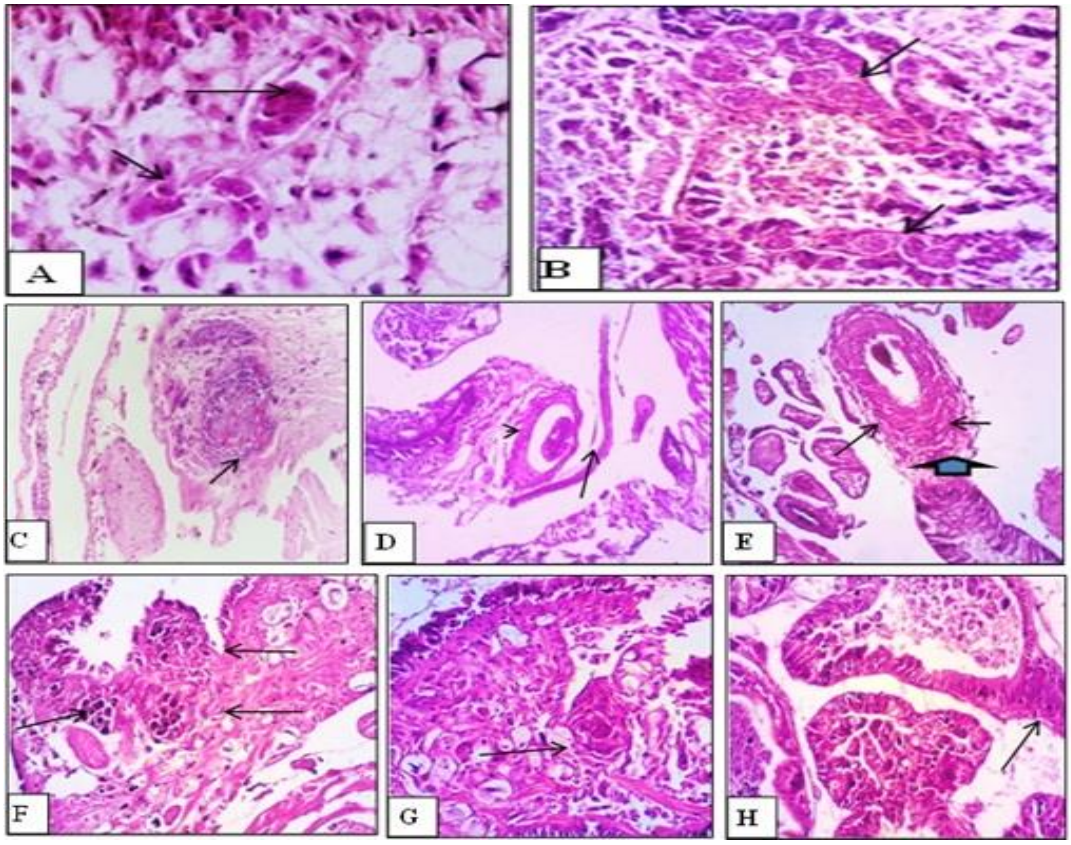

Fig 1(A-H): Histological sections of *B. truncatus* snails infected with *S. haematobium.*
(A)Live *S. haematobium* sporocysts (arrows) with normal development in a moderate susceptible snail.
(B) Developing sporocysts (arrows) between the organs of snail. Note the increase in size of sporocysts and absence of hemocytic response in a moderate susceptible snail.
(C) Dead sporocysts surrounded by hemocytes (arrow) in a moderate susceptible snail from Damietta. (D) Granuloma around a dead sporocyst in a moderate susceptible snail from Damietta and low susceptible snail from Giza consisting of hemocytes (arrow head) and fibers (arrow).
(E)Second type of granuloma around a dead sporocyst in a moderate susceptible snail from Damietta and low susceptible snail from Giza consisting of layers of flattened hemocytes (arrow head) and fibers (arrow) and surrounded by layers of un-flattened hemocytes with no fibers (thick arrow).
(F) Intense diffuse hemocyte aggregation at the penetrating sites of miracidia (arrow) in low susceptible snail from Giza. (G) A loose hemocyte rich nodule (arrow) in the cephalopodal tissues of in low susceptible snail from Giza.
(H) Focal thickening of the stroma among the internal organs (arrow) in low susceptible snail from Giza. X 200.

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In contrast, the snails from Giza group exhibited low susceptibility, and most of sporocysts were dead at 5 WPE. These snails showed an intense diffuse hemocytic reaction at the penetrating sites of miracidia (Fig. 1F). Loose hemocyte rich nodules were detected in the cephalopodal tissues (Fig. 1G). Dead sporocysts were present and appeared as round eosinophilic masses surrounded by several layers of flattened hemocytes. The tissue reaction was in the form of moderate diffuse cellular infiltration in between the organs. The two types of granulomata with the same structure described before surrounding the dead sporocyst were present in between the organs. Hemocyte proliferation with focal thickening of the stroma was present among the digestive glands, the albumin glands and the ovotestis (Fig.1H).

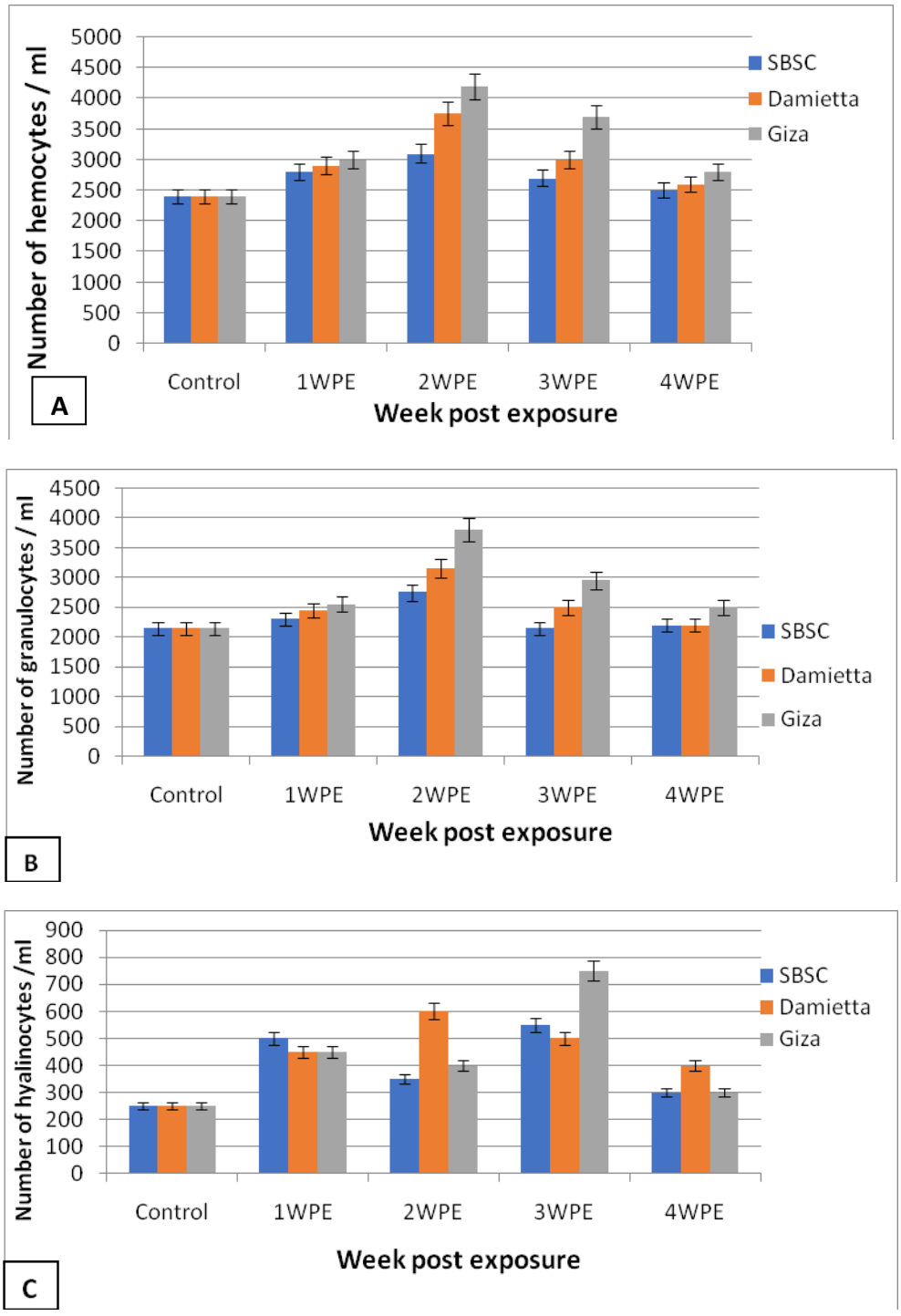

Fig 2: Number of hemocytes (A), granulocytes (B) and hyalinocytes (C) of *B. truncatus* from the different experimental groups after exposure to *S. haematobium* miracidium.

Hemocytes pattern of the snail populations: When comparing the two types of hemocytes in control snail (hyalinocytes and granulocytes) using the unpaired T-test, granulocytes were significantly greater as compared to hyalinocytes ($p<0.001$). Figure (2A) showed that exposure of *B. truncatus* to *S. haematobium* caused gradual increase in the number of circulating hemocytes in the three experimental groups. The increase in number of circulating hemocytes in Damietta and Giza groups became significantly at 1 WPE and 2 WPE, then a gradual decrease occurred. The increasing of hemocytes in Giza group was larger than the two other experimental groups. The number of granulocytes from infected snails of Giza group was significantly elevated than control from 1WPE till 3 WPE (Fig.2B). Indeed, the number of granulocytes in SBSC and Damietta groups increased significantly at 2 WPE, then a gradual decrease occurred (Fig.2B). The number of hyalinocytes from infected snails in the three experimental groups increased as compared with the control. The increase in number of hyalinocytes in Damietta and Giza groups was not statically significant from 1 WPE, but became significant at the 2WPE and 3WPE, then decrease occurred (Fig.2C).

DISCUSSION

Within the genus *Bulinus*, there is a large diversity in the susceptibility of strain to infection by *Schistosoma* parasites. Pathological and genetic aspects of the snail hosts, as well as the genetic feature of the *Schistosoma* parasites, contribute to this diversity as demonstrated in distinct experimental models [33-35]. Until now, most interaction studies have been conducted using *Biomophlaria glabrate- S. mansoni* as an experimental model [36-39; 23; 14]. In addition, there is recent studies have been developed with another important Mollusca host, *B. tenagophila* [40-44; 31]. This experimental model has an important advantage, because the *B. tenagophila* Taim strain is resistant whereas the Cabo Firo strain is susceptible to *S. mansoni* infection, thus it is possible to develop comparative experimental infections within the individual from the same species [43].

After penetration of the snail tissue, *S. haematobium* miracidia undergo morphological and physiological changes, and become primary sporocyst, which then go on to complete their life cycle within the intermediate host. During this process, the sporocysts have to interact with the immune system of the snail connective tissue, including soluble factors and hemocytes. Several authors have seen the presence of an extracellular matrix surrounding and wrapping the sporocysts after penetration of the snail [39; 43; 45].The present study is a comparative histopathological verifying the tissue reactions of *B. truncatus* snail groups from different localities with different degrees of susceptibility to *S. haematobium*. Histopathological examination of the snails obtained from SBSC showed a normal development of the parasites with wide spread of sporocysts in the different organs at 5 WPE. There were neither dead sporocysts nor cellular reactions around the living ones, they could shed high number of cercariae. It appears that there isa tolerance in the snail tissue to the presence, growth and multiplication of sporocystsas [46]. However, Damietta snails could eliminate moderate number of cercariae and some degree of the host tissue reactions was shown by these snails. This was presented by the cellular aggregations surrounding the living sprocysts and by the formation of the granuloma around some dead sporocysts. In spite of the presence of these host reactions, the susceptible *B. truncatus* snail and those of other species were not able to clear the infection [18; 51; 52; 24] Parasitological investigations on different snail species showed within the same species a small number of cercariae were shed from the susceptible offspring because it has a proportion of resistant snails as parents [53;54]. These parasitological results could be clarified by the previous immune reactions in the snail tissues.

The current results also showed a generalized hemocytic infiltration in the moderate susceptible snails from the Damietta group at 5 WPE. This period represents the early phase of enormous colonization of the snail tissue by the cercariae; the extremely pathogenic mobile larvae that are able to do directly ingesting the host tissues. The mechanical and lytic tissue damage caused by the moving cercariae which themselves might be protected by masking the snail antigens might be responsible for this reaction [11; 47]. By this time the hemocytes have been exposed for a long time to non-self and changed self (damaging tissue brought about by getting away cercariae) resulting in a higher state of responsiveness of the hemocytes [10; 48; 18]. At that time, it was guessed that the tissue reactions eliminate the waste products resulting from the parasites, and participate in the healing of the injured tissues [49].

The low susceptible snails from Giza population tested group could eliminate only a few cercariae and showed many forms of tissue reactions against the sporocyst at 5 WPE, including diffuse cellular infiltration with phagocytosis, granuloma formation, hemocyte rich nodules and focal thickening of the stroma.

Phagocytosis was apparent by the presence of remnants of dead forms. It was showed that parasite-amoebocytes contact happened and prompted to phagocytosis and by 48 h only scattered remnants of sporocysts remained [50].

The hemocyte rich nodules are another form of tissue reactions found in this study, mainly in the low susceptible snails and occasionally in the moderate susceptible ones. They were present in the anterior portion of the snails and in between the organs. These nodules were also detected in the strongly resistant *B. glabrata* and *B. tenagophila* [55; 19; 13]

The two types of granulomata formed in the current study around the dead sporocysts were described as type 1 and type 2 granulomata [56].It has been attributed this variability to the individual difference in the host and the parasite[57]. Both types of granulomata appeared in the routinely stained slides as a mixture of hemocytes and fibers. It has been found that the hemocytes in the granuloma had expanded cytoplasmic processes that gave the appearance under light microscope as containing fibers [58].

By observing the tissue reactions of low susceptible snails in this study, the immune responses might be happened by two different mechanisms. One type of defense utilized direct miracidial destruction soon after their penetration. In these snails, the intense hemocytes aggregation and the hemocyte rich nodules were found at the site of the penetration of the miracidia. These reactions may lead to direct miracidial destruction soon after their penetration. In *B. tenagophila*, a diffuse and focal hemocytic infiltration was observed in the cephalopodal tissue of the infected highly resistant snails and found to be associated with rapid parasite destruction after penetration [13]. The second type of the immune reactions demonstrated that the diffuse cellular infiltration, the hemocyte rich nodules and the focal thickening of the stroma were found in the deep tissues. [55; 19] considered these reactions as a delayed development of resistance that happened after spread of sporocysts in the snail tissues. They considered this type of delayed developed resistance represents an alternative sort of host internal defense mechanism against *S. mansoni* miracidia. It happens regardless the evidence suggesting that *S. mansoni* sporocysts can sometimes develop their capacity, in a better way, to interfere with internal defense mechanism of the snail as they grow older [59].

There is a little information about the nature of the factors that determine encapsulation and death of *Schistosoma* sporocysts. It was revealed that encapsulation process occurs in either resistant or susceptible strains, but only results in parasite death in the resistant snail strain [60].It was administrated that sporocyst previously treated with concanavalin Alectin become encapsulated, but it do not die in snails that are usually resistant to infection[61]. Other authors hypothesized that it is necessary to have specific recognition for encapsulation and effective cytotoxic response. It is possible that during hemocyte attachment, a process of cellular activation occurs that triggers the release of peroxidase enzymes and other cytotoxic substances around the parasites as suggested in *B. tenagophila* [43].

The internal defense system (IDS) is stimulated by the excretory secretory products of the penetrating miracidia [62]. It has been identified three subpopulations of hemocytes in hemolymph of *B. glabrata*snails based on their size and ultrastructural aspects [63]. It has been identified subpopulations of hemocytes in hemolymph of *B. alexandrina*, these three hemocytes are designated as round small hyalinocyte cells with circular shape and clear cell membrane, while granulocytes which are characterized by their moderate size with different size granules and amoebocytes are characterized by their large size, extending many pseudopodia and central clear nucleus [64].Three circulating hemocytes subsets in *Biomphalaria* species were identified [31]. In agreement to the present study the *B. alexandrina* hemocytes are classified according to cell size and shape into two cell types, designated as small round hyalinocytes and granular spreading hemocytes [65].

In the current result, the number of circulating hemocytes in infected *B. truncatus* snails increased significantly at 1-2 WPE as compared to normal control and then a gradual decrease occurred. This may be due to that the cells originating within organ would flow with the hemolymph to concentrate themselves at locations of infection. In this way, a higher concentration of hemocytes in the hemolymph of infected snails was observed [32]. The stage of the schistosome infection also is important. The results agreed with authors who found a decrease in hemocyte number in snails with 4-6 WPE, which they attributed to a migration of hemolymph cells to the interior of the tissues [66]. On the other hand, it was observed that significant increase in hemocyte number in snails with 4-6 WPE as compared to normal controls [67].

The present observation showed that the granulocytes were present in significant greater number as compared to hyalinocytes. The results agreed with the authors [32]. It has been found that the successful elimination of potential infective agents requires granulocytes to engulf particles and further eliminate living pathogens through enzymatic or oxidative degradation [68]. However, they thought that hyalinocytes were responsible primarily for wound repair, requiring aggregation at an injury site. Hyalinocytes are smaller spherical cells, unable to adhere to substrates or to emit pseudopods. On other hand, granulocytes are large amorphous cells presenting granules in cytoplasm, which easily adhere to substrates and emit pseudopods [69; 39; 70]. The increase in number of granulocytes from Giza group was more than the two other experimental groups, which may be predisposes Giza snails to exhibit low susceptibility in the present study. Other studies carried out on *B. tenagophila* demonstrate that the temporary reduction in the number of circulating granulocytes results in increased susceptibility to infection by *S. mansoni* [71].

CONCLUSION

In Egypt, *B. truncatus* snails have variant immune response against *S. haematobium* infection.

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REFERENCES

[1] Sluiters JF, Brussaard-Wust C, Meuleman EA. The relationship between miracidial dose, production of cercariae and reproductive activity of the host in the combination *Trichobilharzia ocellata* and *Lymnaea stagnalis*. Zeitschrift fur Parasitenk 1980; 63: 13–26.

[2] Deelder AM, Miller RL, De Jonge N, Krijger FW. Detection of schistosome antigen in mummies. Lancet,1990; 335: 724-725.

[3] Barakat MR. Epidemiology of Schistosomiasis in Egypt: Travel through Time. J.Advan. Res., 2013, 4: 425–432.

[4] Matheson DA, David R, Mark Spigelman, M, Donoghue DH. Molecular confirmation of *Schistosoma* and family relationship in two ancient Egyptian mummies. Yearbook of Mummy Studies, 2014, 2: 39-47.

[5] Rollinson D, Stothard JR, Southgate VR. Interactions between intermediate snail hosts of the genus *Bulinus* and schistosomes of the *Schistosoma haematobium* group. Parasitology, 2001, 123: 245-260.

[6] Davies SJ, Mckerrow JH. Developmental plasticity in schistosomes and other helminthes. Int. J. Parasitol., 2003, 33: 1277- 1248.

[7] Kariuki HC. Ivy JA, Muchiri EM. Sutherland LJ, King, CH. Divergent effects of *Schistosoma haematobium* exposure on intermediate host snail species *Bulinus nasutus* and *Bulinus globosus* from Coastal Kenya. Am. J. Trop. Med. Hyg., 2017 (In press).

[8] Saad AH, Abu El Einin HM, Abdel-Gaber RA, Mansour SM. Diversity and comparative studies on *Bulinus* snails collected from two localities in Egypt. J. Egypt. Soc. Parasitol., 2016 46:509-518.

[9] Amen RI, Ti Jnagel TMG, Van Der Knaap WPW, Meuleman EA, Lange-de Klerk ESM, Siminia T. Effects of *Trichobilharzia ocellata* on hemocytes of *Lymnaea stagnalis*. Dev. Comp. Immunol., 1991, 15: 105-115.

[10] Bayne CJ. Phagocytosis and non-self recognition in Invertebrates. Bioscience, 1990, 40:723-731.

[11] Van der Knaap WP, Loker ES. Immune mechanisms in trematode-snail interactions. Parasitol. Today, 1990, 6: 175-182.

[12] Adema CM, Deutekom-Mulder, EC, Van der KPW, Sminia T. Schistosomicidal activities of *Lymnaea stagnalis* hemocytes: the role of oxygen radicals. Parasitology, 1994, 109: 479–485.

[13] Negrão-Corrêa D, Pereira CAJ, Rosa FM, Martins- Souza RL, Andrade ZA, Coelho PMZ. Molluscan response to parasite: *Biomphalaria* and *Schistosoma mansoni* interaction. J. Invert. Survival., 2007,4: 101-111.

[14] Coustau C, Gourbal C, Duval D, Yoshino TP, Adema CM, MittaG. Advances in gastropod immunity from the study of the interaction between the snail *Biomphalaria glabrata* and its parasites: A review of research progress over the last decade. Fish and Shellfish Immunology, 2015, 1-12.

[15] Anderson RM, Gordon D. Process. influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. Parasitol., 1982, 85: 373-398.

[16] Davies SJ, Mckerrow JH. Developmental plasticity in schistosomes and other helminthes.Int. J. Parasitol., 2003, 33: 1277- 1248.
[17] Zelck, UE, Becker W,Bayne CJ. The plasma proteins of *Biomphalaria glabrata* in the presence and absence of *Schistosoma mansoni*. Dev. Comp. Immunol., 1995, 19:181–194.
[18] Souza CP, Borges CC, Santana AG, Andrade ZA. Comparative histopathology of *Biomphalaria glabrata, B. tenagophilia* and *B. straminea* with variable degrees of resistance to *Schistosoma mansoni* miracidia. Mem. Inst. Oswaldo Cruz, 1997, 92: 517-522.
[19] Borges CM, Souza CP, Andrade ZA. Histopathological features associated with susceptibility and resistance of *Biomphalaria* snails to infection with *Schistosoma mansoni.* Mem. Inst. Oswaldo. Cruz, 1998, 93:117-121.
[20] Lemos QT, Andrade ZA. Sequential histological changes in *Biomphalaria glabrata* during the course of *Schistosoma mansoni* infection. Mem. Inst. Oswaldo. Cruz, 2001, 96: 719-721.
[21] Raghavan N, Miller, AN, Gardner M, FitzGerald PC, Kerlavage AR and Johnston DA Comparative gene analysis of *Biomphalaria glabrata* hemocytes pre-and post-exposure to miracidia of *Schistosoma mansoni*. Mol. Biochem. Parasitol., 2003, 126:181-191.
[22] Souza SD, Andrade ZA. On the origin of the *Biomphalaria glabrata* hemocytes. Mem. Inst. Oswaldo Cruz, 2006, 101: 213-216.
[23] Bayne CJ. Successful parasitism of vector snail *Biomphalaria glabrata* by the human blood fluke (trematode) *Schistosoma mansoni*. Biochem. Parasitol., 2009, 165: 8-18.
[24] Abou-El-Naga IF, Radwan EH. Defense response of susceptible and resistant *Biomphalaria alexandrina* snails against *Schistosoma mansoni* infection. Rev. Biol. Trop., 2012, 60 (3): 1195-1204.
[25] Adema CM, Loker ES. Digenean-gastropod host associations inform on aspects of specific immunity in snails. Dev. Comp. Immunol., 2014, 48(2): 275–283.
[26] Cheng TC, Guida VG. Hemocytes of *Bulinus truncatus* rohlfsi (Mollusca: Gastropoda). J. Inverteb. pathol., 1980, 35: 158-167.
[27] Mandahl-Barth, G. Key to the identification of east and central African freshwater. Snails of medical and veterinary importance. Bull. Wld. Hlth. Org., 1962, 27: 135-150.
[28] Liang YS, Bruce JI, Boyd DA. Laboratory cultivation of schistosome vector snails and maintenance of schistosome life cycles. Proceedings of the First Sino-American Symposium, 1987,1: 34-48
[29] El-Sheikha HM, Hussein HS, Rahbar MH. Clinico-pathological effects of *Schistosoma mansoni* infection associated with simultaneous exposure to malathion in Swiss outbred albino mice. ActaTropica., 2008, 108: 11-19.
[30] Saoud MFA. Comparative studies on the characteristics of some geographical strains of *Schistosoma mansoni* in mice and hamster. J. Helminthol., 1965, 39:101-112.
[31] Martins-Souza RL, Pereira CA, Martins Filho OA, Coelho PMZ, Correa A. Differential lectin labeling of circulating hemocyt from *Biomphalaria glabrata* and *Biomphalaria tenagophila* resistant or susceptible to *Schistosoma mansoni* infection. Mem. Inst Oswaldo Cruz, 2006, 101:185-92.
[32] Souza SD, Andrade ZA. The significance of the amoebocyte-producing organ in *Biomphalaria glabrata*. Mem. Inst. Oswaldo Cruz, 2012, 107(5): 598-603.
[33] Richards CS. Genetics of the host-parasite relationship between *Biomphalaria glabrata* and *Schistosoma mansoni*. Genetics of the host-parasite relationship between host-parasite relationships. Symp. Brithis. Soc. Parasitol., 1976, 14: 45–54.
[34] Basch PF. Intermidiate host specificity in *Schistosoma mansoni*. Exp. Parasitol., 1979, 39:150–69.
[35] Richards CS, Shade PC. The genetic variation in *Biomphalaria glabrata* and *Schistosoma mansoni*. J. Parasitol., 1987, 73: 1146-1151.
[36] Lie KJ, Jeong KH, Heyneman D. Immune responses in parasitic infections (Soulsby, E. J. L., ed.). CRC Press, 1979,211–270.
[37] Spray FJ, Granath, JR. Comparison of hemolymph proteins from *Schistosoma mansoni* (Trematoda)-susceptible and resistant *Biomphalaria glabrata* (Gastropoda). Comp. Biochem. Physiol., 1988, 91: 619–24.
[38] Coustau C, Yoshino TP. Surface membrane polypeptides associated with hemocytes from *Schistosoma mansoni*-susceptible and -resistant strains of *Biomphalaria glabrata* (Gastropoda). J. Invertebr. Pathol., 1994, 63: 82–84.
[39] Bezerra FSM, Nogueira-Machado JA, Chaves MM, Correa RF, Coelho PMZ. Effect of gamma radiation on the activity of hemocytes and the course of *Schistosoma mansoni* in resistant *Biomphalaria glabrata*. Mem. Inst. Oswaldo Cruz, 2003, 98:73–5.

[40] Coelho PMZ, Carvalho OS, Andrade ZA, Martins-Sousa RL, Rosa FM. *Biomphalaria tenagophila/ Schistosoma mansoni* interaction: premises for a new approach to biological control of schistosomiasis. Mem. Inst. Oswaldo Cruz, 2004, 99:109–111.

[41] Coelho JR, Bezerra FS. Compatibility of *Biomphalaria tenagophila* with *Schistosoma mansoni*: a study of homologous plasma transference. Mem. Inst. Oswaldo Cruz, 2006, 101: 111–12.

[42] Martins-Souza RL, Pereira CA, Rodrigues L, Araujo ES, Coelho PM, Correa JA. Participation of N-acetyl-D-glucosamine carbohydrate moieties in the recognition of *Schistosoma mansoni* sporocysts by hemocytes of *Biomphalaria tenagophila*. Mem. Inst Oswaldo Cruz, 2011, 106:88-91.

[43] Nacif-Pimenta R, Mattos ACA, Orfanó AS, Barbosa L, Pimenta PFP, Coelho PMZ. *Schistosoma mansoni* in susceptible and resistant snail strains *Biomphalaria tenagophila*: In vivo tissue response and in vitro hemocyte interactions. Plos One, 2012, 7(9):1-12.

[44] Marques DP, Rosa FM, Maciel E, Negra˜o-Corre^a D, Teles HMS. Reduced susceptibility of *Biomphalaria tenagophila* population to *Schistosoma mansoni* after introducing the resistant taim/ RS strain of *B. tenagophila* into Herivelton Martins Stream. Plos One, 2014, 9:1-5.

[45] Azevedo CM, Borges CC, Andrade ZA. Changes induced in *Biomphalaria glabrata* (Say, 1818) following trials for artificial stimulation of its internal defense system. Mem. Inst. Oswaldo Cruz, 2006, 1: 199–203.

[46] Newton WL. (1952): The comparative tissue reaction of two strains of *Australorbis glabrata* to infection with *Schistosoma mansoni*. J. Parasitol., 38: 362-366.

[47] Souza CP, Cunha RCP, Andrade ZA. Development of *Schistosoma mansoni* in *Biomphalaria tenagophila, Biomphalaria straminea* and *Biomphalaria glabrata.* Rev. Inst. Med. Trop. São Paulo, 1995, 37: 201-206.

[48] Cooper L, Richards C, Lewis F, Minchella D. *Schistosoma mansoni*: relation between low fecundity and reduced susceptibility to parasite infection in the snail, *Biomphalaria glabrata*. Exp. Parasitol., 1994, 79: 21-28.

[49] Vasquez RE, Sullivan JT. Effect of miracidial dose on adoptively transferred resistance to *Schistosoma mansoni* in the snail intermediate host, *Biomphalaria glabrata*. J. Parasitol., 2001, 87: 460-462.

[50] Loker ES, Bayne CJ, Yui MA. *Echinostoma paraensei* hemocytes of *Biomphalaria glabrata* as targets of *Echinostoma* mediate interference with host snails resistance to *Schistosomamansoni*. Exp. Parasitol.,1986, 62: 149-154.

[51] El-Assal FM, Shoukry NM, Abdalla MH, Saad AH. Cellular response to *Schistosoma mansoni* infection in *Biomphalaria alexandrina* strains selected for susceptibility and resistance. J. Egypt. Soc. Parasitol., 2001, 31: 915-938.

[52] Soomro NM, Arijo AG, Qureshi TA, Runham NW, Doenhoff MJ. Pathology of Schistosome infection on host tissue during developmental stages of parasite in vector snails. Int. J. Agri. Biol., 2005,7:133-141.

[53] Rosa FM, Godard AL, Azevedo V,Coelho PM. *Biomphalaria tenagophila*: dominant character of the resistance to *Schistosoma mansoni*in descendants of cross breedings between resistant (Taim, RS) and susceptible (Joinville, SC) strains. Mem. Inst. Oswaldo Cruz, 2005, 100: 19-23.

[54] Abou- El- Naga IF, Eissa MM, Mossallam SF, Abd El-Halim SI. Inheritance of *Schistosoma mansoni* infection incompatibility in *Biomphalaria alexandrina* snails. Mem. Inst. Oswaldo Cruz, 2010, 105: 149-154.

[55] Godoy A, De Souza CP, Guimarães CT, Andrade ZA. Unusual histological findings in *Biomphalaria glabrata* with high degree of resistance to *Schistosoma mansoni* miracidia. Mem. Inst. Oswaldo Cruz, 1997, 92: 121-122.

[56] Lie KJ, Heyneman D. Studies on resistance in snails. Tissue reactions to the *Echinostoma lindoense* sporocysts in sensitized and resensitized *Biomphalaria glabrata.* J. parasitol., (1976), 2: 51-58.

[57] Basch PF. An interpretation of snail-trematode infection rates: specificity based on concordance of compatible phenotypes. Int. J. Parasitol., 1975, 5: 449-452.

[58] Borges CM, Andrade ZA. Extra-cellular matrix changes in *Schistosoma mansoni*-infected *Biomphalaria glabrata*. Mem. Inst. Oswaldo Cruz, 2003, 98:135-139.

[59] Lie KJ, Jeong KH, Heyneman D. Tissue reaction induced by *Schistosoma mansoni* in *Biomphalaria glabrata* as targets of *Echinostoma* mediate interference with host snails resistance to *Schistosoma mansoni*. Exp. Parasitol., 1980, 62: 149-154.

[60] Meuleman EA, Bayne ME, Van Der Knaap WPW. Developmental and Comparative Immunology (Cooper, E. L. Langlet, C. and Bierene, J., eds), 1987, 116–127, Alan Liss.

[61] Boswell CA, Bayne CJ. Lectin-dependent cell-mediated cytotoxicity in an invertebrate model: Con A does not act as a bridge. Immunology, 1986,57: 261–64.

[62] Zahoor Z,Davies AJ,Kirk RSRollinson D, Walker AJ. Larval excretory-secretory products from the parasite *Schistosoma mansoni* modulate HSP70 protein expression in defence cells of its snail host, *Biomphalaria glabrata*. Cell Stress and Chaperones, 2010, 17: 320-328.

[63] Matricon-Gondran M, Letocart M. Internal defenses of the snail *Biomphalaria glabrat*a I. Characterization of hemocytes and fixed phagocytosis. J. Invert. Pathol., 1999, 74:224-34.

[64] Kamel AG, Refaat SM, El-Dafrawy SM, Mohamed AH, Mossalem SHH. The effect of *Schistosoma mansoni* infection on *Biomphalaria alexandina* hematocytes at ultrastructure level. Proc. 4th Int. Cong. Bio. Sci., 2006, 219-26.

[65] Mohamed AH. Sublethal toxicity of Round up to immunological and molecular aspects of *Biomphalaria alexandrina* to *Schistosoma mansoni* infection. Ecotox. Environ. Safe, 2011, 74:754-80.

[66] Granath JR, Yoshino TP. Characterization of molluscan phagocyte subpopulations based on lysosomal enzyme markers. J .Exper. Zool., 1983,226: 205-210.

[67] Abdul-Salam JM, Michelson EH. *Biomphalaria glabrata* amoebocytes: Effect of *Schistosoma mansoni* infection on *in vitro* phagocytosis. J.Invertebr.Pathol., 1980, 35: 241-248.

[68] Sparks AK. Invertebrate Pathology: Noncommunicable Diseases. (1st eds), Academic Press, 1972 New York, USA

[69] Barraco MA, Steil AA, Gargioni R. Morphological characterization of the hemocytes of the pulmonate snail *Biomphalaria tenagophila*. Mem. Inst. Oswaldo Cruz, 1993, 88: 73-83.

[70] Helal EG, El- Dafrawy SM, Mohamed AH, Abou-El-Nour BM,Ibrahim S. Ultrastructural study on *Biomphalaria alexandrina* hemocytes infected with *Schistosoma mansoni* in Egypt and its correlation with nitric oxide level. J. Egypt. Soc. Parasitol., 2014, 44:113– 124.

[71] Pereira CA, Martins-Souza RL, Coelho PMZ, Lima WS, Negrão-Corrêa D. Effect of *Angiostrongylus vasorum* infection on *Biomphalaria tenagophila* susceptibility to *Schistosoma mansoni*. ActaTropica, 2006, 98: 224–233.