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Short communication

Haemolymph Components of Infected & None Infected *Lymnaea snails* with Xiphidiocercariae

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ABSTRACT

Background: In this study the haemolymph components of infected and none infected *Lymnaea gedrosiana* with xiphidiocercaria larvae was compared.
Methods: Five hundred Fifty *Lymnaea* snails were collected from Ilam and Mazandaran provinces, Iran, during 2008-2009. The snails were transported to the lab at Tehran University of Medical Sciences and their cercarial sheddings were studied. Haemolmyphs of snails were extracted and cells were counted using haemocytometer and cell-surface carbohydrate were recognized by conjugated lectin (Lentil). Haemolymph protein concentrations were measured by Bradford protein assay method and soluble protein compositions were determined on sodium dodecyl sulphate polyacrilamide gel electrophoresis (SDS-PAGE).
Result: From the 550 examined *Lymnaea* snails for cercariae, 27 snails were infected with xiphidiocercariae. Mean of haemolymph cells (haemocyte) number were obtained 93480±2.43 (cells/ml) for none infected snails (25 snail) and 124560±2800 (cells/ml) for infected snails (25 snail). Mannose carbohydrate was recognized on haemocyte of none infected and infected snails. Mean of protein concentration of haemolymph plasma was obtained as 1354 ± 160 µg/ml (1.4 mg/ml) for none infected snails (25 snails) and 1802±138 µg/ml (1.8 mg/ml) for infected snail (25 snails). Comparing to none infected snails, the SDS-PAGE results of haemolymph plasma of infected snails, showed an extra protein band (70 kDa). The results showed a significant difference between the amounts and the kinds of proteins in haemolymph of infected and none infected snails.
Conclusion: This information might be useful to understand of parasite detection, adhesion, engulfment and antigen agglutination by snail.
Keywords: *Cercaria,* Lymnaea*, Haemolymph, Xiphidiocercariae*

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Introduction

Cercaria is a larval form of the parasite, developed within the snail. It finds and settles in a selective host and according to species, metacercaria, or adult form will be appeared. Xiphidiocercaria is cercariae with a stylet in the anterior rim of its oral sucker with which it actively penetrates its host. Studies on cercariae infections in freshwater snails help us to understand snail-borne diseases presence and the location of potential transmission sites (1). Several studies have examined the influence of parasites on the host organisms (2-4). Cercariae identification of *Lymnaea* snails may help to explain the geographical distribution of diseases in Iran. Hemolymph analysis of infected snail to parasite also has potential to develop knowledge of parasites immunology.

Therefore, the aim of this study was to compare haemolymph components of infected and none infected *Lymnaea* snails with xiphidiocercaria larvae. These cercariae have been identified in the previous article (5).

Materials and Methods

Cercariae collection and haemolymph extraction from snails

Totally, 550 *Lymnaea gedrosiana* snails were collected from Ilam and Mazandaran provinces, Friedoon-kenar and Ilam rural areas, Iran, by sweeping a net through the agricultures canals and drains during 2008-2009. The snails were transported by wet cotton to the lab at Tehran University of Medical Sciences. In the lab, the snails were maintained in plastic containers. *Lymnaea* snails were fed by lettuce 1–2 times per week and the water in the containers was changed once 24-48 hrs. To collect cercariae, snails were examined by mean of the shedding method and collected cercariae were identified as fresh or stained samples by taxonomic key (6). Haemolymphs of cleaned snails were extracted by capillary tube from the haemocoel of foot-and-mouth part. A haemocoel is a cavity or series of spaces between the organs of snail with open circulatory system (7).

Cell counting and cell- surface carbohydrate recognition

Extracted haemolymph was introduced into one of the V-shaped wells of Neubauer haemocytometer with a capillary tube and determined cell numbers (8). Extracted haemolymphs were centrifuged at 3000 rpm for 5 minute at 4°C in microfuge and haemocytes were collected from the pellet. To recognize of cell-surface carbohydrate, FITC (fluorescein isothiocyanate) -conjugated lectin was used. Then, FITC-lectin was added to the suspension of the cells in the test tubes and added FITC-lectin to the control tubes containing 100 mM inhibitory sugar (mannose). The tubes were incubated at 4°C for 60 min and the cells were washed three times by centrifugation (3000 rpm for 5 min) in PBS. Samples were mounted on slides and studied by fluorescence microscope (9).

Measurement of Protein concentration and detection of protein bands

Extracted haemolymph samples were centrifuged at 8500 rpm for 30 min at 4°C by microfuge and plasma specimens were obtained. Concentrations of total proteins were measured by Bradford assay method. Sodium dodecyl sulphate Polyacrylamide gel electrophoresis (7.5% gel and Coomassie blue staining) was used to separate the components of plasma protein (9).

Statistical methods

The independent *t*-test was used to distinguish the significant difference between two studied groups on the means of cell or protein concentrations in none infected and infected snails.

Results

From the total number of snails, which examined for cercariae, 27 snails were found infected with xiphidiocercaria (Fig. 1). The number of haemocytes was 93480±2043 (Cells/ml) for none infected snails and 124560 ±2800 (Cells/ml) for infected snail. Mannose monosaccharide, CH2OH (CHOH) 4CHO, was detected as surface carbohydrate on the cell at a 1/25 diluted FITC-lectin. Because of covering of FITC-lectin by inhibitory sugar on cells in the control samples, cells were not observable at above dilution under fluorescence microscope (Fig. 2). Total protein concentrations of haemolymph were estimated 1354 ±160ug/ml (1.4 mg/ml) for none infected snails and 1802 ± 138 ug/ml (1.8mg/ml) for infected snails. An extra soluble protein (70 kDa) was detected on SDS-PAGE of haemolymph plasma of infected snails that is presented in Fig. 3. Independent samples *t*-test revealed there was significant difference in cell concentrations between infected snail (M=124560, SD=2800) and none infected (M=93480, SD= 2043) conditions; t (48) = 2.01, t-value = 44.83, $P < 0.05$. There was also significant difference in protein concentration between infected snail (M= 1802, SD= 138) and none infected snail (M= 1354, SD=162) conditions; t (48) = 2.01, t-value = 10.66, $P < 0.05$.

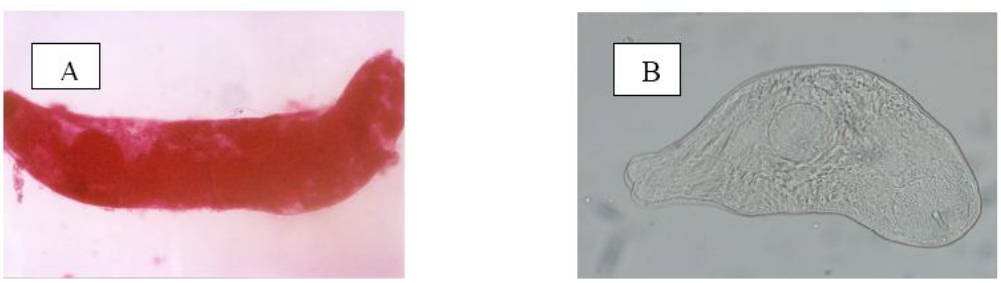

Fig. 1: Xiphidiocercaria larvae from *Lymnaea* snail; A. Sporocyt with developing cercariae; B. Cercaria with stylet in oral sucker

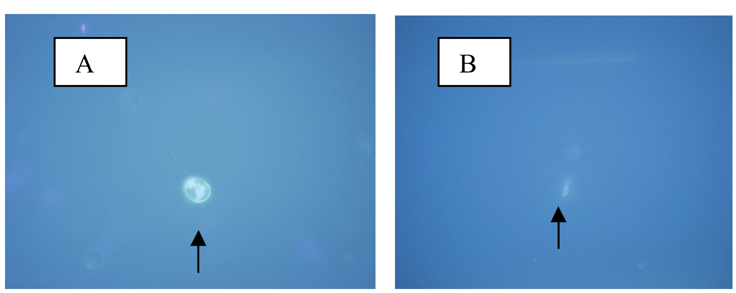

Fig. 2: A. Haemocyte surface with mannose carbohydrate, which was conjugated by FITC-Lentil. **B**. Haemocyte control without surface mannose

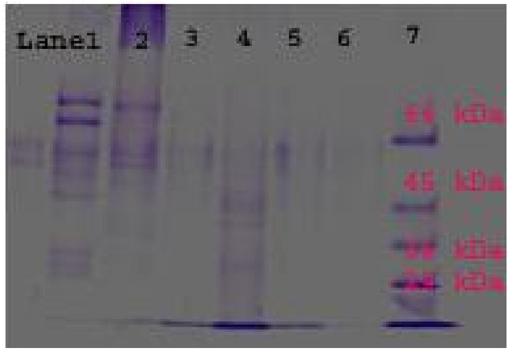

Fig. 3: SDS-PAGE analysis of plasma proteins of extracted haemolymphs from infected (lane 1) and none infected (Lanes 2, 3, 4, 5, 6) *Lymnaea* snail and protein marker (lane 7)

Discussion

In previous studies, it has been shown that xiphidiocercaria larvae belong to Plagiorchidae Trematodes (5). Recently xipidiocercariae has been reported from *Lymnaea natalensis* snail (10). There are few publications about xiphidiocercaria effects on the haemolymph compartments (11, 12); however, various reports have been recorded on the effects of other cercariae on haemolymph of other snails including protein level. *Schistosoma mansoni* infection has increased the total protein concentration of haemolymph of *Biomphalaria alexandrina* (13). In the present study, significant increase of protein concentration may be representing of humoral factors (opsonin and agglutinin) in hemolymph to parasite presence (14).

The use of specific inhibition sugar has been indicated presence of glucose/mannose, galactose, N-acetyl-galactosamine (GalNAc) and N-acetyl-glucosamine (GlcNAc) on the surface of the haemocytes (15). Lectins are responsible for cell surface sugar recognition; therefore, based on the current study, existence of mannose carbohydrate on the cells surface helps them for detection and adhesion to parasite larva through crosslink glycoconjugates (16).

Effects of *Trichobilharzia ocellata* on haemocytes of *Lymnaea stagnalis* with respect to cell number and distribution of subpopulations has been determined (17). The present work reports significantly increase of cell number, which is very important for phagocytosis or engulfment of larval stages of parasite (miracidium, sporocyst, and cercaria) (14).

Two snail populations with respect to haemolymph agglutinins (lectin) from *Lymnaea stagnalis* have been reported (18). Lectins are considered to play main role in humoural immunity of molluscs serving as recognition factors for foreignness (19). SDS-gel-separated protein band from this research might be a member of the lectinic protein family agglutinate, however, need to more characterizations by isolation, purification, and identification.

In conclusion, the significant increase of the cells number and protein concentrations, as well as presence of cell surface mannose and extra soluble proteins in the haemolymph of infected snails with xiphidiocercaria larva might have an important role in parasite detection, adhesion, engulfment / antigen agglutination.

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