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Original Article

Adhesion of Cercaria (Larva of Helminth Parasites) to Host by Lectins- carbohydrates bonds as a Model for Evaluation of *Schistosoma* Entrance Mechanisms in Cercarial Dermatitis

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Abstract

Background: Cercariae (larva of helminth parasites) are covered by a thick glycocalyx coat, which serves as an osmotic protection during their free existence, and contain carbohydrates conjugated as glycoproteins, glycolipids and mucopolysaccharides. Although, limited studies have been made on life cycle of cercariae from fresh water snails, however, carbohydrate studies on cercariae have not been done in Iran so far. This study was made to determine the cercariae specifications from *Lymnaea gedrosiana* and evaluation of surface carbohydrates as receptors for host lectins in a host-parasite relationship system as a model in human schistosomiasis including cercarial dermatitis in Khuzestan Province.

Methods: For this purpose, snails were collected from Dezful region in Khuzestan Province and cercariae were obtained by shedding method and identified by valuable keys. Experimental infection was established in the *Culex pipiens* (Culicidae mosquitoes) larvae for further identification and mode of adhesion. To detect the mode of adhesion, surface carbohydrates of cercariae were detected by lentil (Lens culinaris) lectins.

Results: Examined snails were infected with xiphidiocerceria of trematodes and metacercariae were obtained from *Culex pipiens*. Also, Mannose monosaccharides- CH2OH (CHOH) 4CHO - were detected particularly on the glands of cercariae.

Conclusion: Adhesion of cercariae to their host by lectins-carbohydrates bonds is the first stage of host-parasite relationship. This phenomenon could be happened for animal schistosome's cercaria in cercarial dermatitis.

Keywords: *Xiphidiocercaria, Carbohydrate, Lectins, Cercarial dermatitis*

Introduction

The helminth surface is covered with a layer of carbohydrates conjugated as glycoproteins, glycolipids and mucopolysaccharides. This layer called glycocalyx, is of great importance for the realization of the biochemical and physiological interactions between the helminths and their hosts. In many of the studied helminth species, the origin, nature, morphology and histochemical characteristics of the carbohydrate layer are well précised Cytochemical and autoradiographic investigations have proven that the surface glycoconjugates are mainly of helminth origin but there are data that in some species part of them originates from the host as well. The surface carbohydrate layer has a complex polyanion nature. The anion groups of the glycocalyx bind electrostatically to cations from the environment. The negative charge of the helminth surface plays an important role in the parasite-host interactions as well as in the physiological functions and the immune response. The surface carbohydrates in trematodes, cestodes and nematodes are species- and stage-specifically differentiated. The data are obtained using lectins with known carbohydrate specificity as well as biochemical and immunological tests. In trematodes found the qualitative and the quantitative changes in the carbohydrate compound of the surface glycoconjugates during the different life cycle stages correlated with their adaptation to the specific environment (1-5).

The xiphidiocercariae are asexually produced in sporocysts inside of the hepatopancreas of snails

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of the genus *Lymnaea*. These snails take part in the cycle of the trematodes as the first intermediary hosts. The snails liberate the cercariae, which penetrate and encyst in the larvae or nymphs of aquatic insects. Such insects participate in the cycle as secondary intermediary hosts. Man and animals are definitive host for these parasites.

Snails are first intermediate host of trematode parasites. The larvae of trematodes (cercariae) after developing in the snail tissue for survival and more maturity find the suitable secondary intermediate host or definitive host by means passive transmission (metacercaria) or active penetration, respectively. *Lymnaea* spp. are fresh water snails with various species including *L. gedrosiana, L.auricularia, L.truncatula* and *L. stagnalis,* in Iran (6).These snails are living in the streams, swamps and ditches and are abundant in the highly oxygenated and marginal surface water of agriculture canals of Khuzestan Province in the south west of Iran. Khuzestan Province has many canals and ponds, which are using for bathing, drinking and washing by the people and these places are suitable for living of *Lymnaea* spp. snails. Due to the presence of infected *Lymnaea* spp., water resources could be contaminated by the emerging cercariae including bird schistosomes and therefore consequently cercariae to make an attack to the native residents directly via the skin. The people and especially children swim and play in the rivers or canals, which are used for agriculture purposes. In addition many young children work on the agricultural farms without any protection on their hands or feet areas where animal schistosome cercariae, *Trichobilharzia* sp., can readily penetrate the skin (7).

Although restricted studies have been done on life cycle of trematode cercariae from *Lymnaea* snails, however studies on surface carbohydrates have not been done to date. The aim of this research was to detect the more specifications of xiphidiocercariae (larva of trematodes) from *L. gedrosiana* and evaluation of carbohydrates on their surface as receptors for lectins in this host parasite relationship system as model in cercarial dermatitis.

Materials and Methods

***Collection of Xiphidiocercariae from* Lymnaea gedrosiana** This study was made on *L.gedrosiana* snails in Mazraae district from Ahoudasht region in south of Dezful region (Fig. 1). The snails were collected from the drain, pond, canals and waterway by a wooden handle paddle with 1.5 meter long and net size 30 x 40 cm and were transferred to Dezful Health Research Center as alive. The snails were keeping in aquarium and cercariae obtained by shedding method. In the shedding method, snails were put in the pettry dish containing dechlorinated tap water and were placed against light for two hours or over night in room. Collected cercariae were studied as alive or fixed in hot formalin (5%). Measurements and drawings were made on living or fixed specimens under light cover glass pressure and rather stained with neutral red or azocarmine and identified by key references(8).

Experimental infection of* Culex *larvae* (Culicidae mosquitoes) *by Xiphidiocercaria

Experimental infection was established in the *Culex* larvae for further identification. For this purpose *Culex pipiens* larvae were put in an aquarium with infected snail at room temperature (25C). Periodically, the larvae were observed for checking the penetration of cercariae into the larvae under stereoscope (Fig. 2).

Carbohydrate detection on cercaria surface

To detect of the surface carbohydrate, FITC-conjugated lectins were used. For this purpose, FITC-lentil was added to suspension of whole cercariae as a test tube and added FITC-lectin to a control tube containing 100 mM inhibitory sugar (mannose).The tubes were incubated at 4 for 60 min and washed three times by centrifugation (3000 rpm for two min) in PBS. Samples were mounted on slides and observed under fluorescence microscope (9).

Results

Morphological specifications of cercariae

In the cercariae samples, oral and ventral suckers are almost equal. Penetration gland cells are in several pairs, there is a vertical stylet in oral sucker. Tail, without finefold, and excretory bladder is Y-shaped. Genital primordium is located posteriorly to ventral sucker. Body surface is provided with minute spines. A long esophagus is observed and intestinal caecae reaches to the posterior end of the body. The cercariae were identified as xiphidiocercariae group cercariae belong to Plagiorchiid helminth (Fig. 3, 4).

Penetration into* Culex *larvae by Xiphidiocercaria After two hours from penetration of cercariae into *Culex* larva early metacercariae were observed. Mostly metacercariae were obtained from the gills and the thorax (Fig. 5).

Carbohydrate detection on surface of cercariae Mannose monosaccharide, CH2OH(CHOH) 4CHO, were detected as surface carbohydrates particularly on glands of cercariae at a 1/25 dilution (Fig. 6).

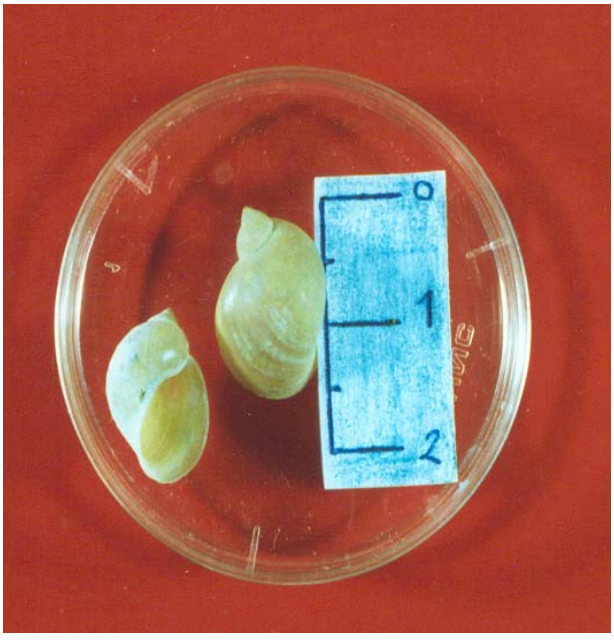

Fig. 1: *Lymnaea gedrosiana* snail from Dezful region in Khuzestan Province (South -West of Iran)

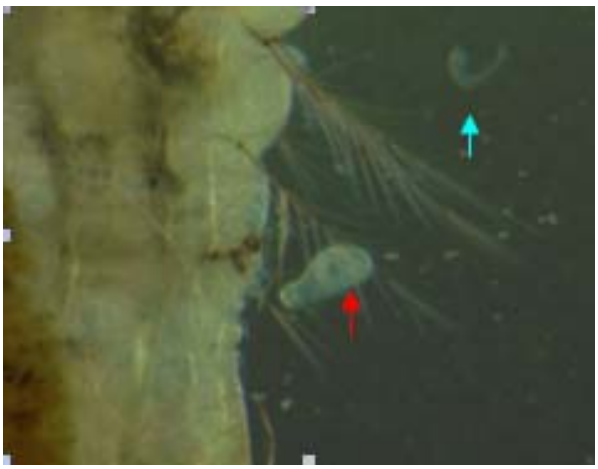

Fig. 2: Entering of xiphidiocercaria cercaria to larva of *Culex pipense* after separation of tail (Blue arrow) from body (Red arrow) of cercaria

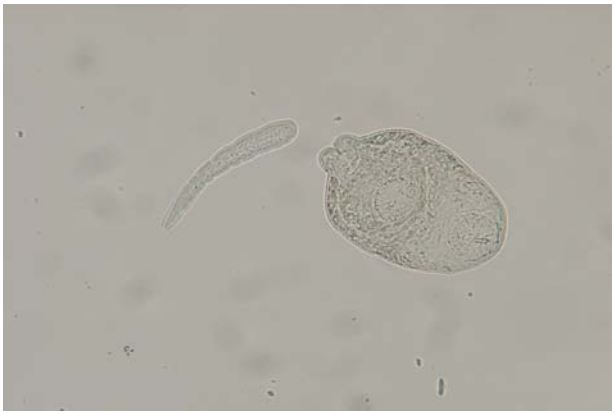

Fig. 3: Xiphidiocercaria with separated tail from body before penetration of cercaria

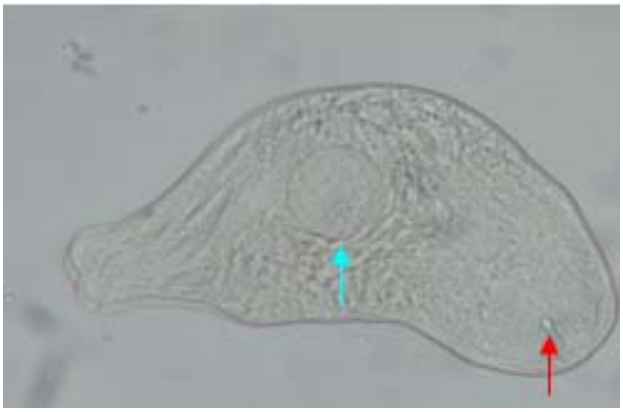

Fig. 4: Xiphidiocercaria with stylet (Red arrow) in the oral sucker and ventral sucker (Blue arrow)

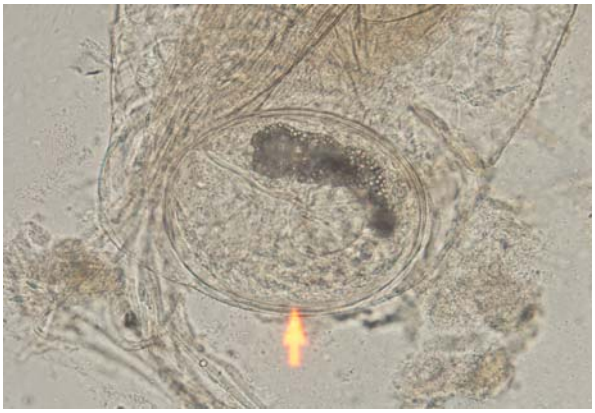

Fig. 5: Metacercaria (Yellow arrow) in culex larva after penetration of xiphidiocercaria

Fig. 6: Detection of mannose saccharid on the xiphidiocercarial glands (yellow arrow) by lectin (lens culinaris)

Discussion

Xiphidiocercaria has been obtained from *Bellamaya bengalensis* snail from Dezful region (10). It seems that these cercariae use the various species of snails as hosts and therefore *L.gedrosiana* may be not a specific host for xiphidiocerceria. This cercaria develops inside the insect larva and is transformed to metacercaria. Our observations showed that *Anopheles'* larvae resistant to penetration of xiphidiocerceria (data not shown) and thus the *Culex pipiens* larva may be specific as secondary host for xiphidiocerceria. Mostly metacercariae were obtained from gills and thorax and this showed that, the second stage of larva needed to highly oxygenated environment to reach to the infective stage for final host.

The results revealed that mannose saccharid were most aboudant on gland and it could be due to diffusion of various carbohydrates from two sides of gland cells.

In trematode parasites, the first larval form, living free in water medium- miracidium- is covered with a thick glycocalyx coat which probably serves as an osmotic protection. The transformation of the miracidia to sporocysts after their penetration into the intermediate host goes together with a considerable thinning of the glycocalyx layer. Next stage of trematode life cycle is cercariae, which spend a short time in an intermediate host and then leave it. They are covered by a thick glycocalyx coat, which serves as an osmotic protection during their free existence (11). The analysis of the carbohydrates residues on the cercaria surface of trematode species, inhabiting the pond snails *Lymnaea stagnalis* shows that they are bound in a different way to lectin probes. On the surface of cercaria of trematodes including *Hypoderaeum conoideum*, *Echinoparyphium aconiatum, Paryphostomum* sp., *Diplostomum pseudospathaceum, Trichobilharzia szidati* a positive reaction with lectins has been observed (12). Probably their surface glycosylation patterns reflect to the interaction with their next host. The L-fucose (6-deoxy-L-galactose) is the most prevalent saccharine in some of developmental stages of schistosome species- miracidia of *S.mansoni* and *T.szidati*, mother and daughter sporocysts of *S.mansoni* and cercariae of *S.mansoni* (13-17).

Lectins are defined as protein or glycoprotein of non-enzymatic natures which do not belong to the immunoglobulin super family and which specifically recognize and reversibly bind to carbohydrates (18).

In conclusion, because carbohydrates such as mannose could be using as receptor for specific lectin from *Culex* larva, we imagine which binding lectin-manose may be cause to adhesion of xiphidiocerceria to *Culex* larvae where this process is first stage of host-parasite relationship. This phenomenon could be happened for *Schistosoma* spp. and human skin including in cercarial dermatitis disease in Khuzestan Province.

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The authors declare that they have no Conflict of Interests.

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