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Antibody response to glycan antigens of hydatid cyst fluid, laminated layer and protoscolex of *Echinococcus granulosus*

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Abstract

Background: Hydatid disease is characterized by long-term growth of hydatid cysts in the human. The glycan antigens have an important role in the immunology of hydatid cyst. In this study immunological reaction of host sera to different glycan antigens of the cyst, has been investigated.

Methods: The antibody responses were tested to glycoprotein and glycolipid of the laminated layer (LL), cyst fluid (CF) and protoscolex (PS) antigens of *E. Granulosus* using ELISA and western immunoblotting tests. Thin-layer chromatography and ß-elimination were used for glycan purification.

Results: Both hydatid cyst and normal human sera reacted with hydatid cyst fluid, protoscolices, laminated layer, glycoprotein and glycolipid antigens. The most antigen-antibody reaction was related to CF and PS antigens, and LL antigens had the minimal reaction with the sera. Thin layer chromatography (TLC) of the antigens showed presence of many glycan bands in the laminated layer.

Conclusion: The parasite may elaborate different glycan antigens in LL to evade host immune response.

Keywords: Echinococcus granulosus, Hydatid cyst, Laminated Layer, Thin-layer chromatography, Enzyme Linked Immunosorbent Assay, ß-elimination

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Introduction

Hydatid disease or hydatidosis is a zoonotic disease caused by Larval stage of *Echinococcus granulosus* which is characterised by long-term growth of the cysts in the intermediate host (herbivores) (1-4). It has distributed on many continents, with the highest prevalence in Mediterranean areas and up to 75% of infected people may remain without symptom for more than 10 years (1, 2, 5-7). Hydatid cyst usually is located in the liver and/or the lung (8-10) and is filled with cyst fluid and protoscolices, the cyst wall which is composed of two layers: germinal layer and laminated layer (11). Each of these sectors (laminated layer, protoscolices and cyst fluid) has their own specific antigens that can be immunogenic or non-immunogenic (12, 13).

A hallmark of *Echinococcus* larva is their ability to survive within their hosts for a long time (14, 15). Despite host immune response, the parasite tries to escape the immune response and also down regulate the host defenses with different strategies such as antigenic mimicry, antigenic depletion and antigenic variation and it seems the immune response regulation in this helminth is beneficial for both the human host and the parasites (15-18). Parasite glycans have an important role in this regulation with "glycan gimmickry" strategy (19, 20). The unusual structure and the host-like glycan antigens of helminths are the other mechanisms to escape from host immune system (19).

Helminths produce different glycoproteins and lipids, and

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↑What is "already known" in this topic:
Hydatidosis, as a zoonotic disease, has distributed on many continents with the highest prevalence in Mediterranean areas. Echinococcus multilocularis has a carbohydrate-rich laminated layer with a crucial role in the establishment of the infection in the mammalian host and protecting the parasite from immunological host reactions.

→What this article adds:
Glycan antigens of hydatid cyst have key roles in host-parasite relationship especially evasion from host immune system.

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Table 1. Mean OD results in ELISA of laminated layer; protoscolex (PS), hydatid cyst fluid (CF), different glycoproteins (GP) and glycolipids (GL) with sera of patients with hydatid cyst and normal human sera

Antigens	OD reaction of Ag with Hydatidosis people sera	OD reaction of Ag with healthy people sera	Subtraction of two ODs	OD negative control
Crude CF	1.95	1.37	0.58	0.13
Crude PS	1.55	1.12	0.43	0.13
Crude LL	1.38	1.21	0.17	0.13
GP of CF	1.85	1.08	0.77	0.13
GP of PS	1.50	0.98	0.52	0.13
GP of LL	0.79	0.74	0.05	0.13
GL of CF	0.37	0.27	0.10	0.20
GL of PS	0.37	0.27	0.10	0.20
GL of LL	0.66	0.55	0.11	0.20

it has been shown that these compounds are used to regulate the host immune system (21, 22). For instance, *Echinococcus multilocularis* has a carbohydrate-rich laminated layer, which has a crucial role in the establishment of the infection in the mammalian host and protecting the parasite from immunological host reactions (23). In this context, it has been shown that in hydatid cyst infection some carbohydrate determinants are specific and immunogenic (24-26) and can immunoregulate the course of infection (27).

Therefore, hydatid cyst glycans have been subjects of many research works recently. In this study immunological reaction of host sera with different glycan antigens of hydatid cyst has been investigated.

Ethics statement

This study was approved by the ethics committee of Isfahan Medical University, and sample collection was obtained with a written informed consent of patients and healthy controls.

Methods

In this descriptive research, the study population consisted of sera of either patient with hydatidosis or normal human sera. Normal sera (n=20) and hydatidosis sera (n=20) were collected from different hospitals in Isfahan, Iran. To prepare the antigens, liver and lung hydatid cyst of sheep were collected from Khomeini-Shahr slaughter house in Isfahan. At first, the hydatid cyst fluid was aspirated with a syringe and checked under the microscope for the presence of protoscolices. Following observation of the protoscolices, the cyst was included in the study. Aspirated cyst fluids were centrifuged for sedimentation of protoscolices. The supernatant was stored at -20 °C as hydatid cyst fluid antigen (CF Ag). Sediment protoscolices were sonicated in PBS and stored at -20 °C as protoscolices crude antigen (PS Ag). Afterward laminated and germinal layers were separated with a forceps, homogenized and sonicated in PBS. The mixture was then centrifuged and the supernatant stored at -20 ℃ as laminated layer crude antigen (LL Ag). Glycoprotein and glycolipid of these antigens were purified by chloroform-methanol extraction (28).

Enzyme Linked Immunosorbent Assay (ELISA) was performed as we published before for proteases(29). Briefly, ELISA plate was coated with different dilutions of antigens. Sodium carbonate 15mM and sodium hydrogen carbonate 35mM to 1L with distilled water was used as coating buffer. The plate was then incubated at 37 °C for one hour and afterward in the refrigerator (4 °C) overnight. The plate was washed three times with washing buffer (0.05% Tween in PBS) and then incubated for 1 h with blocking buffer (5% bovine serum albumin). Hydatidosis patients' sera or normal human sera, diluted in incubation buffer, were added and incubated for One hour at 37 ° C. Plates were washed and a relevant enzyme-conjugated was added (anti-human IgG with HRP) and then incubated at 37 °C for 2 hours). In the next stage the substrate 2, 2 ¢ -azino-bis (3-ethylbenz-thiazoline- 6-sulfonic acid) (ABTS) was added, and the reaction was read in a spectrophotometer at 414 nm.

SDS PAGE and Western immunoblotting were performed for antigens on 12% acrylamide gel under reducing and non-reducing conditions using Bio-Rad mini gel instrument. The gels were either stained with Comassie blue or transferred to nitrocellulose papers (NCP) using BIO-RAD apparatus. The papers were then probed with different sera at appropriate concentrations. Following washing with the buffer, the paper was probed with a secondary antibody against human IgG. Finally, appropriate substrate was added to develop reaction of sera with the bands of antigens.

To separate the sugar from the LL, PS and CF, they were treated with ß-elimination procedure (30) at 37 °C. In this method, 0,32gr NaOH and 0,112gr NaBH4 dissolved in 10cc distilled water and added to different antigens and incubated at 37 °C for 24 hours. The mixtures were then centrifuged, and the pH of the supernatant was changed first to zero and then to seven. The yielded sugar solution was lyophilized and stored at -20 °C (30).

Thin layer chromatography (TLC) for glycan antigens was performed by dissolving them in distilled water, and the samples were added on the silica gel using a Hamilton syringe. Then plate quickly dried and placed in a tank containing mobile solution. The water/ethanol/propanol/ ethanol/ triethylamine (2/3/4/2/0.2) mixture was used as mobile in our study. After TLC, Alkaline silver staining was performed for the glycans. After staining The black spot on white background represents glycan compounds (31).

Results

Results of the ELISA test showed that both sera of patients with hydatid cyst and also normal human sera reacted with hydatid cyst fluid, protoscolices, laminated layer,

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glycoprotein and glycolipid antigens. However, the mean OD of hydatid cyst patients' sera was higher than that of normal sera. The highest difference between OD of hydatid cyst patients and normal sera related to glycoprotein antigens and the lowest difference related to glycolipid antigens. On the other hand, most antigen-antibody reaction was related to CF and PS antigens and LL antigens had minimal reaction with our sera (Table1).

Various above antigens (LL, PS and CF) with different dilutions (1/2 and 1/4) run on 12% SDS-PAGE and stained with Coomassie blue (Figure 1).

Figure 2 shows western immunoblotting of protoscolex (PS), laminated layer (LL) and cyst fluid (CF) probed with sera of patients with hydatid cyst or normal human sear.

Laminin layer (LL), protoscolex (PS) and cyst fluid (CF) glycan antigens following ß-elimination were subjected to TLC. Many glycan bands presented in the laminated layer (Figure 3).

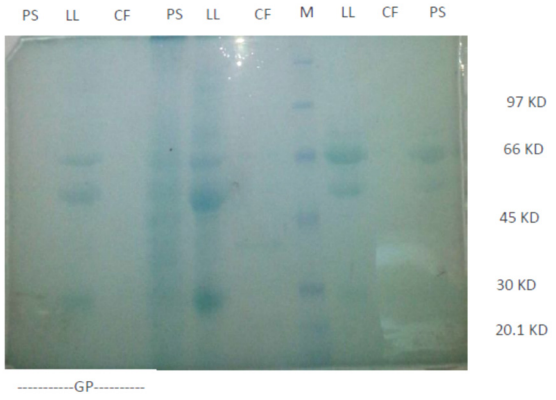


Fig. 1. SDS-PAGE of protein marker (M) and different crude and glycoprotein (GP) antigens: protoscolex (PS), laminated layer (LL), cyst fluid (CF) with different dilutions (1/2 and 1/4 respectively left and right of marker) and following staining with Comassie blue.

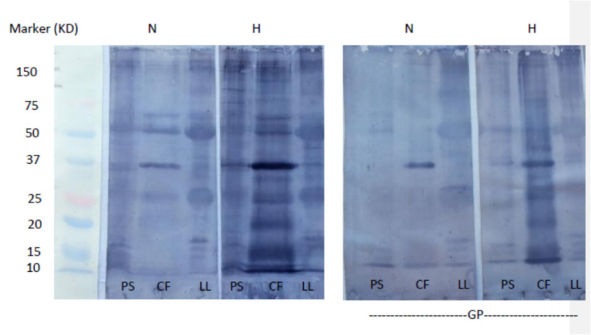


Fig. 2. Western immunoblotting of different crude and glycoprotein antigens: protoscolex (PS), laminated layer (LL) and cyst fluid (CF) probed with sera of patients with hydatid cyst (H) or normal human sera (N).

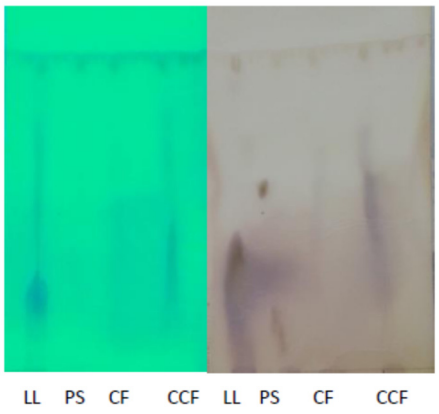


Fig. 3. TLC of laminin layer (LL), protoscolex (PS) and cyst fluid (CF) glycan antigens with condensed crude CF (CCF) antigen as a control. Left image is without staining under UV light and right image is with staining.

Discussion

In our ELISA results, both sera of patients with hydatid cyst and normal human sea cross-reacted with different antigens of E. granulosus. These antigens may have an important role for the parasite to evade from the human immune system. Probably the parasite may elaborate these glycan antigens to raise antibodies that may block the specific sites for effective antibodies (32, 33). So, these antigens may have the potential to abolish production of specific immune responses. In this context, it has been shown that antibodies that Inhibit Malaria merozoite from invasion to erythrocyte are blocked by naturally acquired human antibodies and protozoa can attack to erythrocyte (34).

The laminate layer has a close contact with host tissues. However, according to our ELISA results antibody response to LL to hydatidosis was much lower than a response to protoscolices and cyst fluid. So it is possible that host antigens attach to the laminated layer surface. In agreement with this conception, it has been shown that *E. granulosus* have structural similarity to the host glycoproteins, which they effectively mimic like what we see in the other parasites to evade the immune system (19, 35).

High concentrations of glycol conjugate structure in the surface of hydatid cyst may also suppress several functions of the host immune system such as glycosylphosphatidylinositol and lipophosphoglycan as seen in protozoan parasites (36, 37).

In TLC test, the strip of the LL Ag bands may indicate the presence of a wide range of different sugar in this layer, while PS Ag and CF Ag showed 2 bands or no band, respectively. In agreement with this result it has been shown that laminated layer is highly glycosylated (38-40).

The role of glycans in host immune evasion has also been shown in other helminths such as Schistosoma and filarial parasites (37). The tegument of *S. mansoni* contain an abundance of synthesize and adsorb host glycans molecules that use as a mechanism of immune evasion, and this helminth might disguise itself with the glycans against the attack of immune effectors (41). Filarial nematodes produce phosphorylcholine that anchors on the surface carbohydrate moieties and modulates host immune responses (42).

So in hydatid cyst , glycol conjugates may have a key function in host-parasite interaction such as protecting the parasite by regulating the host's immune responses (19, 20, 38).

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Kanan JH and Chain BM, used the different protein bands of cyst fluid from SDS technique as a dendritic cells maturation and agents to regulate immune responses of the host (43). Also, it has been shown that immune evasion by hydatid cyst related to the production of different antibodies (44, 45). In the other hand, the carbohydrate-rich structure of LL of hydatid cyst is considered as protective material against host attack (46, 47). These observations are in agreement with our findings and confirm that glycan antigens of hydatid cyst have key roles in host-parasite relationship especially evasion from host immune system.

Conflict of Interests: None declared.

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