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A Nonglycosylated 27 KDa Molecule as Common Antigen between Human Breast Cancer and *Echinococcus granulosus* Hydatid Cyst Wall

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Abstract

Background: Hydatid cyst, which has anti-cancer activities, is outwardly covered with the cyst wall. It is in close contact with the host tissues and its molecules presented to the immune system. In this work immunological reaction of the sera of breast cancer patients with the hydatid cyst wall antigens has been investigated. Method: For this purpose, sera of patients with breast cancer, hydatid cyst and normal human sera were collected and their reaction with hydatid cyst wall antigens was tested using western immunoblotting technique. Results: All sera of patients with breast cancer, hydatid cyst and also human normal sera reacted with a band in western immunoblotting. However, sera of patients with breast cancer showed reaction with a 27 KDa band. Results of this work also revealed that this band was not glycosylated and may express only in some stages of breast cancer development. Conclusion: Sera of patients with breast cancer cross reacted with a nonglycosylated antigen of hydatid cyst wall. However, more work is recommended to find if this band involves in anticancer activity of the hydatid cyst.

Keywords

Breast Cancer, Cross Reaction, Hydatid Cyst

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1. Introduction

Hydatid cyst is the larval stage of the tapeworm *Echinococcus granulosus* which is located in human and livestock viscera. Cysts act as space occupying lesion and put pressure on adjacent tissues. Clinical manifestations depend on the location of the cyst. Diagnosis of the disease relies on Para clinical methods including imaging and laboratory immunological techniques. The main therapy is surgery although albendazole is also used for treatment.

The cyst is outwardly covered with a carbohydrate rich material termed laminated layer [1]. Hydatid cyst, like most helminthes, polarizes the host immune response toward a Th2 type response [2]. However, this Th2 shift in hydatid cyst is not as marked as in other helminthes and a Th1 type response may also be detectable [3] [4]. Laminated layer is in close contact with host tissues and must be the major source of hydatid cyst molecules that immune system encounters.

Anticancer effect of hydatid cyst has been shown in cell culture experiments [5] [6] and also in animal model investigations [7] [8] or in human population [9]. However, the mechanism of this effect is still not clarified. Although this anti-cancer activity may be related to share antigens which exist between this parasite and cancers. In this context glycosilated antigens have been shown to exist both in hydatid cyst and some cancers [10]. Because cyst wall is in close contact with host tissues, in this work reaction of sera from patients with breast cancer and cyst wall antigens has been investigated.

2. Materials and Methods

Hydatid cyst antigens: In this descriptive study, sheep hydatid cysts were collected from khomainishahr slaughter house in Isfahan, Iran. The cysts were diagnosed according to their especial appearance in liver or lung and confirmed by observation of protoscolices in microscopic examination. Using sterile needle and syringe, hydatid cyst fluid was aspirated from the cyst and then collected in 50 ml test tubes. One drop of the collected fluid was tested under microscope for presence of protoscolices (**Figure 1**). Hydatid fluid without protoscolices was discarded and ones with protoscolices were centrifuged at 2000 g for 2 minutes and the supernatant was collected as hydatid cyst fluid antigen. In order to make cyst wall antigen the cyst membrane existed from the cyst and then washed and sliced in phosphate buffer saline (PBS). The mixture was then sonicated, centrifuged and the supernatant was collected as cyst wall antigen.

Western immunoblotting: Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was performed for both hydatid fluid and laminated layer antigen and the then transferred on nitrocellulose papers (NCP) as we published before for schistosomes [11]. The antigens in membrane were then coated with sera of patients with breast cancer or hydatid cyst. Normal human serum was used as control. Spare sera of patients with breast cancer were collected with informed consent from clinical laboratory of Saideoshohada hospital in Isfahan, Iran. Spare normal sera were collected from blood bank in Shahrekord, Iran.

Sodium metapenodate treatment of antigens on nitrocellulose paper: To inactivate carbohydrate epitopes, two NCP papers coated with the same antigen (case and control) were prepared. Then both of them washed with 0.1 M sodium acetate (pH 4.5) for couple of minutes. The case paper was then incubated at 37˚C for 1 h with 20 mM sodium m-periodate (Sigma) dissolved in 0.1 M sodium acetate. The control paper was treated with 0.1 *M* sodium acetate in the same conditions. After 2 washes with sodium acetate and one with PBS, the papers were

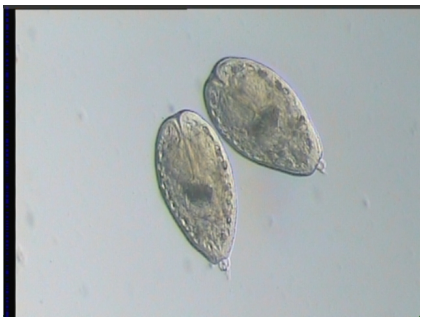

Figure 1. Protoscolices of hydatid cyst observed in hydatid cyst fluid under microscope with 100 times magnification.

treated with 50 mM sodium borohydride (Sigma) in PBS. The reaction was stopped after 30 min at room temperature by 5 washes with 10 mM Tris/HCl (pH 8.0), 150 mM NaCl, and 0.05% Tween-20. Subsequently, the routine western immunoblotting protocol was applied.

3. Results

In western immunoblotting cyst wall antigen and hydatid fluid antigens were probed with pooled sera of patients with breast cancer, hydatid cyst or normal human sera. All sera reacted with a band with molecular weight above 40 KDa band both in cyst wall and hydatid cyst fluid. However, breast cancer sera reacted with a 27 KDa band in laminated layer (**Figure 2**). To find if all individual sera of patients reacted with the 27 KD band, the laminated layer was probed with sera of 10 individual patients with breast cancer. It was revealed that the reaction was not consistent in all patients' sera (**Figure 3**). To figure out if the reaction to cyst wall antigens related to glycan epitopes, cyst wall in NCP paper was treated with sodium metaperiodate and then probed with pooled sera of patients with breast cancer. The results showed that while the 27 KD band was sodium metaperiodate resistant other bands disappeared following sodium metaperiodate treatment (**Figure 4** & **Figure 5**).

4. Discussion

Results of this work revealed that sera of patients with breast cancer cross reacted with a nonglycosylated 27 KD band of hydatid cyst wall.

Immunological cross reaction between antibody against hydatid cyst and antibody against other parasites or antibody of patients with noninfectious diseases has been reported [12]. Common antigens among parasites have been reported, for example, it has been shown that *Taenia crassiceps* which is an animal infection and *Taenias olium* which is a human infection have some share antigens [13]. In another investigation immunological cross reaction among different helminthes antigens has also been reported [14]. Immunological cross reaction

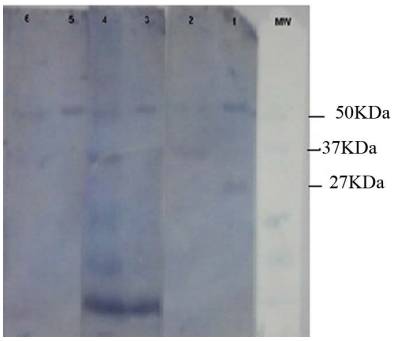

Figure 2. Western immunoblotting of cyst wall (lanes 1, 3 and 5) or hydatid cyst fluid (lanes 2, 4 and 6) probed with pooled sera of patients with breast cancer (1 and 2), hydatid cyst (3 and 4) or normal human sera (5 and 6). MW stands for molecular weight marker.

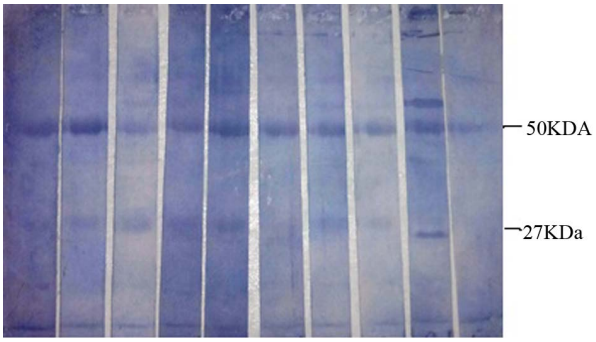

Figure 3. Western immunoblotting of the hydatid cyst wall antigens probed with sera of 10 individual patients with breast cancer.

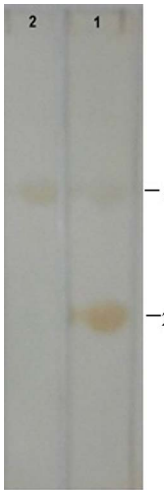

Figure 4. Western immunoblotting of the hydatid cyst wall antigens probed with the pooled sera of patients with breast cancer (1) or pooled normal human sera (2) before treatment with sodium metaperiodate.

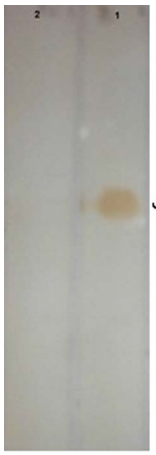

Figure 5. Western immunoblotting of the hydatid cyst wall antigens probed with the pooled sera of patients with breast cancer (1) or pooled normal human sera (2) after treatment with sodium metaperiodate.

between hydatid cyst antigens and other infections such as amoebic liver has also been reported [15].

Presence of share antigens between tumors and components of nervous system has been reported in the previous studies [16]. Also immunological cross reaction between *Besnoitia besnoiti* antigens and anti *Neospora caninum* has been shown [17].

It has been shown that much of the immunity to helminthes in mammalian hosts directed against glycan epitopes [18]. In hydatid cyst, it has also been shown that carbohydrates have an important contribution in host immune responses [19] [20]. So glycan epitopes may be responsible for majority of cross reactions that exist between hydatid cyst and other infectious and non-infectious agents. Although antibody response to helminthes is mostly directed against helminthes glycan epitopes, in our work sera of patients with breast cancer cross react with a peptide band of hydatid cyst. So it is interesting that a peptide molecule of the hydatid cyst laminated layer cross reacts with the sera of patients with breast cancer. It would be very worthwhile to sequence this peptide band in order to find if there is a similar molecule on the surface of cancer cells.

Results of the present investigation also revealed that reaction of sera of 10 patients with breast cancer reacted with the 27 KDa band of hydatid cyst wall was not consistent among all individuals. Some sera showed strong

reaction while others showed some weaker reaction. So it seems that this peptide antigen expresses in specific stage in breast cancer development. Therefore more investigations are needed to find if there is a correlation between expression of 27 KDa band and the stage of the cancer.

Finally anti-cancer effect of hydatid cyst has been shown in different investigations [5]-[10] [21]-[25]. So it would be worthwhile to search whether this 27 KDa molecule involves in anti-cancer activities of the hydatid cyst or not.

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Disclosure

None of authors have conflict of interest.

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List of Abbreviations

NCP: Nitrocellulose paper.
PBS: Phosphate buffer saline.
SDS PAGE: Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis.