Antigen Similarity In Hydatid Cyst Wall And Human Bone Tumours: A Short Report

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Short report

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Abstract

Background: less studies have been done on bone cancers which are complex despite lower incidance. Hydatidosis is a parasitic disease that may influence host immunity by mimicking cancer cells antigens. So, this study aimed to elavuate the similarity of the immunogenic antigens between hydatid cyst and different bone cancers.

Method: Cyst wall of hydatid cysts were collected and their antigens were separated with SDS-PAGE gel electrophoresis (SDS-PAGE). Serum samples obtained from patients with bone cancers and the anitigenicity of isolated anitgens were evaluated inwith *E. granulosus*(Larval form)infection and healthy individuals using western-blot approaches.

Results: The crude extract of the laminated layer showed two specific antigens, 53 KDa and 70 KDa, after stainging the membrane with Coomassie blue. Both antigens reacted with the serum of different bone cancers but only the 53 KDa band reacted with all sera.

Conclusion: It seems people with bone tumours may have extra antibodies in their serum comparing to healthy and hydatidosis which may be an autoantibodies; and the presence of this antibody against 70 KDa band protein in sera of patients with various types of bone cancers, may be helpful in diagnostic test or designing of preventive approaches in future.

Background

Bone tumours are biologically diverse and complex, although bone metastases or secondary bone cancers are very common after lung and liver cancer. Fortunately, primary bone cancers, which originates in the bone, are rare [1] thus, less studies focued on primary bone cancers compared to other cancers [2]. Primary bone cancers are classified into 15 different categories including cartilage, osteogenic, fibrogenic, fibrohistiocytic, hematopoietic, giant cell, notochordal, smooth muscle, vascular, lipogenic and neurological, Ewing sarcoma / primary neuroectodermal tumour, miscellaneous tumours, and joint lesions [3, 4, 5]. Osteosarcoma (35%), chondrosarcoma (30%), and Ewing sarcoma (16%) are the most common forms of bone cancers. Malignant fibrocystic histiocytoma, fibrosarcoma, chordoma, and giant cell tumours accounts as a less prevalent tumours which compromise 1–5% of all primary malignant bone tumours [6]. Hydatid cyst, which is the larval stage of the cestode worm *Echinococcus granulosus*, is a common parasitic infection affecting human and animals. E.granulous cyst composed of different layers in which the last external layer, laminated layer (LL), has a carbohydrate-rich structure that protects the parasite against the destruction by host immune system molecules [7]. Cancer cells and parasites shared some common features and the presence of similar antigens between them has been reported; in he other hand, it has been postulated that some parasites may have anti-cancer activities due to having similar antigene there fore, administrating the paprasite anitgens as target for cancer immunotherapy have been gain the attraction [8] Some parasites such as *Trypanosoma cruzi (T. cruzi)*, *Toxoplasma gondii (T. gondii)*, *Toxocara Canis*, *Acanthamoeba castellani*, and *plasmodium Yoelii* have been showed

to have a anticancer activity in the experimental animals [9, 10, 11] henece some parasites such as *T. cruzi*, *Echinococcus granulosus (E. granulosus) and T. gondii* shared a similar carcinogen antigenes such as the presence of the cancer-associated mucin-type O-glycans in hydatid cyst [12, 13]. So it is suggested that *E. granulosus* larva may induce anticancer effect through activating the host immune response [14], for example, a mucin-type-O-glycans (glycosylated 27 kDa molecule) pf this helminth is similar to human breast cancer antigens and can introduce to the host immune system for later protection against cancer [15]. In liver and lung hydatidosis, induce seceration of cytokines (IFN, TNF-α, and IL-6) [16] contributing host immune defence against tumours. Also, several *Echinococcus* antigens can induce antibody-mediated immunity, that can establish non-specific immunity against some cancer [17]. Inquiries show an immunological association between the laminated layer of hydatid cyst and different cancer cells, therefore in this study, antigens similarity between hydatid cyst and different bone cancers were evaluated.

Method

Patients and sera

Patients with different type of bone cancers which their dieases have been confirmed by clinical and paraclinical tests including CT SCAN, MRI, and finally physician decision, have been enroleed in the study . Blood samples were taken from pateints, centrifuged at 5000 g for 15 min, and the sera was separated, and stored at −20°C. Initially, the serum samples were examined by Human IgG ELISA (yekta-tajhiz) kit to find *E. granulosus* antibodies and positive samples were excluded, finially about 53 samples were included in this study.

Antigen preparation

The hydatid cysts of sheep were collected from a slaughterhouse and the laminated layer detached easily from the fibrous layer of the hydatid cyst with a sterile forceps, following washed several times in phosphate-buffered saline (PBS) crushed and sonicated at150-W ultrasonic disintegrator 15 min on ice. These procedures were done in a sterile situation as much as possible and crude antigens were stored at -20℃ [18].

SDS-PAGE

1 µg of LL of hydatid cyst antigens mixed with 4X SDS sample buffer,loaded on double %12 polyacrylamide gel and electrophoresis was performed in a vertical electrophoresis chamber (Bio-Rad tank -80Amp, 10V, 90min). One of these gels stained with Coomassie blue and the other used for western blot.

Western blot

pooled serum of 10 patients with *E. granulosus* infection and 10 healthy individuals without antibodies of the parasite in their serum, were considered as a positive and negative control and case serums

composed of 53 patients with various bone cancers. The resulted SDS-PAGE gel electrophoresis was transferred to nitrocellulose paper (Sartorius 0.45 μm) by electrophoretic transfer in a Hoeffer Miniblotter at 100 mA/gel for 2 h, then the nitrocellulose membrane blocked with 5% bovine serum albumin (BSA) and incubate in 0.3% Tween 20 for 1 h, then washed in PBS / Tween 0.1%, and finally pooled sera of bone cancer patients, hydatid cyst, and normal human sera were added, separately. Peroxidase reaction with 0.06% (w/v) diaminobenzidine tetrahydrochloride in 50 mM Tris–HCl (pH 7.6) and 0.03% (v/v) H2O2 visualized different bands and the reaction was stopped after 5 min with distilled water [12].

Result

Demographic data of patients with different bone cancers with negative anti-Echinococcus IgG are summarized in Table 1. The crude extract of the laminated layer on SDS-PAGE displayed 53 KDa and 70 KDa bands after Coomassie blue staining (Fig. 1A). In western immunoblotting of cyst wall antigens, a 50 KDa band was seen while pooled serum of hydatidosis and healthy people's sera were added but both 53 and 70 KDa bands were also seen in a case of adding pooled serum of bone cancer patients (Fig. 1B).

Table 1
Clinical and pathological data of bone cancer patients

Characteristics		*Number of patients (n = 53)*
Gender	Female	24 (45.28%)
	Male	29(71.54%)
Age	> 20 years old	14 (26.4%)
	20–40 years old	26(49%)
	40–60 years old	7(13.2%)
	≤ 60 years old	4 (7.5%)
Malignant tumour	Osteosarcoma	11(34.37%)
	Ewings sarcoma	7(28.12%)32 (60%)
	Chonderosarcoma	9(21.87%)
	Other Malignant tumour	5(15.42%)
Benign tumour	Osteochondroma	5(23.80%)
	Giant tumour	6(28.57%)21 (40%)
	Exostosis	5(23.80%)
	Other benign tumour	5(23.80%)
Chemotherapy	Yes	15(30%)
	no	35(70%)

Discussion

Epidemiological studies have demonostrated that that infection with helminthic parasites is associated with a low incidence of allergy/asthma and autoimmunity in developing countries [19]. Henece, helminthic therapy, using an experimental type of immunotherapy, can be also target to autoimmune diseases and immune disorders through deliberate encountering with helminthic antigens [19, 20]. In this study, hydatid cyst antigens specially laminated layer antigens reacted with pooled sera of bone cancer patients and it is observed that people with bone tumours had an extra antibodies in their serum comparing to healthy and hydatidosis which may contributes to the a possibility of developing autoantibodies or extra antibodies in people with this type of tumour. It has been shown that mucin-type O-glycan structures of cancers are also expressed by helminths, for example, the Tn antigen was shown in larval stages of *E. granulosus* seems to have an important functions in the interaction of parasites with their hosts [21, 22]. In immunology of helminths' infection, a 53 KDa antigen of hydatid cyst wall that reacted with human sera probably has an important role in evasion from the host immune system [23]. In our work, a 53 KDa antigen cross-reacted with healthy, hydatidosis, and bone cancer people which may leads to envaide from the human immune system and block the site of effective antibodies such as IgE (blocking antibodies) [24]. Identification of tumour-associated antigens or the corresponding autoantibodies in the body fluids as potential noninvasive biomarkers could be an effective approach for early detection and monitoring of cancer. An overview of the differentially expressed protein, antigen, and autoantibody biomarkers was presented as clinical appraches for early detection of lung cancer [25]. The presence of antibodies against 70 KDa band protein, in sera of patients with various types of bone cancers was observed, whereas normal populations do not exhibit such antibodies, which can be helpful for diagnosis of bone cancers in future. For example, H3.3 G34W/R/V mutant-specific antibodies proposed to be helpful for the diagnosis of giant cell tumours of bone and its variants [26]. Recent studies in Europe and Central Asia have shown that there is a connection between hydatids and the lifetime of persons with tumours,also, the median survival rate for patients with liver cancer and hydatid cysts were 17 months, while the surivial rate of patients with advanced hepatocellular carcinoma (HCC) patients was only 3–5 months [27]. In thiscurrent study, the antigen of laminated layers of hydatid cysts induce an antibody which could render the prarasite infection along with attenuating the tumore proression suggesting the preventive effect of induced antibody against tumore development.. Recently most researches are focued on the immunotherapy of helminth for treatment of bone tumours and other forms of cancer [22]. However further studies are needed for proper isolation and administration ofthis hydatid cyst antigen on animal models. Scientific evidence indicated that parasite infections interfere with tumour growth either in the human population or experimental animals and it appears that parasitic antigens may be used for cancer-targeted therapy in the future. Immunized mice with hydatid cyst antigens and passive transfer of spleen cells of infected mice, had shown lower growth of melanoma tumour on their skin in contrast with non-immunized mice [28, 29]. However, there are few studies on the effect of parasitic antigens on cancer treatment, we are still in the early stages of understanding this relationship.

Conclusion

It seems that people with bone tumours may have extra antibodies in their serum cin comparsion to healthy and hydatidosis which may be as autoantibodies.The presence of this antibody against 70 KDa band protein in sera of patients with various types of bone cancers, may be helpful for diagnosis of cancer in future or prevent the tumour development.

Declarations

Ethics approval and consent to participate

The study was approved by the Institutional Research Ethics Committee School of Medicine of Iran with code number: IR.IUMS.REC.1397.1283. All participants were informed before surgery and accepted the informed consent.

Consent for publication

Not applicable

Availability of data and material

Not applicable

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

All authors reviewed and approved the final manuscript. All authors read and approved the final manuscript

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Figures

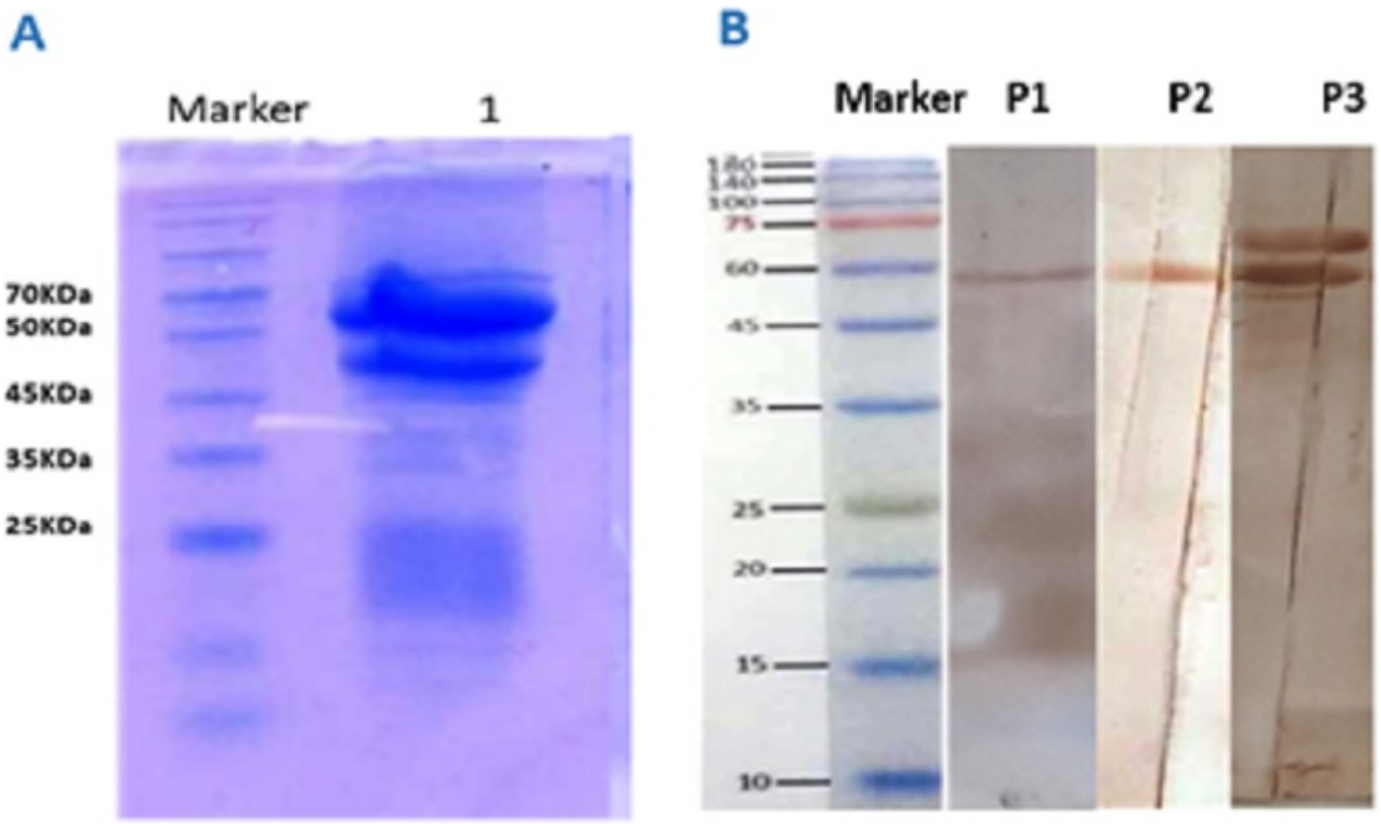

Figure 1

A-SDS-PAGE of laminated layer suspension stained with Coomassie blue. Cullum 1: 1 µg LL suspension, respectively. B. Echinococcus Western Blot IgG. Cyst wall antigens were probed with pooled different sera. P1: healthy individuals, P2: patients with hydatid cyst, and P3: patients with different bone cancers.

Supplementary Files

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