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ORIGINAL ARTICLE

Sero-epidemiological value of some hydatid cyst antigen in diagnosis of human cystic echinococcosis

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Abstract Cystic echinococcosis (CE) is a severe zoonotic disease which affects both human and animals. The disease has a considerable economic and social impact, because it has numerous complications leading to important disabilities and even death. CE is a widespread chronic endemic helminthic disease caused by infection with metacestodes of tapeworm *Echinococcus granulosus*. This study was conducted to diagnosis human CE by hydatid cyst antigens from camels and sheep. Hydatid fluid and protoscoleces crude antigens corresponding to camel and sheep were resolute by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions and the protein bands of different antigens were exposed to infected patients serum CE through western blot (WB) assay. The camel hydatid fluid antigen revealed five polypeptide bands of 18–98.8 kDa by SDS-PAGE while sheep hydatid fluid antigen revealed four polypeptide bands of 20–100 kDa. Immune reactive bands were obtained through WB ranged from 25 to 125 kDa. The study showed prominent immune reactive bands of 92, 52.2 and 35.7 kDa which may helpful in diagnosis of human CE.

Keywords Cystic echinococcosis (CE) · Human · Camel · Sheep · SDS-PAGE · Western blot (WB)

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Introduction

Human cystic echinococcosis (CE) is a serious life-threatening neglected zoonotic disease that occurs in both developing and developed countries and is recognized as major public health problem (Mandal and Mandal 2012). The geographical distribution of the members of genus *Echinococcus* is characterized by the presence of different strains existing in each country (Rausch 1995; Eckert and Deplazes 2004). The genetic characterization of the members of genus *Echinococcus* in different animals; sheep, pig, cattle and camel were also based on morphology, host specificity and molecular characteristics (Zhang et al. 2006; Tawfeek et al. 2009).

The diagnosis of human CE is usually established by a combination of imagistic methods; Ultrasound, CT scan, X-ray, MRI and serological immunodiagnosis (Constantea and Ciobanca 2007). Specific antigens of *Echinococcus granulosus* hydatidosis of camel and equines were characterized through SDS-PAGE, the antigens showed common shared protein bands at the level of 66, 55, 45 and 29 kDa. The WB assay recognized two polypeptides at 80 and 150 kDa which might be diagnostic bands in the two intermediate hosts (Mahmoud et al. 2008). IgG anti-echinococcus was determined by ELISA and WB and confirmed the positive results done by imagistic methods (Ciobanca and Junie 2011). The diagnostic efficacy of crude sheep hydatid cyst fluid (HCF), antigen B and its subunit (12 kDa) to detect IgG antibodies in CE patient serum was evaluated using ELISA assay, it was concluded that sheep was the most closely related isolate to human at antigen B level and recommended to use anti-HCF IgG ELISA for initial screening in large seroprevalence studies (Tawfeek et al. 2011).

In Egypt, several studies have been done on hydatidosis in both man and livestock for estimation of the prevalence of

infection rates in camels, donkeys, horses, pigs and sheep which were 40, 7.69, 6.25, 0.92 and 0.77 % respectively (Derbala and Zayed 1997). The SDS-PAGE and WB analysis of human and camel hydatid fluid antigens revealed immune reactive 50 kDa band which was prominent in serum of patients (El Zayyat et al. 1999). Moreover, the electrophoretic make up of camel protoscoleces PCAg and camel hydatid fluid HFC Ag showed common bands of 205, 191 and 98 kDa and the WB analysis revealed three polypeptides of PC and HF Ag by rabbit anti PC and HFAg of molecular weight 191, 149 and 45 and 190, 149 and 33 kDa, respectively (Abdel-Rahman et al. 2003).

The performance of western blot assay (WB) using native antigen B which prepared from sheep hydatid cyst fluid (HFS) for diagnosis of human hydatidosis was evaluated, 8, 16 and 24 kDa subunits of antigen B was detected and the 8 kDa subunit, was highly recommended for the confirmatory diagnosis of hydatidosis (Sarkari et al. 2007). WB assay was stated as more confirmatory test than indirect haemagglutination assay (IHA) and the WB IgG technique indicated the presence of immune reactive bands against *E. granulosus* and *Echinococcus* multilocularis: 7, 12, 15, 24, 26 and 28 kDa in the patient serum samples and was considered specific for cystic echinococcosis (Logar et al. 2008).

However, it is still need of confirmation of clinical diagnosis and epidemiological surveys in detection of human Cystic Echinococcosis (Ito et al. 2003); So that the aim of the present work was to determine the antigenicity and sero-epidemiological value of some hydatid cyst antigens from camels and sheep in diagnosis of human Cystic Echinococcosis.

Materials and methods

Hydatid cysts collection

The hydatid cysts were collected from lung and liver of naturally infected camels and sheep slaughtered at Cairo abattoir. The cysts kept in ice boxes and immediately transferred to the laboratory. The fertile hydatid cysts that possess viable protoscoleces were chosen and marked according to the procedures of Himonas et al. (1994). The protoscoleces and hydatid fluids of each cyst were collected aseptically and preserved in normal saline at −20 °C for antigen preparation.

Antigens preparation

Protoscoleces crude antigen

The crude antigen of protoscoleces for both camels (PCAg) and sheep (PSAg) origin were prepared as the methods described by Gasser et al. (1988).

Hydatid fluid crude antigens

The hydatid cysts fluids were aseptically aspirated from fertile unilocular hydatid cysts of camel and sheep. The collected fluids of each animal type; HFC and sheep HFS were centrifuged separately at 12,000 rpm for 15 min at 4 °C. The supernatant fluids were dialyzed against 0.1 M phosphate buffer solution (PBS, pH 7.2) at 4 °C, transferred to sterile tube as HFC and HFS crude antigens and stored at −20 °C until use (Nasrieh and Abdel-Hafez 2004).

Detection of antigen protein concentration

The protein concentration of the both protoscoleces and hydatid fluid antigen was determined using the method of Lowry et al. (1951) with slight modification.

Serum of human cystic echinococcosis

Two serum samples were obtained from two clinic patients admitted to the Department of Tropical Medicine, Faculty of Medicine, Ain Shams University. The patients complained acute abdominal pain and showed soft abdominal masses (hydatids) of moderate size being confirmed by abdominal sonography and serologically using ELISA test. The present history of the two patients showed no other parasitic infection or chronic diseases. Stool examination and urine and blood analysis were done for confirmation.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

The assay was adopted for PC, PS, HFC and HFS antigens as the procedures of Laemmli (1970). The molecular weight marker was included into the gel. After separation, the gel was fixed in methanol and stained with silver stain according to the method of Wray et al. (1981).

Western immunoblot (WB)

Immunoblot assay was utilized to identify the immune reactive components. The assay was carried out as the method described by Towbin et al. (1979), briefly, the protein bands of different antigens were electrophoretically transferred from SDS-PAGE to nitrocellulose sheet and the human sera were diluted in 5 % BSA in 0.3 % PBS-T, and then a nitrocellulose sheet was exposed to the diluted patient sera for one hour for detection of specific diagnostic antigens of human CE.

The molecular weight of polypeptide bands obtained by SDS-PAGE and western-blot were calculated using image software; www.bio-rad.com/prd/en/US/LSR/PDP/Image-Lab-Software.

Results

Electrophoretic assay (SDS-PAGE)

The electrophoretic profiles of camel and sheep hydatid fluids and protoscoleces crude antigens were assayed by SDS-PAGE; the electrophoretic pattern of camel hydatid fluid antigen (HFC) was separated into five bands with the molecular weights: 98.8, 61.2, 50.7, 23 and 18 kDa. While, camel protoscoleces antigen (PC) revealed seven bands with molecular weights of 141.7, 137.5, 99.7, 84, 60.5, 51.5 and 30.4 kDa. The electrophoretic profile of the sheep hydatid fluid antigen (HFS) showed four bands with molecular weights of 100, 60, 49.5 and 20 kDa, at the same time, the electrophoretic profile of the sheep protoscoleces antigen (PS) resulted in five bands with molecular weights of 103.1, 99.1, 96.5, 50.1 and 28.3 kDa (Table 1; Fig. 1).

Western immunoblot analysis (WB)

The immunoblot reactions were detected by WB assay where, the different separated protein bands of crude antigens (HFC, PC, HFS and PS) were passed against infected human patient's antisera. The recognized immunoreactive bands were: 125, 92, 52.2, and 33 kDa for HFC antigen, 92 and 45.5 kDa for PC antigen, 100, 52.2 and 35.7 kDa for HFS antigen and 35.7 and 25 kDa for PS antigen (Table 1; Fig. 2).

Discussion

Evaluation of cystic fluid and protoscoleces as alternative source of antigen by using the immune diagnostic methods is important tool in sero-diagnosis of cystic echinococcosis infection (Mahmoud et al. 2008; Youssef et al. 2010). In the present study, 4 types of antigens (hydatid fluid and protoscoleces of both camel and sheep) were investigated with respect to the epidemiology of *E. granulosus* in human, camels and sheep. Also, the broad immunogenicity of some molecules detected in the current research raise the chance to be used as immunodiagnostic tools in human infections with hydatidosis.

Extraction of special protein fraction using SDS-PAGE electrophoretic analysis to use as antigens derived from cyst fluid (Heath et al. 1992) and protoscoleces (Hernandez and Nieto 1994) could be able to induce accurate diagnosis for hydatid infected animals and man. In our investigation SDS-PAGE of the 4 antigen types revealed separated similar or nearly identical protein molecular bands between hydatid fluid antigens 100 and 98.8; 61.2 and 60; 50.7 and 49.5 kDa from camel and sheep, respectively. Also, camel and sheep protoscoleces Ag revealed relatively similar

Table 1 Electrophoretic separation camel and sheep hydatid antigens (SDS-PAGE) followed by subsequent western blot assay (WB)

	Camel		Sheep	
	Hydatid fluid antigen (HFC)	Proto-scoleces antigen (PC)	Hydatid fluid antigen (HFS)	Proto-scoleces antigen (PS)
SDS-PAGE	Five bands of MW: (98.8, 61.2, 50.7, 23 and 18) kDa	Seven bands of MW: (141.7, 137.5, 99.7, 84, 60.5, 51.5 and 30.4) kDa	Four bands of MW: (100, 60, 49.5 and 20) kDa	Five bands of MW: (103.1, 99.1, 96.5, 50.1 and 28.3) kDa
Western-blot	Four bands of MW:(125, 92, 52.2 and 33) KDa	Two bands of MW:(92 and 45.5) kDa	Three bands of MW: (100, 52.2 and 35.7) kDa	Three bands of MW: (35.7 and 25) kDa

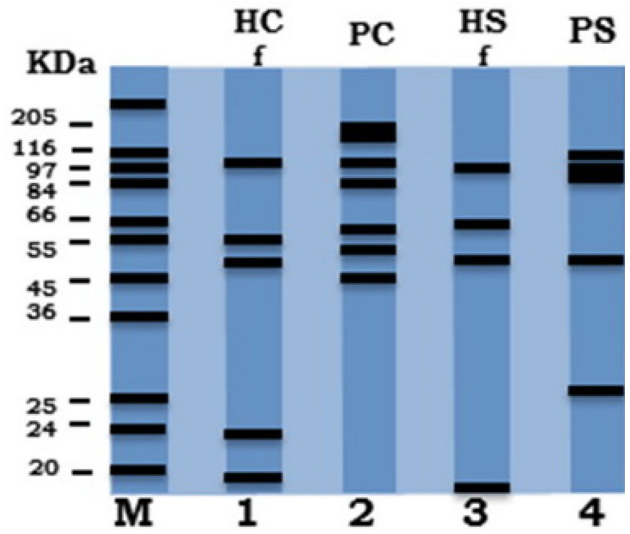

Fig. 1 Comparative electrophoretic profile (SDS–PAGE): camel hydatid fluid (HC) *lane 1*, camel protoscoleces extract (PC) *lane 2*, sheep hydatid fluid (HS) *lane 3*, sheep protoscoleces extract (PS) *lane 4* and molecular weight standards in kDa *lane M*

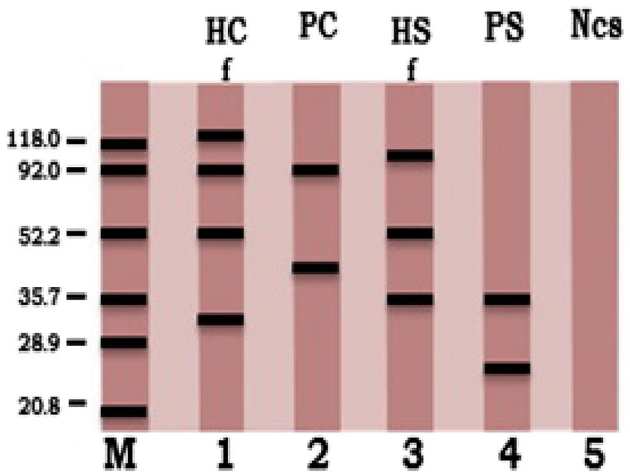

Fig. 2 Immunoreactive *bands* identified by infected human serum with hydatid cyst through western blot assay (WB): camel hydatid fluid (HC) *lane 1*, camel protoscoleces (PC) *lane 2*, sheep hydatid fluid (HS) *lane 3*, sheep protoscoleces (PS) *lane 4* and negative control serum (Ncs) *lane 5* and molecular weight standards in kDa *lane M*

molecular bands: 99.7 and 99.1; 51.5 and 50.1 kDa. The similarity between hydatid fluid antigens and between protoscoleces antigens obtained from different animal species was accepted as obtained by many other studies (Mahmoud et al. 2008; Derbala and Zayed 1997; El Zayyat et al. 1999).

The immune reactive bands detected by the western immunoblot against infected patient's serum with hydatidosis revealed one similar band 92 kDa in both camel hydatid fluid and protoscoleces antigens and also one similar band of 35.7 kDa in both sheep hydatid fluid and protoscoleces antigenss. At the same time, one similar bands of molecular weights 52.2 kDa in camel and sheep hydatid fluid antigens; while, no sharing similar bands were found in PC and PS. The present results were in agreement with (El Zayyat et al. 1999), who detected a band of 50 kDa in running WB using camel hydatid antigens. On the other hand some studies showed high molecular weight band above 60 kDa in WB analysis (Mahmoud et al. 2008; Abdel-Rahman et al. 2003), while other ones showed low molecular weight ones, below 30 kDa (Ciobanca and Junie 2011; Tawfeek et al. 2011; Sarkari et al. 2007).

It is obvious from this study and many authors' results which showed different data of human cystic *Echinococcus* infection that CE is still a public health problem and predominantly in many countries (Schantz 1991; Siracusano et al. 2008; Huang and Zheng 2012). Therefore, clinicians and health authorities should pay greater attention to CE and should make additional efforts to decrease or even eliminate this disease in the future.

Conclusion and recommendations

It could be concluded that immunological characterization of both camel and sheep hydatid fluid and protoscoleces antigens, beside the immune-reactive study may be helpful for diagnosis of hydatidosis in human. However, immunological procedures for the detection of antibodies to hydatid infection need epidemiological studies so effective measurements might become implemented for a better control of this zoonotic Cystic Echinococcosis which should be considered and need further examination and treatment.

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