**ORIGINAL ARTICLES**

**Does the Origin of Hydatid Cyst Antigen Affect Diagnosis of Human Hydatidosis?**

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**ABSTRACT**

Hydatidosis is an important public health problem worldwide and one of the most important zoonotic diseases in Egypt. Several serological diagnostic methods were adopted worldwide depending usually on antigens of animal origin. The present study investigates if the origin of hydatid cyst (HC) antigen affects diagnosis of human hydatidosis using enzyme linked immuno-transfer blot (EITB) technique. Fractionation of fertile HC fluid antigens (FHCFA) of human and camel origin and reaction of different fractions on nitrocellulose strips (NC) versus infected human and camel sera revealed four specific protein fractions at MW of 38, 36, 18 and 8 kDa. Protein fraction of 18 kDa proved to be highly species specific as it did not react after treatment of human origin antigen with infected camel sera and vice versa. It did not cross react with antibodies (Ab) in sera of patients infected by *Schistosoma mansoni*, Virus hepatitis C and enteric protozoa and it did not cross react with Ab in sera of gastro-intestinal nematodes (GIN.), *Monezia trigonophora* and *Cysticercus dromedary* infected camel using EITB technique. In conclusion, protein fraction of 18 kDa is considered to be highly species specific hydatid antigen. This fraction succeeded in excluding cross reacted antibodies in sera of examined patients, the matter which recommended using this human origin antigen only for accurate diagnosis of hydatidosis in suspected patients.

**Key words:** Hydatid, Antigen, EITB, Human, Camel.

**Introduction**

Hydatidosis is an important public health problem worldwide and one of the most important zoonotic diseases in Egypt (Ibrahim *et al.*, 2007). It is considered as a serious parasitic disease of humans caused by the larval stage of *Echinococcus granulosus* (El Shazly *et al.*, 2007). The disease is of global distribution with cysts developed in almost all body organs (Eckert and Deplazes, 2004). Humans are accidental intermediate hosts infected by handling soil, dirt or animal hair containing eggs. Eggs hatch in the small intestine releasing the oncosphere which migrates via the portal system to various organs; mainly the liver; differentiating into the metacestode stage. The infection is a zoonosis, normally maintained in dogs and sheep in close association with humans (*E. granulosus*), or in foxes or wild canines and rodents (*E. multilocularis*) (Eckert and Deplazes, 2004).

Human hydatidosis is typically asymptomatic due to the slow growth of metacestodes. Clinical symptoms usually do not become evident until 10 years or more after initial infection (Sako *et al.*, 2011). Early diagnosis and treatment are important for reduction of morbidity and mortality (Sarkari *et al.*, 2007). The disease is usually diagnosed in patients using imaging techniques as ultrasonography or others (Sako *et al.*, 2002); this primary diagnosis needs to be confirmed by serological tests since the clinical signs of the disease are non-specific (Doiz *et al.*, 2001).

Identification of sensitive and specific methods for immunodiagnosis of hydatid cysts (HC) is affected by the degree of sensitivity and specificity of the used antigens. These methods are able to exclude false negative or false positive reactions caused by infection with other cestoda or even other helminthes (Sadjjadi *et al.*, 2007). Several HC antigens have been used in serodiagnosis of human hydatidosis (Carmena *et al.*, 2006).

Sabry (2007) and Osman (2006) proved that fertile hydatid cyst antigen (FHCA) is considered to be the most specific HC antigen in comparison with germinal layer and protoscolices antigens. Fractionation of fertile Hydatid cyst fluid antigen (FHCFA) revealed 12 protein fractions. Seven of these fractions at MW 12-38 kDa, reacted specifically with sera of HC experimentally infected rabbits as well as sera of surgically proved HC infected patients using enzyme linked immuno-transfer blot (EITB) technique (Sabry, 2007). Fertile hydatid cyst fluid antigen (FHCFA) of 8 kDa, of 29 kDa and that of 34 kDa bands exhibited high specificity than sensitivity.

In previous studies; Osman (2006), Sabry (2007) and Abdel-Hameed (2009) used HC have used HC antigens of animal’s origin of animal’s origin for the serodiagnosis serodiagnosis of human hydatidosis. Genetically, Oudni *et al.*, (2004) found a high degree of genetic similarity between HC of sheep, cattle and...
human origin. Moreover, the ovine and human samples were highly similar, while the cattle samples were slightly different genetically. They concluded that human are mostly infected by parasites originating from sheep. On the contrary, Azab et al. (2004) reported low level of genetic heterogeneity between HC isolated from human, camel and pig. They concluded that human and camel isolates were the most related pair, and human cases in Egypt are of camel/dog strain.

The present study aimed to identify specific and sensitive protein fraction from fertile hydatid cysts via SDS-PAGE & EITB techniques and investigating if the origin of the antigen (from human or camel) can affect sensitivity and specificity of diagnosis of hydatidosis in human patients.

Materials And Methods

Fertile hydatid cysts fluid antigen (FHCFA):

- Hydatid cysts (HC) of human and animal origins were collected. Intact unilocular hydatid cyst was freshly obtained from the lung of 21 years old female after surgical operation at Kasr-Al-Aini Hospital, Cairo University. She was diagnosed radiologically and pathologically. Similar cysts were collected from the lung of freshly slaughtered camels at Cairo abattoir. Cystic fluid was aspirated; presence of protoscolices; indicating vitality was evaluated microscopically.
- Cysts fluid were collected; clarified by centrifugation at 5000 rpm for 15 min at 4°C and dialyzed against 5mM Tris-Hcl (pH 7.4) for 48 hr at 4°C, after determination their protein content by method of Lowry et al. (1951). Antigen was allocated into 1ml vial and stored at –20°C until use (Ito et al., 1999).

Fractionation of FHCFA using SDS-PAGE:

The prepared antigens were resolved using 1.5mm thickness Sodium dodecyl Sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (1970) in 12 % polyacrylamide gel slabs in Tris-glycine buffer, pH 8.3 under reducing conditions. The stacking gel consisted of 5 % acrylamide prepared in 12.5 mM Tris-HCL buffer (pH 6.7) (Sigma chemical Co.). Pre-stained low molecular weight (MW standard) was employed (Sigma). The comb was adjusted as one small well for standard and one large for the sample.

Electrophoretic transfer of protein fractions onto nitrocellulose sheet:

Electrophoresis transfer of fractionated proteins onto nitrocellulose sheet (NC) for electro-immuno transfer blot technique (EITB) was performed according to Towbin et al. (1979) using transfer buffer (25 mM tris-base, 192 mM glycine, 20% (v/v) methanol at (pH 8.3). Transferring was carried out at 10V, 100 mA overnight at 4°C for initial determination of protein bands on NC membrane. The membrane was washed by distilled water and stained with Ponceau-S stain (0.2% Ponceau-S in 3% Trichloroacetic acid) (Sigma). Excess dye was washed off by distilled water.

Determination of FHCFA specific protein fractions using EITB:

EITB technique was applied in order to determine the specific diagnostic fractions of FHCFA, according to Towbin et al. (1979). Longitudinal NC strips (15 x 0.5 cm) for each antigen type (human & animal) were evaluated. Strips were cut out and allowed to react versus the tested serum samples at 1:100 dilution, 0.5ml of sera/strip. The used conjugate was horseradish peroxidase conjugated anti-protein A (Sigma Immunochemicals), at 1:1000 in 3% BSA/PBS. The used substrate is 4-chloro-1-naphthol. Fractions reacted versus reference positive sera and in the same time did not react versus negative control ones; were considered as specific protein fractions. Specificity of the tested fraction was evaluated according to Abdel-Rahman et al. (1998), as the ability of the tested fraction to detect its target antibody from different antibodies of other parasites using the following equation:

Specificity % = (T – P) / T X100 / 100.

Where T = No. of tested sample & P = No. of positive samples

This was at standard serum dilution, while sensitivity is the ability of the tested antigenic fractions to detect its target antibody in samples infected by the parasite at standard serum dilutions (percentage of positive sera among the total number of the positive samples).
Tested serum samples:

Human sera:

Five samples of hydatidosis patients proved sonographically and pathologically; 10 samples of *Schistosoma mansoni* infected patients and of patients with viral hepatitis C. All patients had free stool from other parasitic infections. Moreover; 10 samples of enteric protozoa (*Giardia* and *Entameba* species) infected patients as well as sera of 10 healthy individuals from Kasr-Al-Aini Hospital, Cairo University, Ethical approval of the study was given by the Ethics Committee of Cairo University of Medical Sciences and consents were obtained from participants.

Animal samples:

Ten sera of infected camels with hydatid cyst; 10 samples from camels with gastrointestinal nematode infection; 10 samples from camels with *Moniezia trigonophora* infection and 10 samples of *Cysticercus dromdari* infected camels were selected from large number of samples collected after slaughtering in Cairo abattoir. Post mortem data of most of these animals could be obtained after evisceration; concerning the presence of hydatid cyst and other different tissue parasites. Moreover10 samples of healthy non-infected camels were examined.

Fecal samples were examined parasitologically in order to determine the parasitic infection in each case, by one or more method including direct smear method according to the technique of WHO (1983). Concentration flotation technique using saturated sodium chloride solution according to Wattal *et al.* (1986), while the large eggs were diagnosed via two successive sieve system (Fluke finder, Moscow, ID) according to Welch *et al.* (1987). Collected blood samples were used for separation of the required serum.

Results:

Fractionation of HCFA of human and animal origin and reaction of these bands versus known hydatid infected human and camel sera using EITB technique as in (Table I) and (Plate I), revealed that protein fractions corresponding to molecular weights of 38, 36, 18 and 8 kDa reacted specifically in NC strip of human origin versus infected human sera (lane 2) and in antigen of camel origin versus infected camel sera (lane 5). The fraction corresponding to 18 kDa proved special interesting characters as it proved to be species specific. This fraction did not react specifically after treatment of FHCFA of human origin versus positive serum sample of camels (lane 3) and that of camel origin did not react specifically from camel origin versus positive patient samples (lane 4) (Table 1). Other nonspecific bands at 105 kDa, 12 and 8 kDa reacted using negative serum samples (lane No. 6-9).

<table>
<thead>
<tr>
<th>Band No.</th>
<th>Different Reacted Protein Fractions on NC Strips</th>
<th>FHCFA of Human origin reacted versus (kDa)</th>
<th>FHCFA of Camel origin reacted versus (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Human sera</td>
<td>Camel sera</td>
<td>Human sera</td>
</tr>
<tr>
<td>1</td>
<td>+ ve</td>
<td>- ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>2</td>
<td>- ve</td>
<td>- ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>3</td>
<td>+ ve</td>
<td>- ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>4</td>
<td>- ve</td>
<td>- ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>5</td>
<td>+ ve</td>
<td>- ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>6</td>
<td>- ve</td>
<td>- ve</td>
<td>+ ve</td>
</tr>
</tbody>
</table>

Species specificity and sensitivity of the protein fractions at 18 kDa were evaluated using different known human and camel serum samples (Table2). Concerning the band specificity, no false positive or negative results were determined after treatment of FHCFA of human origin versus other infected sera including *Schistosoma mansoni*, Virus hepatitis C and samples of patient harboring enteric protozoa (*Giardia* and *Entameba*) species (10 samples each). In the same time 2 serum samples of tested camels cross reacted versus the 18 kDa fraction of camel origin which reduced the specificity to 80 and 90% in camels sera infected by *Moniezia* and *Cysticercus dromdari* respectively, while no cross reactions versus Ab of gastrointestinal nematode in sera of infected camels.

At the same time, this protein fraction showed absolute sensitivity as that of human origin reacted specifically versus human hydatidosis infected patients (100% sensitivity) and in the same time did not react by any percentage versus hydatidosis infected camel sera. The same phenomenon was recorded using NC strips carrying fractionated FHCFA of camel origin using EITB technique as described in (Table 2).
Table 2: Specificity & Sensitivity of 18 kDa Fertile Hydatid cysts Protein Fractions of Human and Camel Origin in Diagnosis of Infection in Selected Serum Samples Using EITB Technique (1:100 serum dilution).

<table>
<thead>
<tr>
<th>Type of tested sera</th>
<th>History of the selected serum samples</th>
<th>No. of samples</th>
<th>Tested eluted concentrated Protein fraction of</th>
<th>Human origin</th>
<th>Camel origin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Specificity</td>
<td>Human sera</td>
<td>10</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Schisto.mansoni infected patients</td>
<td>10</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Virus hepatitis C. infected patients</td>
<td>10</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Enteric Protozoa infected patient*</td>
<td>10</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Camel sera</td>
<td>10</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>GIT Nematode infected camel*</td>
<td>10</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Moneziza infected camel</td>
<td>10</td>
<td>0</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Cysticercus infected camel</td>
<td>10</td>
<td>0</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Mean Specificity</td>
<td>Human sera</td>
<td>60</td>
<td>100</td>
<td>93</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Surgical proved HC infected human sera</td>
<td>5</td>
<td>5</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Sera of healthy persons</td>
<td>10</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Camel sera</td>
<td>10</td>
<td>0</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Hydatid infected at P.M.</td>
<td>10</td>
<td>0</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Parasite free young camel sera</td>
<td>10</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Mean Sensitivity</td>
<td>35</td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*GIT Nematode Infection: Gastrointestinal Nematode Infection.
* Enteric Protozoa infected patient: (Giardia & Entamoeba).

Plate 1: Recognition of Specific Fertile Hydatid Cysts Fractions of Human and Camel Origin Using EITB Technique.
- Lane (1): Low molecular weight marker.
- Lane (2): NC strip of human origin reacted with hydatidosis infected patient sera.
- Lane (3): NC strip of human origin reacted with hydatidosis infected camel sera.
- Lane (4): NC strip of camel origin reacted with hydatidosis infected patient sera.
- Lane (5): NC strip of camel origin reacted with hydatidosis infected camel sera.
- Lane (6): NC strip of human origin reacted with healthy human sera.
- Lane (7): NC strip of human origin reacted with healthy camel sera.
- Lane (8): NC strip of camel origin reacted with healthy human sera.
- Lane (9): NC strip of camel origin reacted with healthy camel sera.
Discussion:

Human cystic echinococcosis is recognized globally as an increasing major zoonotic disease with high prevalence all over the world. Yearly infected people with *E.granulosus* are 2.7 million (WHO, 2001 and da Silva, 2010). Hydatidosis remains as a significant health problem in Arab countries (Ahmadi, 2004). Its distribution is related to underdeveloped countries especially in rural communities where man remains in close contact with dogs (definitive host). Man is infected accidentally by ingestion of food or water contaminated with dog feces containing the infective eggs (Nasser-Moghaddam et al., 2011). Dogs are infected by eating infected carcasses containing the hydatid cyst (Moro and Schantz, 2009).

Diagnosis of hydatidosis is still problematic (Sako et al., 2011). In human; diagnosis is based mainly on imaging studies; which are unable to distinguish lesions from abscesses or neoplasm; and immunodiagnostic procedures. This led to difficulties in terms of interpreting disease status (progression or regression) (Bresson-Hadni et al., 2006 and Ozsoy et al., 2011).

Searching for highly sensitive and specific antigens is the greatest challenge to overcome unsatisfactory performance of currently available immunological assays (Carmena et al., 2006). Serological tests such as immunoelectrophoresis, double diffusion in agar, or indirect hemagglutination are being replaced by more sensitive assay methods such as enzyme-linked immunosorbent assay (ELISA), immunoblot (IB), and indirect immunofluorescent antibody test (IFA) (Virginio et al., 2003). The main problems for the serodiagnosis of cystic echinococcosis are often the unsatisfactory performance of the available tests and the difficulties associated with the standardization of antigenic preparations and techniques (Doiz et al., 2001). To overcome these drawbacks, highly sensitive and specific antigens and antigenic components derived from different developmental stages of *E. granulosus* must be available (Carmena et al., 2006). In this respects several studies identified more specific and sensitive fractions for diagnosis of infection in human and animals (Sako et al., 2002; Sarkari et al., 2007 & Rahimi et al., 2011).

Several previous studies used antigens obtained from animal hydatid cysts for diagnosis of human infection (Sabry, 2007). This unspecific antigenic origin plays a role in the inaccurate diagnosis of the disease for some degrees. From three known different crude hydatid antigens (germinal, protoslices and fluid antigens) HC fluid antigens proved to be the most reliable one for serodiagnosis (Zhang et al., 2003; Osman, 2006 and Sabry, 2007).

The present study succeeded in determination of four protein fractions at MW of 38, 36. 18 and 8 kDa in both FHCFA obtained from human or camel origins. These specific fractions were previously obtained by other studies (Ito et al., 1999; Sabry, 2007 and Tappe et al., 2008). Also, this study revealed that 18 kDa fraction was considered to be the most species specific antigen. It was highly specific and sensitive to the identified host. This means that it was specific and sensitive for Ab present in patient’s sera if it was separated from HC of human origin and by the same way it was specific for Ab in camel sera if it was separated from HC extracted from camel. These findings goes with those of Rahimi et al. (2011) who showed that the highest sensitivity for diagnosis of hydatidosis was seen with antigen B prepared from human liver cysts (97.8%). This maximal sensitivity was followed by antigen B isolated from those of sheep liver and lung cysts. The least sensitivity was found with antigen B prepared from bovine lung cysts. The highest specificities (97.1%) were observed with antigen B isolated from human liver cysts followed by those of sheep and goat liver cysts while the lowest specificity was seen with antigen B isolated from bovine lung cysts. Moreover, Poretti et al. (1999) revealed that fraction at 8 kDa was highly specific in differentiation between infection by cystic and alveolar hydatidosis. At the same time, this fraction (8 kDa) revealed no cross-reactivity with any sera from patients with cysticercosis, other parasitic diseases, hepatoma, or healthy controls as genus-specific for echinococcus (Ito et al., 1999). Dreweck et al. (1997) mentioned also that the band fractions of 16-18 appeared lower in specificity (81.66%) and sensitivity (92.0%) than the other tested fractions which may be due to the difference in the strain of the used hydatid fluid antigens.

The present study revealed that 18 kDa protein fraction did not cross react with patient sera containing antibodies of other parasitic infestations such as *Giardia, S.mansoni, Entamoeba histolytica* and hepatitis C virus infection. This agreed with Sabry (2007) who explained that the marked specificity & sensitivity of this fraction demonstrated this antigen as a high valuable purified antigen helping in accurate diagnosis of hydatid infected animals without reaction with other parasitic infections which could be diagnosed in these animals. In the present study; cross reaction of this fraction in case of camel sera infected by *Cysticercus dromdari* and *Monezia* may be related to the presence of un-apparent true infection in these camels or as some characters related to camel serum antibodies.

In conclusion, the present study recommended using of hydatid cyst antigen of human origin for accurate specific diagnosis of infection in suspected patients. The protein fraction of 18 kDa is the only fraction able to identify the specific anti-hydatid cyst Ab in sera of infected patients without cross reaction with other antibodies in the tested sera. This facilitates early diagnosis of the disease which is essential to improve the prognosis of the infected patients as mentioned by Fujimoto et al. (2005). Further work is running now aiming at
recombination of this fraction after specific propagation using PCR for the production of enough amount of this fraction. The produced fraction will be evaluated for diagnosis in more wide scale with separate trail for vaccination against infection in further work in the near future by the authors.

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