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Cross-reacting and specific antigenic components in cystic fluid from metacestodes of *Echinococcus granulosus* and *Taenia solium**

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Abstract: Sera from confirmed patients of 5 hydatidosis, 67 neurocysticercosis and 89 other parasitic diseases were tested for specific antibody (IgG) levels by ELISA to cystic fluid antigens from metacestodes of *Echinococcus granulosus* (HF) and *Taenia solium* (CF). All hydatidosis sera reacted positively to both HF and CF while neurocysticercosis sera did in 49.3% to HF and 85.1% to CF. The frequencies of cross-reactions were lower in other parasitic diseases to both antigens. By SDS-PAGE, protein bands of 64, 35, 22 and 7 kilodaltons (kDa) were found common in HF and CF. SDS-PAGE/immunoblot exhibited that hydatidosis sera reacted crossly to CF at 135, 110, 100, 86, 64, 45, 39, 35 and 24 kDa bands while neurocysticercosis sera did to HF at 135, 100, 86, 64, 52, 39, 35, 29 and 24 kDa bands. These results indicated that protein bands of 135, 100, 86, 64, 39, 35 and 24 kDa were major common components in HF and CF. Protein bands of 7 kDa in HF and 15, 10 and 7 kDa in CF did not react crossly and were specific components in respective antigens.

Key words: Hydatidosis, cysticercosis, *Echinococcus granulosus*, *Taenia solium*, serologic diagnosis, ELISA, antigen

INTRODUCTION

Non-specific positive reactions are frequent in antibody tests for the diagnosis of parasitic diseases. Causes of the non-specific reactions are not so simple. As host factors, diseases of immune apparatus such as monoclonal gammopathy *etc.* and liver diseases or chronic infections with elevated serum levels of immunoglobulins are important. As parasite factors, however, sharing common antigenic proteins or common antigenic determinants at different proteins are considered to be important in eliciting cross-reactions between taxonomically related or unrelated species of parasites(Schantz and Gottstein, 1986).

A classical example of the common antigenic protein is antigen 5 in HF of *E. granulosus*. Capron *et al.*(1967) firstly reported that arc 5 in immunoelectrophoresis (IEP) was species-specific in *E. granulosus* hydatidosis. The responsible antigen which made the arc 5 with patients sera was purified by Oriol *et al.*(1971). Since then, biochemical and immunologic properties of the antigen 5 were studied (Bout *et al.*, 1974; Pozzuoli *et al.*, 1975; Dottorini and Tassi, 1977; Piantelli *et al.*, 1977; Yarzabal *et al.*, 1977). This highly immunogenic, thermolabile lipoprotein had molecular weight over 400 kDa when

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measured by gel filtration; it composed of 67/69 kDa subunits as observed by SDS-PAGE and further subdivided into 47 and 27 kDa which were probably linked by disulfide bonds. Antigen A is found at the internal layer of germinal membrane and parenchyme of protosclices of hydatid cyst.

Unlike Capron *et al.*(1967) considered, however, antigen 5 was later found not only in cystic fluid of *E. granulosus* but also in *E. multilocularis* metacestodes(Yarzabal *et al.*, 1977), cystic fluid of *T. hydatigena* metacestodes (Valera-Diaz *et al.*, 1977). Thereafter, arc 5 in IEP (=antigen 5) was confirmed in human *E. vogeli* hydatidosis, *T. ovis* cysticercosis in sheep and human *T. solium* cysticercosis (Varela-Diaz *et al.*, 1978; Yong and Health, 1979; Schantz *et al.*, 1980).

Whatever the cross-reacting components are, serologic cross-reactions between *T. solium* cysticercosis and hydatidosis have long been observed by either indirect fluorescent antibody test (Rydzewski *et al.*, 1975) or indirect hemagglutination test (Schantz *et al.*, 1980) or ELISA (Diwan *et al.*, 1982). Based on the hitherto known facts on the cross-reactions, antigen 5 may be one of the common antigenic proteins which is responsible for the cross-reactions. But there should be more components in HF and CF which elicits the cross-reactions.

This study was undertaken to observe the frequency of serologic cross-reactions between hydatidosis and *T. solium* cysticercosis by ELISA when crude HF and CF were used as antigen and to find the common and specific antigenic components in the both antigens by SDS-PAGE/immunoblot.

MATERIALS AND METHODS

1. Antigens

(1) Cystic fluid of *T. solium* metacestodes

Cystic fluid was collected from *T. solium* metacestodes which were naturally infected in a pig. The procedure of fluid collection was described in Choi *et al.*(1986). In brief, the metacestodes were delivered out from fibrous host wall in pig muscle and washed in physiologic saline (4°C) for 4 times. Drops of saline on surface of the worms were removed by rolling on a filter paper. CF was collected by puncturing wall of the worm with a fine-tipped forceps; let CF flow into a beaker through a funnel. CF was kept at −40°C until used. The protein content was 5 mg/ml as measured by Lowry *et al.*(1951).

(2) Hydatid fluid

From an imported case of *E. granulosus* hydatidosis (Lee *et al.*, 1986) a lung mass was surgically removed. About 60 ml of HF punctured aseptically. Protoscolices were confimed. After centrifugation, HF was measured for its protein content (0.05 mg/ml). HF was concentrated by filtering through colloidon bag (Sartorius, SM 13200) to make protein content 2.0 mg/ml.

2. Patients sera

(1) Hydatidosis

Sera from 5 surgically confirmed *E. granulosus* hydatidosis were used. They were all Korean workers returned home from either Saudi Arabia or Libya. Case history of 3 patients were recorded in Korean literature (Lee *et al.*, 1986; Jeon *et al.*, 1988; Huh *et al.*, 1988). Hydatid cysts were surgically confirmed in liver (3 patients), lung (2) and abdominal cavity (together with liver involvement).

(2) Neurocysticercosis

A total of 67 sera from confirmed neurocysticercosis patients was used. Diagnosis was made as Cho *et al.*(1986) described. In brief, patients of surgically confirmed neurocysticercosis, biopsy-proven, specific IgG antibody positive patients of neurocysticercosis with brain CT of multiple low densities and neurologic patients with typical brain CT findings with positive antibody tests were included. Specific antibody (IgG) levels in sera ranged absorbance (abs.) 0.03 to 1.81 by ELISA.

(3) Other parasitic diseases

Sera from 29 paragonimiasis patients, 25 sparganosis, 25 clonorchiasis and 10 taeniasis were tested. Patients of paragonimiasis and

clonorchiasis were diagnosed by positive antibody levels to homologous antigens by ELISA. Sparganosis patients were surgically confirmed. Taeniasis were confirmed by observing proglottids after chemotherapy (*T. saginata* infections). All sera were kept at −40°C until used.

3. ELISA

Specific antibody (IgG) test for *E. granulosus* hydatidosis and *T. solium* cysticercosis were done as described by Cho *et al.*(1986). HF and CF were coated in polystyrene microtiter plate wells respectively in protein concentration of 2.5 μg/ml overnight at 4°C. Patients sera were reacted in dilution of 1:100 for 2 hours at 36°C. Peroxidase-conjugated antihuman IgG (heavy- and light-chain specific, Cappel) in dilution of 1:10,000 was reacted for 2 hours. Substrate containing *o*-phenylene diamine was reacted at 20°C for 30 minutes. Abs. was read at 492 nm. Cut-off value of positive reaction was abs. 0.25 for hydatidosis and abs. 0.18 for cysticercosis.

4. SDS-PAGE

Method of Laemmli (1970) was followed. Briefly, using 10~15% linear gradient gel, HF and CF in reducing conditions were electrophoresed with standard proteins until dye front reached the bottom of 9 cm long separating gel (Cho *et al.*, 1987). The gel was stained with 0.125% Coomassie brilliant blue R-250 solution unless used for immunoblot.

5. Immunoblot

The method of Tsang *et al.*(1983) was followed as Cho *et al.*(1987) described. After SDS-PAGE of HF and CF, the proteins in the gel were transferred to nitrocellulose paper by electrophoresis for 2 hours at 1 mA/100 V. The paper was cut longitudinally and each strip was reacted sequentially with 1:100 diluted patient serum and 1:1,000 diluted peroxidase-conjugated antihuman IgG for 1 hour respectively. After washing, substrate containing 3,3′-diaminobenzidine and H_2O_2 was reacted for 15 minutes. The reaction was stopped by washing them 3 times with distilled water.

RESULTS

1. Cross-reacting patterns by ELISA

All hydatidosis sera reacted positively to both HF and CF antigens (Table 1, Figs. 1 and 2). Mean±standard deviation(SD) of abs. was 0.92 ±0.44 to HF and 0.41±0.14 to CF.

Sera from neurocysticercosis patients showed positive reactions to HF in 49.3% and their mean abs. was 0.33±0.29 while reaction to homologous CF antigen was positive in 85.1%

Table 1. Positive reactions of hydatidosis, neurocysticercosis and other parasitic diseases to HF and CF by ELISA

Disease category	No. of cases	No. (%) of positive reactions to	
		HF	CF
Hydatidosis	5	5(100)	5(100)
Neurocysticercosis	67	33(49.3)	59(85.1)
Paragonimiasis	29	1(3.4)	2(6.9)
Sparganosis	25	2(8.0)	3(12.0)
Clonorchiasis	25	0(0)	1(4.0)
Taenia saginata infection	10	3(30.0)	5(50.0)

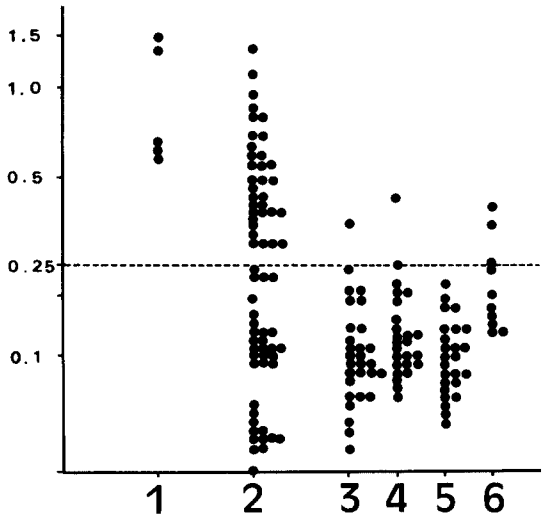

Fig. 1. Specific antibody (IgG) levels to hydatid fluid (HF) as measured by ELISA in disease groups. Dotted line at abs. 0.25 is cut-off value of the positive reaction. 1. Hydatidosis, 2. Neurocysticercosis, 3. Paragonimiasis, 4. Sparganosis 5. Clonorchiasis and 6. *Taenia saginata* infection.

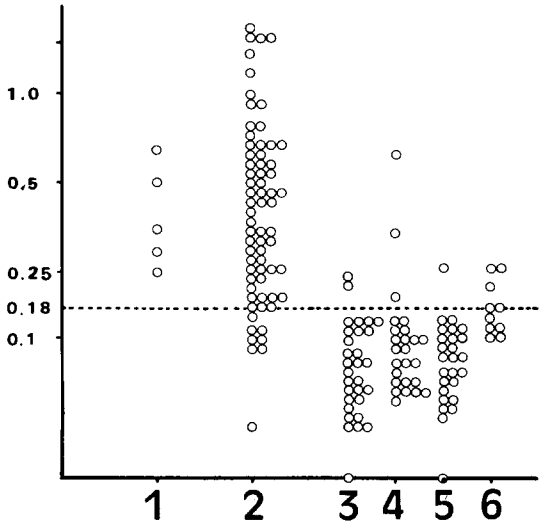

Fig. 2. Specific antibody (IgG) levels to cystic fluid of *T. solium* metacestodes(CF) as measured by ELISA in disease groups. 1. Hydatidosis, 2. Neurocysticercosis, 3. Paragonimiasis, 4. Sparganosis, 5. Clonorchiasis, and 6. *T. saginata* infection.

and their mean abs. was 0.42±0.31 (Table 1, Figs. 1 and 2).

Out of 89 sera from other parasitic diseases, 6 sera (6.7%) reacted positively to HF while 11 cases (12.4%) did to CF. Of them cestode infections such as sparganosis and taeniasis showed higher frequency of cross-reactions than in paragonimiasis and clonorchiasis.

2. Protein bands in SDS-PAGE

In SDS-PAGE of HF, at least 19 bands were shown(Fig. 3). Of them, 82, 64, 52, 35, 24, 22 and 7 kDa bands were stained more darkly than the remaining bands and constitute major bands.

CF showed at least 23 bands. Of them, 10 bands of 94, 64, 48, 45, 42, 39, 35, 24, 15, 10 and 7 kDa bands were major bands.

When compared each other by molecular weight markers, bands of 64, 35, 22 and 7 kDa were found in both HF and CF (Fig. 3).

3. Cross-reacting components in HF and CF

All sera from hydatidosis reacted to HF components of 145, 140, 135, 125, 117, 110, 100, 86, 64, 52, 45, 39, 35, 29 and 24 kDa.

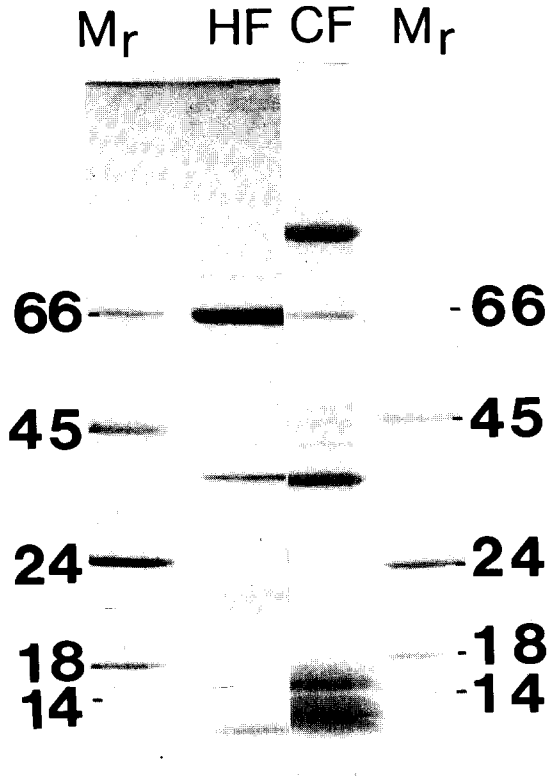

Fig. 3. SDS-PAGE findings of HF and CF in 10~15% linear gradient gels at reducing condition. Coomassie brilliant blue R-250 stained. M_r: Molecular weight markers in kilodaltons (kDa).

In addition, in 2 patients (Cases 3 and 5 in Fig. 4), bands of 22, 17, 12 and 7 kDa in HF were also reacted (Fig. 4). To HF components proteins, 8 neurocysticercosis sera reacted to 135, 100, 86, 64, 52, 39, 35, 29 and 24 kDa but not to 22, 17, 12 and 7 kDa (Fig. 4).

To CF, 20 neurocysticercosis sera showed positive reactions at 135, 130, 110, 105, 86, 72, 64, 57, 52, 45, 39, 35, 24, 22, 15, 10 and 7 kDa bands (Fig. 5). Sera form 5 hydatidosis showed individual variations in reactions to CF components. Case 5 in Fig. 5 did not show any reaction. In Case 4, only faint reaction at 94 kDa was observed. In Case 3, bands of 35 and 86 kDa in CF were reacted. In 2 patients (Cases 1 and 2), many bands in CF were reacted; 135, 110, 100, 86, 64, 45 and 35 kDa

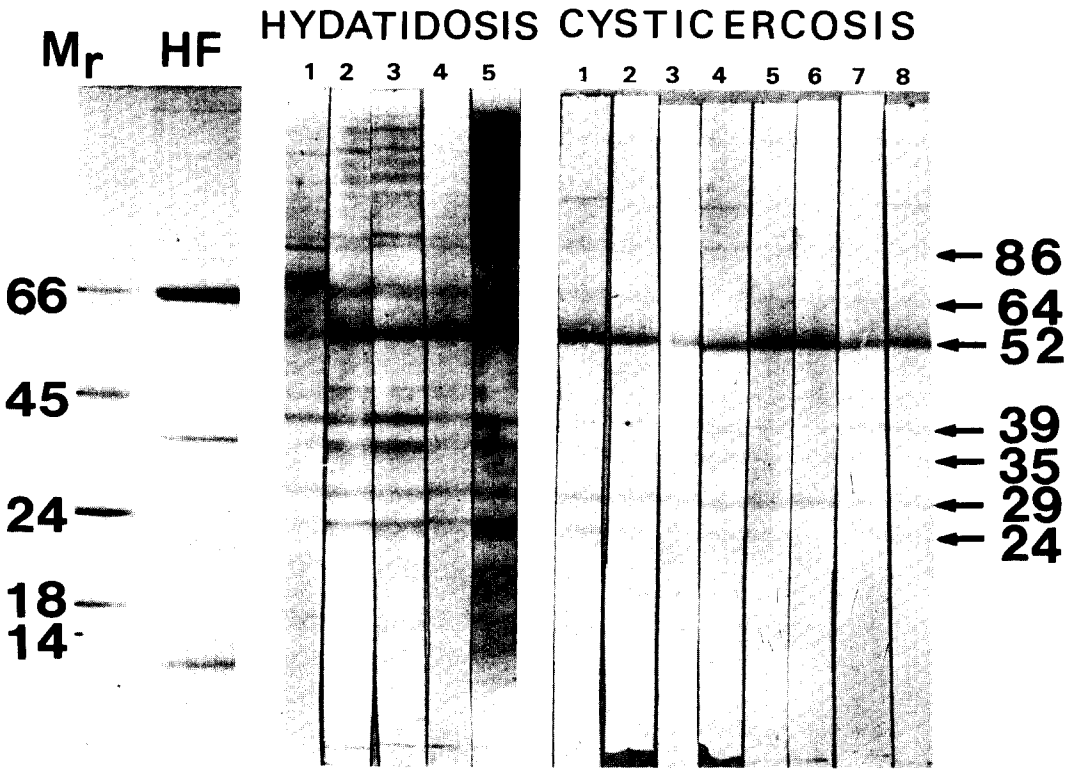

Fig. 4. SDS-PAGE/immunoblot findings to HF as antigen in sera of hydatidosis and neurocysticercosis patients. M_r: Molecular weight markers in kDa. Numbers in right column are those of cross-reacting components in kDa.

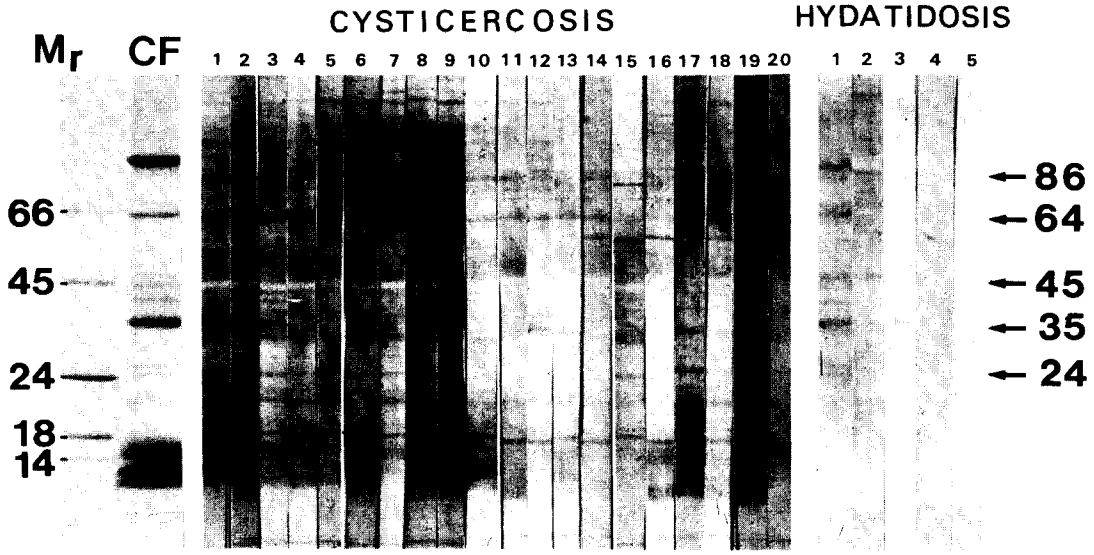

Fig. 5. SDS-PAGE/immunoblot findings to CF in sera of neurocysticercosis and hydatidosis patients. M_r: Molecular weight markers in kDa. Numbers in right column are those of cross-reacting components in kDa.

bands showed clear reactions. In Case 1, bands of 39 and 24 kDa in CF reacted additionally. But none of hydatidosis sera reacted to 22, 15, 10 and 7 kDa bands in CF.

DISCUSSION

This study confirmed that serologic cross-reactions between hydatidosis and cysticercosis are very frequent when respective cystic fluid are used as antigens in ELISA. The antigenic component which caused cross-reaction is not a single but multiple when observed by SDS-PAGE/immunoblot. Reviewing shortly the previous reports on this subject, Gottstein *et al.* (1986) showed that all protein bands in SDS-PAGE except 26 and 8 kDa in crude extract of *T. solium* metacestodes were cross-reacting with hydatidosis sera. Cho *et al.* (1987) showed that 13 of 24 cysticercosis sera reacted crossly with 52 and 27 kDa bands in HF. Kim and Yang (1988) reported that hydatidosis sera reacted to 104, 82, 72, 59 and 34 kDa bands in crude extract of *T. solium* metacestodes. Larralde *et al.*(1989) observed that almost all bands in SDS-PAGE of CF and HF reacted crossly with sera from hydatidosis and cysticercosis: only the frequency of the reaction was different by band. Therefore, they suggested an immunoplotting method to differentiate hydatidosis from cysticercosis by analysing the reacted band patterns in CF and HF respectively. Trial of immunoplotting by them illustrated dramatically the high frequency of cross-reactions and the resulting difficulty in interpretation and differential diagnosis. In SDS-PAGE of CF and HF, however, polypeptide bands of lower than 16 kDa were not separated in the report of Larralde *et al.*(1989). Importantly, the low molecular weight band of 8 kDa in HF was claimed to be a species-specific while higher molecular weight proteins of 14~40 kDa and 62/52 kDa were cross-reacting not only with cysticercosis but also with other parasitic diseases (Maddison *et al.*, 1989).

This study also revealed that high molecular weight bands above 86 kDa and 64/52 kDa and 39~24 kDa in HF were highly antigenic in hydatidosis, but also cross-reacting with cysticercosis sera. Of them, 64/52 kDa and 39 kDa in HF were subunits of antigen 5 (Pozzuoli *et al.*, 1975; Di Felice *et al.*, 1986; Shepherd and McManus, 1987; Maddison *et al.*, 1989).

Though antigen 5 has not been isolated and characterized in CF, there are many evidences indicating the presence. For example, patients sera of cysticercosis may make arc 5 with HF in IEP. Subunit of antigen 5, 64 kDa have been repeatedly recognized in SDS-PAGE of CF either in reducing or non-reducing conditions. The 64 kDa component was proved very antigenic in SDS-PAGE/immunoblot when CF or crude extract of *T. solium* metacestodes were reacted with cysticercosis sera (Grogl *et al.*, 1985; Cho *et al.*, 1987; Joo *et al.*, 1987). Furthermore, some of hydatidosis sera reacted to 64 kDa in SDS-PAGE/immunoblot when CF was used as antigen (Fig. 4).

The 8 kDa band in SDS-PAGE of HF (Maddison *et al.*, 1989) is regarded as a subunit of antigen B of Oriol *et al.*(1971) as shown by Piantelli *et al.*(1977). The subunit was described as 7 kDa band in this study. The reason why only 1 of 3 subunits of the antigen B was separated was not understandable yet. But Maddison *et al.*(1989) showed the 8 kDa in HF was specific antigen in the diagnosis of hydatidosis. Our results coincides with them in high specificity of the 8 kDa though the sensitivity was low. Antigen B-like, 150 kDa, antigenically thermostable protein with subunits of 15, 10 and 7 kDa was purified in CF (Cho *et al.*, 1988). Unlike the structural similarities of 150 kDa protein in CF with antigen B in HF, hydatidosis sera did not react to the subunits of the protein in SDS-PAGE/immunoblot. The 150 kDa protein in CF was antigenic to cysticercosis srea (Figs. 4 & 5).

It seems too early to conclude that the antigen 5 in both CF and HF are cross-reacting and strong antigen whereas 150 kDa protein in CF and HF (antigen B) are structurally similar

but antigenically specific each other. But the above seems a plausible hypothesis concerning the major antigens in HF and CF. Probably because of the quantitative differences of antigen 5 between HF and CF and because of relative low sensitivity of IEP itself arc 5 may be diagnostic for hydatidosis though not always specific. The merit of low sensitivity of IEP would disappear or be reduced when sensitive serologic techniques such as ELISA or immunoblot is undertaken. The 150 kDa protein in CF seems to be specific for cysticercosis but the sensitivity is still questionable.

An ideal, single protein antigen with high sensitivity and specificity for serologic differentiation of hydatidosis and cysticercosis seems far from reality yet as Larralde *et al.*(1989) observed. Antigen of Rhoads *et al.*(1985) which was purified partially to contain a certain proportion of 65 kDa and 9.5~16 kDa (maybe antigen 5 and antigen B) could be a second choice.

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=國文抄錄=

包蟲 및 有鉤囊尾蟲 囊液에 있어서 共通抗原 및 特異抗原 分劃

中央大學校 醫科大學 寄生蟲學敎室 및 漢陽大學校 醫科大學 寄生蟲學敎室*
孔 允·姜信榮·趙昇烈·閔得映*

사람의 包蟲症과 有鉤囊尾蟲症을 血淸學的으로 診斷하는 데에는 交叉反應이 相互 頻繁히 일어나 臨床像이나 其他 病歷 등을 基礎로 하여야 鑑別診斷이 可能하다. 이 硏究는 血淸學的 診斷上 두가지 疾患에서 交叉反應을 일으키는 抗原分劃을 觀察하고 또 包蟲症과 有鉤囊尾蟲症에 特異하게 反應하는 分劃이 各 囊液中에 있는지를 觀察하기 爲하여 實施하였다.

사우디·아라비아와 리비아에서 勤務하고 歸國한 다음 發病한 包蟲症 患者 5例의 血淸과 有鉤囊尾蟲症 患者 67例, 其他 肝吸蟲症, 肺吸蟲症, 스파르가눔症, 無鉤條蟲感染者 89例 血淸을 包蟲과 有鉤囊尾蟲 囊液을 抗原으로 各各 酵素免疫測定法(ELISA)으로 特異抗體價(IgG)를 測定하였다. 그 結果 包蟲 囊液에 對하여 包蟲症 血淸은 全例에서, 有鉤囊尾蟲症 血淸은 49.3%에서, 其他 寄生蟲症 血淸은 5.6%에서 陽性反應을 보였다. 有鉤囊尾蟲 囊液에 對하여 包蟲症 血淸은 全例가, 有鉤囊尾蟲症 血淸은 85.1%가, 其他 寄生蟲症 血淸은 12.3%가 陽性反應을 나타내었다.

包蟲 囊液과 有鉤囊尾蟲 囊液을 10~15% linear gradient gel에서 SDS-PAGE를 實施한 바 包蟲 囊液에는 모두 19個 分劃이, 有鉤囊尾蟲 囊液에는 23個 分劃이 나타났고 그 中 64, 35, 22, 7 kDa 分劃이 두가지 囊液에 모두 나타나고 있었다. SDS-PAGE로 分離한 各囊液 分劃을 抗原으로 包蟲症과 有鉤囊尾蟲症 患者 血淸을 反應시키고 反應分劃을 發色시킨 바(면역얼룩법, immunoblot), 包蟲症 患者血淸은 包蟲 囊液의 145, 140, 135, 125, 117, 110, 100, 86, 64, 52, 45, 39, 35, 29, 24, 22, 17, 12 및 7 kDa 分劃에 反應하였고 有鉤囊尾蟲 囊液에 對해서는 135, 110, 100, 86, 64, 45, 39, 35 및 24 kDa 分劃에 反應하였다. 또 有鉤囊尾蟲症 患者血淸은 有鉤囊尾蟲 囊液의 135, 130, 110, 105, 86, 72, 64, 57, 52, 45, 39, 55, 24, 22, 15, 10 및 7 kDa 分劃에 反應하였고 包蟲囊液에는 135, 100, 86, 64, 52, 39, 35, 29 및 24 kDa 分劃에 反應하였다.

以上의 結果에서 包蟲 및 有鉤囊尾蟲 囊液에는 SDS-PAGE로 분리되는 分劃中 135, 100, 86, 64, 39, 35 및 24 kDa 分劃이 交叉反應에 관여하는 共通抗原 分劃으로 判斷되며 包蟲 囊液의 7 kDa 및 有鉤囊尾蟲 囊液의 囊液의 15, 10 및 7 kDa 分劃은 特異抗原 分劃으로 생각된다.