기 생 충 학 잡 지 제28권 제2호
The Korean Journal of Parasitology
Vol. 28, No. 2, 101-107, June 1990

Component proteins in cystic fluid of *Taenia solium* metacestodes collected surgically from neurocysticercosis patients

Yoon Kong, Shin-Yong Kang and Seung-Yull Cho
Department of Parasitology, College of Medicine,
Chung-Ang University, Seoul 156-756, Korea

Abstract: Surgically collected cystic fluid of *Taenia solium* metacestodes from patients of intracranial cystic lesion were compared in their protein composition with those from naturally infected pigs in Cheju Do, Korea and Ecuador. In non-denaturing discontinuous-polyacrylamide gel electrophoresis(disc-PAGE), no discernible differences were recognized in banding patterns between the cystic fluids from Cheju Do and Ecuador, and between the cystic fluids from pigs and human lesions except wider bands that corresponded to human albumin and γ-globulin (in 4 of 9 patients). In reducing SDS-PAGE, bands in the cystic fluid from Ecuador showed the same banding pattern with that from Cheju Do but two bands of 21 and 17 kDa were stained darker. Cystic fluids from patients revealed the same protein compositions of the major protein bands of 94, 64, 15, 10 and 7 kDa as in the cystic fluid of pig origin, but human albumin (66 kDa), heavy and light chains of gamma globulin (55 and 22.5 kDa) were contaminated in 4 of 9 cystic fluids. Human CSF proteins seem to have been contaminated during cystic fluid collection. In any cystic fluid from patients, the major protein component was 150 kDa which was subdivided into 15, 10 and 7 kDa in reducing SDS-PAGE.

Key words: *Taenia solium* metacestodes, human neurocysticercosis, disc-PAGE, SDS-PAGE, protein composition, cystic fluid

INTRODUCTION

In serodiagnosis of human cysticercosis, cystic fluid of *Taenia solium* metacestodes is now regarded as more sensitive antigen than the crude extracts of parenchymal tissue of the metacestodes or adults (Choi *et al.*, 1986; Larralde *et al.*, 1986; Bailey *et al.*, 1988). The protein composition of the cystic fluid have been studied to find out the major antigenic proteins, thus to obtain better diagnostic antigen. Choi *et al.* (1986) reported that the cystic fluid was composed of 6 bands of which C band was major protein comprising about 70% when observed by disc-PAGE and densitography. Cho *et al.* (1988) showed that the 150 kDa protein of C band was composed of 3 subunit polypeptides of 15, 10 and 7 kDa when observed by reducing SDS-PAGE. In addition to C band protein, SDS-PAGE of the cystic fluid revealed 94, 64, 48, 39 and 21 kDa bands which were also antigenic (Cho *et al.*, 1987; Kong *et al.*, 1989).

Antigenic protein composition in the cystic fluid may be variable by batch of collection. Yakoleff-Greenhouse *et al.* (1982) reported that a pooled hyperimmune serum reacted differently with parenchymal extracts from 15 batches of *Taenia solium* metacestodes. In double immunodiffusion(DID) or immunoelectrophoresis(IEP),

1-4 precipitin lines were formed by batch of the parenchymal extract. They regarded this difference in number of precipitin lines as antigenic variation or diversification. However, DID or IEP were relatively insensitive tool for serologic reactions. Therefore, absence of precipitin line in a certain component of the parenchymal extract may result from low quantity as well as absence of the antigenic protein. In order to observe the difference in antigenic protein composition, therefore, direct observation of component protein is desirable as well as indirect serologic reactions.

From June 1985 to December 1989, a total of 31 samples of cystic fluid, which was collected surgically from human intracranial lesions, was requested to examine whether the fluid comes from *T. solium* metacestodes or not. To answer the question, we used the fluid as antigens in enzyme-linked immunosorbent assay (ELISA) and measured the difference in serologic reactivities of *Cysticercus*-specific IgG antibody (Cho *et al.*, 1986) by reacting the antigen with known sera of human cysticercosis and normal control together with phosphate buffered saline (PBS) control. By this kind of back titration, 9 of 31 samples were confirmed to contain significant amount of antigenic protein of *T. solium* metacestodes. Higher absorbance to known cysticercosis serum and low absorbance to PBS blank and normal control serum were the criteria of presence of antigenic protein in the cystic fluid.

The above serologic titration provide indirect evidence of the presence of antigenic protein in the fluid. In addition, we thought it necessary to observe the protein composition. Therefore, we undertook disc-PAGE and SDS-PAGE of the surgically collected cystic fluids and compared the findings with those from cystic fluid of *T. solium* metacestode of naturally infected pigs.

MATERIALS AND METHODS

1. Cystic fluids of *T. solium* metacestodes from pigs

Two naturally infected pigs with *T. solium* metacestodes were collected at the municipal abattoir of Salinas city, Ecuador in August, 1989. Cystic fluid were collected as described by Choi *et al.* (1986) and pooled. Cystic fluid was also collected from the metacestodes of a heavily infected pig muscle in Cheju Do, Korea in January, 1990. Protein content as measured by Lowry *et al.* (1951) was 5.3 mg/ml in cystic fluid from Ecuador and 6.0 mg/ml in that from Cheju Do, Korea.

2. Surgically collected cystic fluids from human brain lesions

Short clinical history of 9 patients whose cystic fluid were examined were as follows. Case 1 (lane 3 of Figs. 1 & 2, our reference No. HSC-875), KWY, 63 year-old male was admitted to Kangnam Hospital due to right hemiparesis for several months. Brain computerized tomography (CT) revealed large encapsulated, decreased density on left frontal lobe. Under the impression of brain abscess, the cyst was punctured, However, clear fluid was drained instead of pus from the lesion. The cystic fluid was said to be contaminated with barium saline during probing the extent of the brain lesion. The pathology of removed cyst wall was *Cysticercus cellulosae*. Serum level of *Cysticercus*-specific IgG antibody was abs. 0.58 by ELISA when undertaken as described by Cho *et al.* (1986) (cut-off abs. for positive reaction, abs. 0.18).

Case 2, HKL, 50 year-old male was admitted to Kwangju Christian Hospital due to symptoms of intracranial hypertension for 3 years. Brain CT showed 7×5 cm sized cystic mass on middle cranial fossa with scattered small calcifications in right occipital lobe. Craniotomy revealed racemose cysticercus filled with clear fluid. ELISA for specific (IgG) antibody showed abs. 0.56 (positive, +) in serum and 0.75(+) in cerebrospinal fluid(CSF).

Case 3, YKR, 54 year-old female was admitted to Yongsan Hospital of Chung-Ang University with the chief complaints of personality change and headache. Details of the history were

recorded elsewhere(Jang *et al.*, 1987). In brain CT, scattered calcifications were associated with large cystic mass at temporal lobe. The worm was racemose cysticercus and 39 ml of cystic fluid was collected. After the surgery specific (IgG) antibody level in serum was 0.26(+) and 0.52(+) in CSF by ELISA. After praziquantel treatment the level was elevated to 0.88 in serum and 1.30 in CSF.

Case 4, 38 year-old male patient named JDK was suffered from intracranial hypertension and surgically treated at Seoul National University Hospital by removing racemose cysticercus from lateral ventricle. The antibody level in serum was 0.40(+) at the time of operation.

Case 5, MKK, 61 year-old male, ill of intracranial hypertension, memory and speech disturbances was surgically treated for neurocysticercosis at Seoul National University Hospital. Cystic lesion in brain CT had been turned out to be a racemose cysticercus. At the time of surgery, the antibody levels in serum was 0.61 (+) and 1.19(+) in CSF.

Case 6, JSK, 28 year-old female complaining headache and visual field defect was surgically treated for neurocysticercosis at Chunnam National University Hospital. Exactly after the surgery, antibody levels in serum and CSF were all negative but turned to positive after the praziquantel treatment in serum(abs. 0.20).

Case 7, HKL, 42 year-old male was sick for headache, vomiting and seizures for 2 years. Preoperative serologic test for *Cysticercus*-specific (IgG) antibody was positive in serum (abs. 0.32). Surgery revealed highly degenerated racemose cysticercus.

Case 8, 35 year-old male whose name was SMK had been ill of generalized seizures, headache and left visual defect. Brain CT revealed huge right temporo-parietal cystic mass. Serum level of specific(IgG) antibody was once negative(abs. 0.15) and once positive(abs. 0.28). Cystic fluid of 9.2 ml was surgically aspirated at the Severance Hospital, Yonsei University.

Case 9, KHT, 38 year-old female had been suffered from seizure attacks. Brain CT revealed high and low densities at right fronto-temporal area. Surgery, done at Yongsan Hospital, Chung-Ang University, revealed racemose cysticercus at subcortical region. A total of 4.5 ml of cystic fluid was collected. Protein content of each cystic fluid was measured by Lowry *et al.*(1951).

3. Disc-PAGE

Non-denaturing disc-PAGE was done by the method of Davis(1964) in 7.5% polyacrylamide gel and 2.5% stacking gel using Tris-glycine buffer (pH 8.3). Protein amount of 75 μg of each sample was loaded. DC power was adjusted to 1.5 mA per tube for stacking gel and to 3 mA for separating gel. Gels were stained with 0.125 % Coomassie brilliant blue R-250 overnight and destained by methanol/acetic acid solution.

4. Reducing SDS-PAGE

Technique of Laemmli(1970) was used. Vertical electrophoresis system of 17×12 cm was used. Separating polyacrylamide gel of 9 cm long and 1.5 mm thick was prepared in 1.5 M Tris buffer (pH 8.8) containing 0.4% SDS. Linear gradient gel of 10~15% was used. Stacking gel of about 1 cm long was 3% polyacrylamide gel in 0.5M Tris buffer (pH 6.8) containing 0.4% SDS. Cystic fluid and CSF content(75 μg/ml of protein) were treated at 95°C for 5 minutes with the same amount of sample buffer containing 0.125 M Tris, pH 6.8, 20% glycerol, 4.5% SDS and 10% 2-mercaptoethanol. Electrophoresis was done at 30 mA for 3~4 hours. Staining and destaining were done as described in disc-PAGE.

RESULTS

1. Protein content and antigenicity of cystic fluid

As shown in Table 1, protein content in the cystic fluid was in range of 0.70~1.76 mg/ml. The antigenicity of the cystic fluid were shown by abs. to positive reference serum (mean abs. 1.00±0.03 in routine ELISA for specific IgG antibody test using 2.5 μg/ml of porcine cystic fluid as antigen by Cho *et al.*, 1986), negative reference serum and PBS blank. Cases 1, 3, 7, and 11 show high abs. of 0.28~0.49 to PBS

Table 1. Protein content and result of antigenicity of cystic fluid which were collected surgically from human brain lesions

Case No.	1	2	3	4	5	6	7	8	9
Our Ref. No.	895	4953	802	1692	1702	2976	5718	2922	7310
Lane in Figs. 1 & 2	3	4	5	6	7	8	9	10	11
Protein content(mg/ml)	0.96	1.35	1.76	0.67	1.59	1.30	0.70	1.00	1.59
*Abs. by ELISA to									
(+) ref. serum	0.68	1.28	1.58	1.20	1.03	1.02	N.N.**	1.05	1.00
(−) ref. serum	0.36	0.09	0.38	0.02	0.35	0.03	N.D.	0.17	0.64
PBS blank	0.39	0.06	0.34	0.00	0.28	0.00	N.D.	0.00	0.49

* At cystic fluid dilution of 1 : 2,000
** N.D. : Not done

blank because they contained certain amount of human IgG in the cystic fluid.

2. Findings in disc-PAGE

Major component of protein in all cystic fluid (lanes 1-11) was band C whatever the sources of the fluid were (Fig. 1). Cystic fluid from natu-

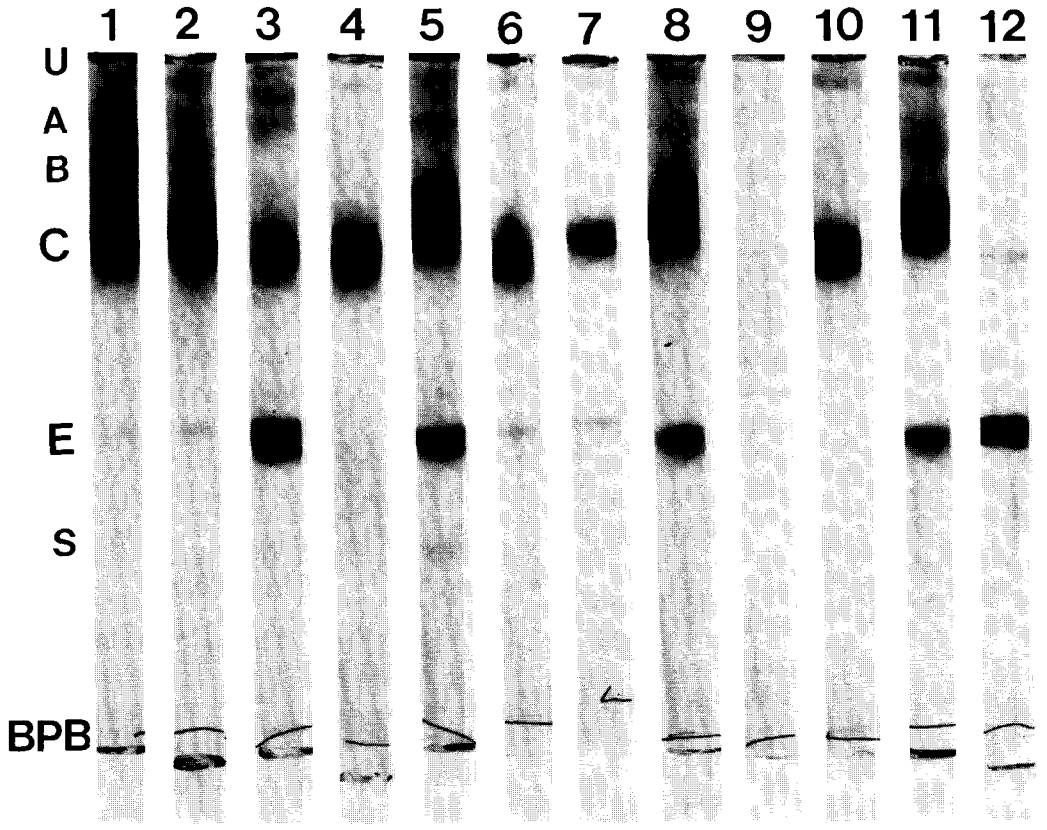

Fig. 1. Disc-PAGE findings of cystic fluid(CF) from pigs and human, and normal CSF at 7.5% gel concentration. 1: CF from Ecuador, 2: CF from Cheju Do, 3: HSC*-895, 4: HSC-4953, 5: HSC-802, 6: HSC-1692, 7: HSC-1702, 8: HSC-2976, 9: HSC-5718, 10: HSC-2922, 11:HSC-7310, 12: Normal CSF
* Our reference file of Human Serum Collection

rally infected pigs in Ecuador(lane 1) and Cheju Do, Korea (lane 2) revealed 2~3 bands above the Band C. Below the C band, faintly stained E band was recognized in lanes 1, 2, 4, 6, 7 and 10. Cystic fluid from human cases (lane 3, 5, 8 and 11) showed darker E bands. Electrophoresis of normal CSF (lane 12) revealed that human albumin corresponded to E band and γ-globulin to C band of the cystic fluid from pigs.

3. Findings in reducing SDS-PAGE

Fig. 2 showed the banding patterns of cystic fluids from pigs (lanes 1 & 2), from human brain lesions (lanes 3-11) and of human CSF (lane 12). Cystic fluid from naturally infected pigs from Ecuador and Cheju Do, Korea showed almost same banding patterns of major proteins such as 94, 64, 39, 34, 15, 10 and 7 kDa. Protein bands of swine cystic fluid from Ecuador showed darker bands of 21 and 17 kDa bands than that from Cheju Do.

Cystic fluid collected surgically from human brain lesion showed also major protein bands of 94, 64, 15, 10 and 7 kDa. Especially, major bands of 15, 10 and 7 kDa were recognized even in cystic fluid of Case 7 (lane 9) though other bands were hardly recognizable. These major bands of 15, 10 and 7 kDa were major components in all cystic fluid whatever the sources were.

In Cases 1, 3, 5 and 9 (lanes 3, 5, 7 and 11), 66 kDa band of human albumin was darkly stained wich corresponded to albumin in CSF (lane 12). Of these 4 cases, 2 (lanes 3 and 5) showed clearly the heavy and light chains of 55 and 22.5 kDa bands. In Case 3 (lane 5) unrecognizable band of 35 kDa was additionally observed, which were found in cystic fluid of Cases 5 and 9 (lanes 7 and 11) without the bands of heavy and light chain bands of IgG.

DISCUSSION

This study demonstrates clearly that the major antigenic protein of 150 kDa (Band C) was common in cystic fluid of *T. solium* metacestodes

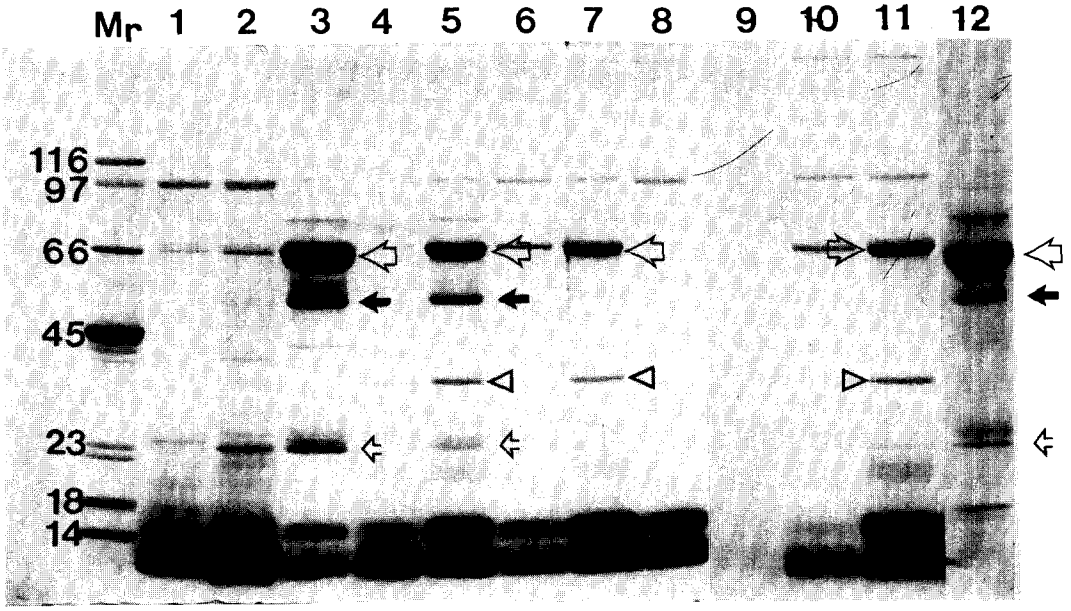

Fig. 2. SDS-PAGE findings of CF from pigs and human and normal human CSF. Linear gradient gel of 10~15% was used for protein separation. M_r: Molecular weight in kDa. Lane 1~12: Same as described in Fig. 1. ⇦: Human albumin ⬅: Heavy chain of γ-globulin ◁: Light chain of γ-globulin ◁: Unidentified band

whatever the sources were. When observed by reducing SDS-PAGE, immunoaffinity-purified band C of 150 kDa protein is divided into 3 subunits of 15, 10 and 7 kDa polypeptides(Cho *et al.*, 1988). This major protein is also known to be antigenically specific, not reacting with patients sera from *E. granulosus* hydatidosis (Kong *et al.*, 1989). Band C protein of 150 kDa showed the same Rf value with γ-globulin in human CSF when observed by disc-PAGE in 7.5% gel. However, as shown in Fig. 2, subunits of 15, 10 and 7 kDa were not superimposed with any subunit of human CSF proteins.

Other major antigenic proteins such as 94 and 64 kDa in reducing SDS-PAGE (Grogl *et al.*, 1985; Joo *et al.*, 1987; Cho *et al.*, 1987), were common in cystic fluid of both pigs and human sources. Of them, 64 kDa protein was superimposed with human albumin, therefore, its presence was masked in SDS-PAGE in 4 of 9 cystic fluid from human brain lesion while all human cystic fluid showed clearly 94 kDa protein in SDS-PAGE.

Out of 9 cystic fluid from human neurocysticercosis, 4 comes from pathologically confirmed racemose cysticercus. The fluid from racemose cysticercus showed no differences in composition of major antigenic proteins such as 94, 64, 15, 10 and 7 kDa which were found in cystic fluid from infected pigs. Biochemical differences between cystic fluid from racemose cysticercus and *T. solium* metacestodes were not discernible as the major antigenic proteins were concerned. Rhoads *et al.* (1985) described the identical lipoproteins in cystic fluid of *Taenia hydatigena* metacestodes. So far it seems that protein composition in cystic fluid is generic or higher taxa characteristics rather than variable within the *T. solium* metacestodes population(Yakoleff-Greenhouse *et al.*, 1982).

Four of 9 cystic fluid of human sources showed a certain degree of non-specific reactions for human IgG in ELISA(Table 1) even when PBS blank was reacted. That means the fluids contained some amount of human IgG, which was confirmed in SDS-PAGE(Fig. 2) of those 4 fluid. Human CSF component in cystic fluid would be contaminated during surgical collection. However, in the present study, we can not definitely determine whether CSF component can permeate naturally into cystic fluid through cystic wall of the metacestodes. To this point, naturally infected *T. solium* metacestodes in pigs are not uniform in their physiologic status (Cervantes-Vazquez *et al.*, 1990; Correa *et al.*, 1987). But it is still questionable whether the large molecules can move into the cystic space of the metacestodes according to aged and degenerated stages.

REFERENCES

Bailey, G.G., Mason, P.R., Trijssenar, F.E.J. and Lyons, N.S. (1988) Serological diagnosis of neurocysticercosis: evaluation of ELISA using cyst fluid and other components of *Taenia solium* cysticerci as antigen. *Trans. Roy. Soc. Trop. Med. Hyg.*, **82**:295-299.

Cervantes-Vazquez, M., Correa, D., Merchant, M., Hicks, J.J. and Laclette, J.P. (1990) Respiratory changes associated with the *in vitro* evagination of *Taenia solium* cysticerci. *J Parasitol.*, **76**:108-112.

Correa, D., Laclette, J.P., Rodriguez-del-Rosal, E., Merchant, M. and Flisser, A.(1987) Heterogeneity of *Taenia solium* cysticerci obtained from different naturally infected pigs. *J. Parasitol.*, **73**:443-445.

Cho, S.Y., Kang, S.Y. and Kim, S.I. (1987) Analysis of antigen specificity using monoclonal and polyclonal antibodies to *Cysticercus cellulosae* by enzyme-linked immunoelectrotransfer blot technique. *Korean J. Parasit.*, **25**:159-167.

Cho, S.Y., Kim, S.I., Kang, S.Y., Choi, D.Y., Suk, J.S., Choi, K.S., Ha, Y.S., Chung, C.S. and Myung, H. (1986) Evaluation of enzyme-linked immunosorbent assay in serological diagnosis of human neurocysticercosis using paired samples of serum and cerebrospinal fluid. *Korean J. Parasit.*, **24**:25-41.

Cho, S.Y., Kim, S.I., Kang, S.Y. and Kong, Y. (1988) Biochemical properties of a purified protein in cystic fluid of *Taenia solium* metacestodes. *Korean J. Parasit.*, **26**:87-94.

Choi, B.K., Kim, S.I., Kang, S.Y. and Cho, S.Y. (1986) Evaluation of antigens from different parts

of *Cysticercus cellulosae* in serological diagnosis of human cysticercosis. *Chun-Ang J. Med.*, **11**: 135-146.

Davis, B.J. (1964) Disc electrophoresis II. Method and application to human serum proteins. *Ann. N. Y. Acad. Sci.*, **121**:404-427.

Grogl, M., Estrada, J.J., McDonald, G.M. and Kuhn, R.E. (1985) Antigen-antibody analyses in neurocysticercosis. *J. Parasitol.*, **71**:433-442.

Jang, J.S., Hwang, S.N., Choi, D.Y., Cho, S.Y. and Kim, S.I.(1985) A case of racemose cysticercus infection. *J. Korean Neurosurg. Soc.*, **15**:841-847 (in Korean).

Joo, K.H., Kang, S.H., Lee, J.S. and Rim, H.J. (1987) Studies on the demonstration of species specific and cross reactive components of vesicular fluid and parenchymal antigens from *Cysticercus cellulosae*. *Korea Univ. Med. J.*, **24**:139-150.

Kong, Y., Kang, S.Y., Cho, S.Y. and Min, D.Y. (1989) Cross-reacting and specific antigenic components in cystic fluid from metacestodes of *Echinococcus granulosus* and *Taenia solium*. *Korean J. Parasit.*, **27**:131-137.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature(London)*, **227**:681-685.

Larralde, C., Laclette, J.P., Owen, C.S., Madrazo, I., Sandeval, M., Bojalil, R., Sciutto, E., Contreras, L., Arzate, J., Diaz., M.L., Govenzensky, T., Montoya, R.M. and Goodsaid, F. (1986) Reliable serology of *Taenia solium* cysticercosis with antigens from cyst vesicular fluid: ELISA and hemagglutination tests. *Am. J. Trop. Med. Hyg.*, **35**:965-973.

Lowry, O.H., Rosenbrough, N.J., Lewis-Farr, A. and Randall, R.J. (1951) Protein measurement with Folin-phenol reagent. *J. Biol. Chem.*, **163**:265-275.

Rhoads, M.L., Murrel, K.D., Dilling, G.W., Wong, M.M. and Baker, N.F. (1985) A potential diagnostic reagent for bovine cysticercosis. *J. Parasitol.*, **71**: 779-787.

Yakoleff-Greenhouse, V., Flisser, A., Sierra, A. and Larralde, C(1982) Analysis of antigenic variation in cysticercosis of *Taenia solium*. *J. Parasitol.*, **68**:39-47.

=국문초록=

인체 유구낭미충증 환자에서 수거한 낭미충 낭액의 성분 단백질의 양상

중앙대학교 의과대학 기생충학교실
공 윤 • 강신영 • 조승열

포도낭미충증을 포함한 유구낭미충증 환자의 신경외과적 수술 과정에서 수거한 낭미충 낭액의 성분 단백질을 비교 검토하였다. 7.5% gel에서 Disc-PAGE와 reducing condition의 10~15% gel에 SDS-PAGE를 실시하여 다음과 같은 결과를 얻었다.

1. 7.5% gel에서 실시한 Disc-PAGE 소견상 제주도와 Ecuador에서 수집한 돼지의 유구낭미충 낭액은 U, A, B, C, E, S의 6개 band로 구성되어 있었고 그 중 band C가 주 구성 성분이었다. 그리고 인체에서 수집한 낭액은 band C가 주 구성 성분이었고 경우에 따라 band E가 주 구성 성분으로 관찰되고 있었다. 정상 뇌척수액은 band C, E 위치에서 γ-globulin과 albumin이 보이고 있었다.

2. 10~15% gel에서 실시한 SDS-PAGE상 제주도 낭액은 최소 25개 이상의 subunit로 구성되어 있었고 그 중 96, 64, 48, 39, 34, 24, 15, 10 및 7 kDa band가 주 구성 성분이었다. 그리고 Ecuador 감염 돼지의 유구낭미충 낭액은 전체적으로 비슷했으나 48, 39, 34, 24 kDa band가 희미한 반면 21, 17 kDa가 진하게 염색되었다. 인체 뇌낭미충증 환자에서 수집한 낭액들은 15~21개 정도의 band가 보이고 있었다. 그 중 94, 64, 15, 10 그리고 7 kDa band는 모든 낭액에 공통적으로 포함되어 있었다. 한편, 정상 뇌척수액의 SDS-PAGE에서는 77, 66 (albumin), 55 (heavy chain of γ-globulin), 22.5 (light chain of γ-globulin) 그리고 17 kDa band가 보였다. 환자의 낭액은 77, 66, 55, 22.5 kDa에서 진하게 염색되는 경우가 있었다.

이상의 결과로 인체 유구낭미충증에서 수집한 낭액에도 15, 10, 7 kDa의 subunit로 구성된 150 kDa의 band C protein이 주 성분으로 구성되어 있으며 수술장에서 수집하는 과정에서 뇌척수액이 섞이는 경우가 많음을 알 수 있었다.

[기생충학잡지, 28(2):101-107, 1990년 6월]