

Comparative evaluation of indirect immunofluorescent antibody test with enzyme-linked immunosorbent assay in serodiagnosis of human neurocysticercosis

Kee-Seon Eom, Seung-Yull Cho* and Han-Jong Rim**

Department of Parasitology, College of Medicine, Chungbuk National University, Cheongju 360-763, Department of Parasitology, College of Medicine, Chung-Ang University, Seoul 156-756, and Department of Parasitology**, College of Medicine, Korea University, Seoul 110-522, Korea*

Abstract: The applicability of indirect immunofluorescent antibody test (IFAT) was compared with enzyme-linked immunosorbent assay (ELISA) in sera from 163 cases of confirmed neurocysticercosis, 101 other neurologic and parasitic diseases and 100 normal controls. As antigen, frozen sections of a *Taenia solium* metacestode from a human brain was used in IFAT and cystic fluid was used in ELISA. For the detection of specific IgG antibody, IFAT was equally sensitive (89.6%) and specific (85.1%) as ELISA. The antibody titers by IFAT were correspondingly increased with mean absorbance of ELISA. The corresponding rate of positivity in the two techniques was 90.8%. Except for the difficulty in detecting antibodies in cerebrospinal fluid (CSF), IFAT was concluded to be very useful for the serodiagnosis of human neurocysticercosis.

Key words: indirect immunofluorescent antibody test, enzyme-linked immunosorbent assay, neurocysticercosis, serodiagnosis

INTRODUCTION

Indirect immunofluorescent antibody test (IFAT) has been applied to the serodiagnosis of neurocysticercosis. Used antigens were sections of hog cysticerci (Konovalova, 1973; Gonzalez-Barranco, 1978), human cysticerci (Kang *et al.*, 1987) or adult *Taenia saginata* (Rydzewski *et al.*, 1975). Reported sensitivities of IFAT by them were 80~95.8% in 28~35 confirmed cases of cysticercosis. The main problem of IFAT is subjective interpretation in defining positive reaction; but with experience, IFAT is very simple and one of the most sensitive and least variable method just as the case of ELISA (Flisser and Larralde, 1986). Until now, however, there have not been enough

data which evaluated IFAT in sufficient number of confirmed cysticercosis cases.

The present study was performed to evaluate comparatively the IFAT with ELISA in the serodiagnosis of human cysticercosis.

MATERIALS AND METHODS

1. Subjected cases

Sera from 364 subjects were tested. The patients consisted of 163 confirmed neurocysticercosis, 44 other neurologic diseases, 26 intestinal cestodiasis, and 31 trematodiasis cases. A total of 100 medical students of Korea University, who were 22~24 years old, 81 males/19 females showing negative reactions to intradermal test for *Paragonimus* and *Clonorchis*, were used as non-infected control. Neurologic dis-

eases other than neurocysticercosis consisted of 11 cases of meningitis or tuberculous meningitis, 3 cerebrovascular accidents (CVA), 5 brain tumors or granulomas, 2 subarachnoidal hemorrhages and 23 cases showing CNS symptoms including Shy-Drager syndrome, arachnoid cyst, multiple sclerosis, porphyria, Behçet's disease, encephalitis, tuberculoma and unknown neurologic diseases.

All neurocysticercosis patients with neurologic problems clinically were examined by brain computerized tomography (CT). They revealed abnormal findings which were typical or atypical of neurocysticercosis. Twenty five cases were confirmed by biopsy of skin nodule or surgery of brain lesions. Serologic tests for specific IgG antibodies in serum or CSF were strong back up of the diagnosis in all neurocysticercosis patients.

By ELISA, 145 cases were IgG antibody positive both in serum and CSF, while remaining 18 showed positive reaction in CSF but negative in sera.

2. IFAT

Used antigen was frozen sections of bladder wall of a *Taenia solium* metacestode which was removed surgically from the fourth ventricle of a human brain. Fluorescein isothiocyanate-conjugated goat anti-human IgG (H & L chains) (molar F/P ratio 4.6; specific antibody concentration 1.0~2.0mg/ml, Bio-Yeda Ltd., Israel) was used. Optimal dilution of fluorescent conjugate was 1 : 32 as determined by checkerboard titration. Test procedures were followed those of Wilson *et al.* (1974) and Kang *et al.* (1987). Antigen was incubated with two-fold diluted sera for 30 minutes and after wash, the conjugate was incubated for 30 minutes in a humid chamber.

Antigenic localities on the frozen sections appeared at inner surface of the bladder wall, tegument and subtegumental cells in the order of both frequency and intensity of apple-green fluorescence. Extension of yellow-green fluorescence at entire inner surface of the bladder wall, in serum dilution of 1 : 16 or

higher, was regarded as positive reaction.

3. ELISA

Procedures described by Cho *et al.* (1986) were followed. Cystic fluid of *Taenia solium* metacestodes was used as antigen at concentration of 2.5µg/ml in carbonate buffer. Serum was diluted to 1 : 100 and peroxidase-conjugated goat anti-human IgG (H & L chains) (Cappel, U.S.A.) was diluted to 1 : 5,000. Absorbance at 492 nm was read, and absorbance of 0.18 or higher was regarded as positive as described by Cho *et al.* (1986).

RESULTS

Out of 163 neurocysticercosis patients, specific IgG antibody test by IFAT was positive in 146 patients (sensitivity 89.6%) when serum titer of 1 : 16 or higher was regarded as positive. In 145 positive reactors in serum by ELISA, 138 (95.2%) were also positive by IFAT, while in 18 negatives by ELISA only 8 (44.4%) were positive by IFAT. Of 100 non-infected controls, 3 cases (serum titer of 1 : 16) in IFAT and 5 cases (absorbance 0.20~0.25) in ELISA showed positive reaction (Table 1).

As shown in Fig. 1, levels of specific IgG antibody as shown by absorbance in ELISA and serum dilutions in IFAT were clearly correlated in neurocysticercosis cases. Mean absorbance by ELISA in 1 : 8 serum titer by IFAT was 0.19. In case of serum titers of 1 : 16, 1 : 32, 1 : 64, 1 : 128, 1 : 256 and 1 : 512, mean absorbances by ELISA were 0.26, 0.44, 0.66, 0.71, 1.07 and 1.24 respectively.

Table 1. Results of serum IFAT in 163 confirmed neurocysticercosis patients and 100 normal control cases

| Group | No. exam. | No. posit. | (%) |
|-------------------------------|-----------|------------|--------|
| Neurocysticercosis | | | |
| Serum(+)/CSF(+ or -) by ELISA | 145 | 138 | (95.2) |
| Serum(-)/CSF(+) by ELISA | 18 | 8 | (44.4) |
| Subtotal | 163 | 146 | (89.6) |
| Control (non-infected) | 100 | 3 | (3.0) |

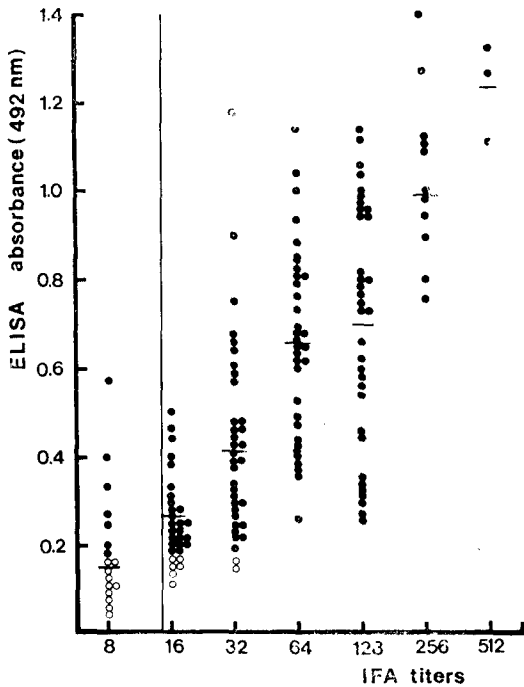


Fig. 1. Relations between absorbance by ELISA and IFAT titers in detecting specific IgG antibody in sera of 163 confirmed neurocysticercosis cases. Open circle (○): serum (-)/CSF (+) cases by ELISA.

In 17 patients with serum IFAT titer of 1 : 8, 7 were positive by ELISA (41.2%). In 30 patients with IFAT titer of 1 : 16, 24 (80.0%) were positive by ELISA and in 35 patients with IFAT titer 1 : 32, 33 (94.3%) were positive by ELISA. The remaining 81 patients with IFAT titers of 1 : 64 or higher showed positive reactions by ELISA without exception.

Table 2 shows the incidence of cross reactions for specific IgG antibody in sera of other neurologic or parasitic diseases. IFAT was more specific especially for excluding other parasitic infections. *Taenia solium* and *T. saginata* infections revealed higher incidence of cross reactions.

By ELISA, the levels of specific IgG antibody in cross reacted cases were absorbance 0.20~0.31 in other neurologic diseases, and 0.21~0.32 in *T. solium*, 0.21~0.26 in *T. saginata*, 0.18~0.34 in *Diphyllobothrium latum*, 0.20 in

Table 2. Comparative incidence of cross reactions by ELISA and IFAT for specific IgG antibodies in sera of other neurologic and parasitic diseases

| Category | No. cases | No. of positive cases by | |
|-------------------------------|-----------|--------------------------|----------|
| | | ELISA (%) | IFAT (%) |
| Other neurologic diseases | 44 | 2 (4.5) | 2 (4.5) |
| <i>Taenia solium</i> | 5 | 4(80.0) | 2(40.0) |
| <i>Taenia saginata</i> | 7 | 3(42.9) | 2(28.6) |
| <i>Diphyllobothrium latum</i> | 14 | 2(14.3) | 2(14.3) |
| <i>Paragonimus westermani</i> | 11 | 1 (9.1) | 0 (0.0) |
| <i>Clonorchis sinensis</i> | 20 | 3(15.0) | 0 (0.0) |

Paragonimus westermani, and 0.19~0.25 in *Clonorchis sinensis* infections. Those levels were just above the cut-off value of the positive reaction.

By IFAT, the levels of antibody in cross-reacted cases to parasitic infections were 1 : 16 or 1 : 32 without exception.

DISCUSSION

In IFAT, different antigens have been used in serodiagnosis of cysticercosis. The adults of *T. solium* or *T. saginata*, or *T. solium* metacystodes from pork or human are used in paraffin sections, particle of worms or in frozen sections (Konovalova, 1973; Gonzalez-Barranco, 1978; Kang *et al.*, 1987; Rydzewski *et al.*, 1975). In this study, frozen sections of *T. solium* metacystode from a human brain were used as described by Kang *et al.* (1987). The sensitivity of IFAT in this study, 89.6%, was not so different from those of previous reports (80.0~95.8%).

In ELISA, on the other hand, many different antigens such as extracts of parenchymal tissue of *T. solium* metacystodes, cystic fluid, or extracts of adult *T. solium* or *T. saginata* were used as antigens. The cystic fluid is now regarded as a more sensitive and specific antigen than any other antigens (Choi *et al.*, 1986; Larralde *et al.*, 1986; Bailly *et al.*, 1988). Using this antigen, our ELISA data showed 89.0% sensitivity in 163 confirmed cases of neurocysticercosis when only sera were tested.

The corresponding rate of the two serologic techniques was 90.8% (148/163) when positive or negative results were combined. These results suggest that IFAT is equally sensitive to ELISA in the serodiagnosis of cysticercosis. Because CSF samples can not be applied in IFAT, the sensitivity of the two serologic techniques using CSF were not compared. Unless CSF is sufficiently concentrated, IFAT could not be applied to measure the specific IgG antibody in CSF.

Flisser *et al.* (1980) and Espinoza *et al.* (1982) described that antigen B was most frequently recognized by infected human sera. Antigen B was fibronectin-rich in connective tissue of the bladder wall or scolices (Guerra *et al.*, 1982). The relations of positively reacting antigenic components and antibody binding sites in IFAT should be elucidated in the future.

Of other parasitic infections tested by IFAT and ELISA in this study, adult cestode infections showed higher cross reactivity with cysticercus antigen than trematode infections. Gonzalez-Barranco (1978) also described a weak cross-reaction in all of 5 cases of *T. saginata* infection. In this study, adult *T. solium* infection showed relatively high cross reactivity in both IFAT and ELISA. Whether the cases of adult *T. solium* infection were associated concomitantly with cysticercosis was not determined. Therefore, it is very difficult at this moment whether the positive reactions in these cases are really cross reaction.

In conclusion, IFAT was found equally sensitive and specific as ELISA in the diagnosis of human neurocysticercosis using serum samples.

REFERENCES

Baily, G.G., Mason, P.R., Trijssenar, F.E.J. and Lyons, N.F. (1988) Serological diagnosis of neurocysticercosis: evaluation of ELISA using cyst fluid and other components of *Taenia solium* cysticerci as antigens. *Trans. Roy. Soc. Trop.*

- Med. Hyg.*, 82:295-299.
- 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(1):25-41.
- 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. *Chung-Ang J. Med.*, 11(2):135-146.
- Espinoza, B., Flisser, A., Plancarte, A. and Larralde, C. (1982) Immunodiagnosis of human cysticercosis: ELISA and immunoelectrophoresis. In: *Cysticercosis "Present state of knowledge and perspectives."* A. Flisser, K. Willms, J.P. Lacleste, C. Larralde, C. Ridaura and F. Beltran (eds.). Academic Press, pp.163-170.
- Flisser, A. and Larralde, C. (1986) Cysticercosis. In *Immunodiagnosis of parasitic diseases. Vol. I. Helminthic diseases.* K. W. Walls, P. M. Schantz (ed.). Academic Press, pp.109-161.
- Gonzalez-Barranco, D., Sandoval-Islas, M.E. and Trujillo-Valdes, V.M. (1978) Indirect immunofluorescence reaction in cysticercosis. *Archivos de Investigacion Medica*, 9:51-58.
- Guerra, G., Flisser, A., Canedo, L. and Lacleste, J.P. (1982) Biochemical and immunological characterization of antigen B purified from cysticerci of *Taenia solium*. In *Cysticercosis "Present state of knowledge and perspectives."* A. Flisser, K. Willms, J.P. Lacleste, C. Larralde, C. Ridaura and F. Beltran (eds.). Academic Press, pp.437-451.
- Kang, O.J., Eom, K.S. and Rim, H.J. (1987) Serodiagnosis of human cysticercosis using sectioned antigen of *Taenia solium* cysticerci in an IFAT. *Korea Univ. Med. J.*, 24(1):85-97. (in Korean with English abstract).
- Konovalova, L.M. (1973) Development and application of new methods for serodiagnosis of cysticercosis in man. *Med. Parazitol. Parazitar. Bol.*, 42:536-542.
- Rydzewski, A.K., Chisholm, E.S. and Kagan, I.G. (1975) Comparison of serologic tests for human cysticercosis by indirect hemagglutination, indirect immunofluorescent antibody, and agar gel precipitin tests. *J. Parasit.*, 61(1):154-155.

Wilson, M., Sulzer, A.J. and Walls, K.W. (1974)
Modified antigens in the indirect fluorescence test

for schistosomiasis. *Am. J. Trop. Med. Hyg.*,
23(6):1072-1076.

＝국문초록＝

뇌낭미충증의 혈청학적 진단에 있어서 간접 형광항체 반응 및 효소연결성 면역흡착 검사의 비교 평가

忠北大學校 醫科大學 寄生蟲學教室

嚴 基 善

中央大學校 醫科大學 寄生蟲學教室

趙 昇 烈

高麗大學校 醫科大學 寄生蟲學教室

林 漢 鍾

뇌낭미충증의 면역혈청학적 진단에 있어서 간접 형광항체법의 유용성을 평가하기 위하여 효소연결성 면역흡착 검사와 비교 검토하였다. 검사대상자는 확진된 뇌낭미충증 환자의 혈청 163예, 다른 뇌신경 증상 환자, 조충 및 흡충류 감염자 101예 및 건강인 대조군 100예로서 모두 364예이었다. 간접 형광항체 반응에는 인체 유구낭미충의 낭벽 항원을, 효소연결성 면역흡착 검사에는 낭액 항원을 사용하여 혈청내 특이 IgG 항체를 조사한 결과 두 방법간의 민감도 및 특이도에 큰 차이가 없었으며, 양성 및 음성의 동일한 혈청을 검사하였을 때 낭미충증 혈청의 90.8%가 서로 합치되어 밀접한 연관성을 나타내었다. 또한 장내 조충 감염증의 경우 두 방법 모두에서 높은 교차반응을 나타내었으나 간접 형광항체반응의 특이성이 더 좋았으며 특히 간접 형광항체 반응은 흡충류 감염자 혈청에서 교차반응을 나타내지 않았다.

이와 같은 결과는 혈청만을 사용하였을 경우 간접 형광항체 반응의 민감도나 특이도가 효소연결성 면역흡착 검사와 차이가 없으며 뇌낭미충증의 진단에 매우 유용함을 나타내고 있었다.