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# Detection of Antibodies in Serum and Cerebrospinal Fluid to *Toxoplasma gondii* by Indirect Latex Agglutination Test and Enzyme-Linked Immunosorbent Assay*

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**Abstract:** Sensitivity of anti-*Toxoplasma* antibody (IgG) test by enzyme-linked immunosorbent assay (ELISA) was evaluated in comparison with indirect latex agglutination (ILA) using 2,016 paired human samples of serum and cerebrospinal fluid (CSF). The samples were collected from neurologic patients in Korea with mass lesions in central nervous system (CNS) as revealed by imaging diagnosis (CT/MRI). When the sera were screened for anti-*Toxoplasma* antibody by ILA, 76 cases (3.8%) were positive (1 : 32 or higher titers). In the paird samples of CSF, no positive reactions were observed. When ELISA was performed using PBS extract of Percoll purified tachyzoites as antigen, cut-off absorbance was determined as 0.40 for serum and 0.27 for CSF tests. The antibody positive rates by ELISA were 7.0% in serum and 5.6% in CSF. Of them, 40 cases (2.0%) showed positive reactions in both serum and CSF. The antibody positive rates were higher in groups older than 40 years. The rates were higher in male (4.7% by ILA, 8.3% by ELISA) than in female (2.2% by ILA, 5.0% by ELISA). The rates in CSF showed no such sex difference. ELISA showed twice higher positive rates when serum was tested, and was sensitive enough to detect specific antibodies in CSF. Etiologic relations between positive antibody tests and CNS lesions remained unknown.

**Key words:** Toxoplasmosis, *Toxoplasma gondii*, serological diagnosis, ELISA, indirect latex agglutination, neurologic disease

## INTRODUCTION

Toxoplasmosis is a typical zoonosis caused by *Toxoplasma gondii* (Protozoa: Apicomplexa) which infects mammals and birds with very low host specificity (Choi *et al.*, 1987). *T. gondii* is a worldwidely distributed etiological agent. It has been known that 20~30% of human and several ten percentages of animals are infected with *T. gondii*, but the rate of infection varies widely by geographic region. Human toxoplasmosis is generally benign in healthy

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persons, but it can be serious in the cases of immunocompromised patients of AIDS, organ transplantations or other causes of immunodeficiency as well as in children of congenital infections. In these patients, CNS involvement is common.

Since Soh *et al.* (1960) reported first the seropositive rate of 5.6% in 373 Koreans by skin test using toxoplasmin, prevalence of *Toxoplasma* antibodies in Korea has been surveyed in both human and animal population (Moon, 1965; Choi, 1969; Choi *et al.*, 1982, 1983, 1984, 1985, 1987 & 1989; Kim & Choi, 1983). Most of these surveys were performed to detect anti-*Toxoplasma* antibody in sera by ILA. Using ILA as an antibody test, however, the levels in CSF have not been measured probably because of its low sensitivity.

Because CSF is now available increasingly in Korea as test materials for the screening of parasitic diseases of the neurologic patients, a sensitive method measuring the anti-*Toxoplasma* antibody levels in CSF becomes more required. ELISA seems the best candidate for such need. The present study was designed to evaluate ELISA as a method of anti-*Toxoplasma* antibody tests in serum and CSF in comparison with ILA. In addition, the prevalence of anti-*Toxoplasma* antibody in the neurologic patients in Korea was estimated.

## MATERIALS AND METHODS

**Antigen:** Diagnostic antigen was prepared from tachyzoites of the RH strain of *T. gondii* harvested from peritoneal exudates of mice that had been infected 4 days before. The parasites were separated from peritoneal exudate cells by 40% Percoll centrifugation. And the purified tachyzoites were washed and suspended in phosphate-buffered saline (PBS, pH 7.4). They were disrupted by sonication three times for 30 sec each. After centrifugation at 13,000 rpm for 5 min, the supernatant was stored at −20°C until used.

**Sera and CSF:** A total of 2,016 pairs of serum and CSF was subjected to the antibody tests for toxoplasmosis. These human samples were collected and stored at −20°C at the Department of Parasitology, College of Medicine, Chung-Ang University from 1986 to 1991 to test the anti-cysticercus antibody by ELISA in patients with neurologic manifestations. Main symptoms of the patients were seizures, headache and hemiparesis. Most of the patients were examined by brain CT or MRI which revealed one or multiple low densities or nodules, hydrocephalus, *etc.* Patients of AIDS or organ transplantation were not included in this study.

**Indirect Latex Agglutination Test:** ILA tests for toxoplasmosis were performed by the method of Choi *et al.* (1989). Briefly, sera or CSF were diluted 2-folds serially in a U-shaped 96 microtiter plates in PBS buffer, reacted with sensitized latex antigen (Eiken, Japan) for 16 hr at room temperature. Antibody titers were determined by the last dilution number of serum or CSF which precipitated latex agglutination of middle class dispersions. In serum test, agglutination at dilution of 1:32 or higher were regarded as positive.

**Enzyme-Linked Immunosorbent Assay:** ELISA for toxoplasmosis was performed essentially according to the procedure of McLaren *et al.* (1978). Two hundred μl of antigen diluted in carbonate buffer (pH 9.6) (protein concentration of 2.5 μg/ml) were coated to 96 well polystyrene EIA plate (Costar) overnight at 4°C. After washing the wells with PBS containing 0.05% Tween-20 (PBS-Tween) for 3 times, 200 μl of 1:100 diluted sera in PBS-Tween and CSF were incubated for 2 hr at 37°C. After washing, 200 μl of 1:1,000 diluted peroxidase-conjugated goat antihuman IgG (Fc specific, Cappel) in PBS-Tween were incubated for 2 hr at 37°C. After final washing 200 μl of substrate solution made of 1 ml of 1% *o*-phenylene diamine, 50 μl of 30% $H_2O_2$ and 99 ml of distilled water were added. The reaction was stopped by adding 20 μl of 5 N $H_2SO_4$ after 25 min incubation at 25°C. The absorbance was read at 490 nm with an ELISA reader (Dynatech).

## RESULTS

### 1. Cut-off absorbance of ELISA in anti-*Toxoplasma* antibody test

When all of 2,016 serum samples were screened by ILA at dilution of 1:8, a total of 188 samples showed agglutination (Table 1). These 188 sera were tested again by ILA at 2-fold dilutions from 1:8 to 1:1,024. Of them, 76 cases (3.8%) showed positive reactions of 1:32 or higher titers. CSF samples of serologically positive cases were tested by ILA for *Toxoplasma* antibody, which showed no positive reaction with 4 cases of 1:16 titers (Table 2).

Absorbance for anti-*Toxoplasma* antibody (IgG) was observed in 245 serum samples by ELISA. The tested serum samples included the above mentioned 188 sera with titers over 1:8, and additional 57 sera selected randomly of which ILA titers were below 1:4. Absorbance by ELISA was plotted against ILA titers as shown in Fig. 1. Antibody levels by ELISA in serum samples elevated significantly by paired t-test in sera of ILA 1:32 group ($p<0.05$). Based on these results, the cut-off absorbance for positive reactions by ELISA was determined to be 0.40 which was the mean absorbance of ILA 1:32 titer group. In the case of CSF, absorbance for anti-*Toxoplasma* antibody (IgG)

**Table 1.** Frequencies of serological ILA titers over 1:8

| Titer | No. of cases | | |
|---|---|---|---|
| | Male | Female | Total |
| 1:8 | 48 | 22 | 70 |
| 1:16 | 25 | 17 | 42 |
| 1:32 | 28 | 6 | 34 |
| 1:64 | 9 | 5 | 14 |
| 1:128 | 7 | 2 | 9 |
| 1:256 | 6 | 0 | 6 |
| 1:512 | 3 | 2 | 5 |
| 1:1,024* | 6 | 2 | 8 |
| Total | 132 | 56 | 188 |

* Titers of 1:1,024 or higher

**Table 2.** ILA titers of CSF of which the serum titers are 1:1,024 or higher

| Titer | No. of cases |
|---|---|
| 1:2 | 2 |
| 1:4 | 1 |
| 1:8 | 2* |
| 1:16 | 4** |
| Total | 9 |

* Serum titer of one case is 1:32
** Serum titer of one case is 1:512

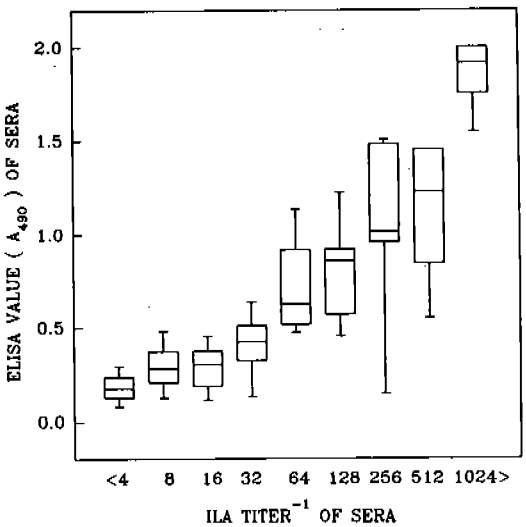


**Fig. 1.** Relations between anti-*Toxoplasma* antibody (IgG) in sera by ELISA and the ILA titers.

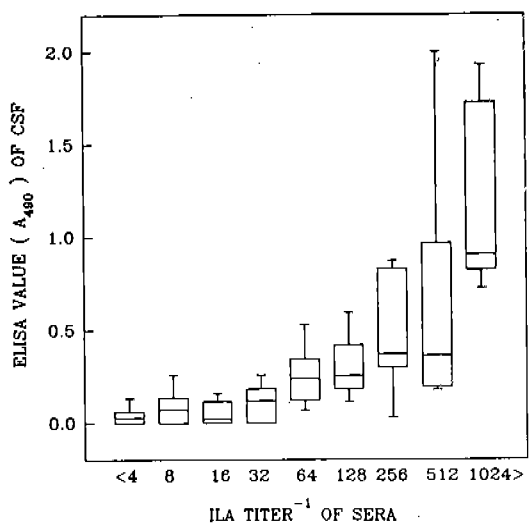


**Fig. 2.** Relations between anti-*Toxoplasma* antibody (IgG) in CSF by ELISA and the ILA titers.

by ELISA was elevated significantly in ILA titers in paired serum samples were 1:64($p<0.01$). The average absorbance in serum ILA titer of 1:64 group was 0.27 which was regarded as cut-off absorbance for the positive reaction in CSF (Fig. 2).

### 2. Comparative sensitivities of ILA and ELISA in anti-*Toxoplasma* antibody test

The cut-off absorbance for anti-*Toxoplasma* antibody by ELISA was applied in the assay results of 2,016 sera and CSF. The positive reactions were compared with that obtained by ILA (Table 3). While ILA found 76(3.8%) of positive reactions in sera assay and none in CSF, ELISA detected 142(7.0%) of positive in sera assay and 112(5.6%) of positive in CSF assay (Fig. 3). Of 1,940 seronegative cases by ILA, 84(4.3%) were positive in serum and 83(4.3%) were positive in CSF by ELISA. Of

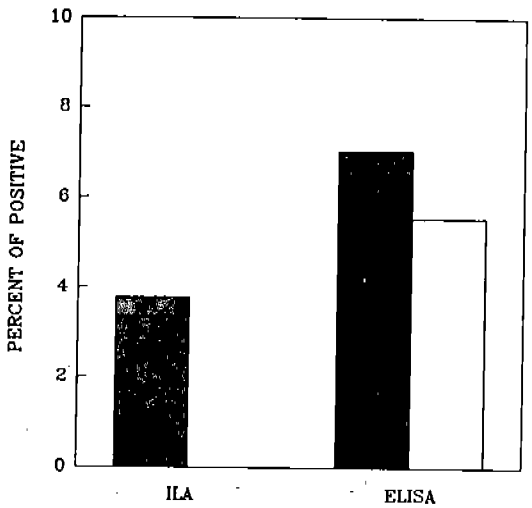


**Fig. 3.** Comparison of percent of positive between ILA and ELISA. ▪, serum and □, CSF.

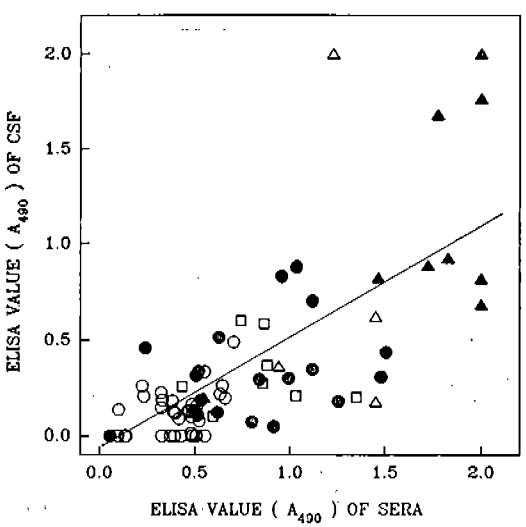


**Fig. 4.** Correlationship between ELISA values of the sera and CSF among ILA positive cases. ○, 1:32; ●, 1:64; □, 1:128; ▪, 1:256; △, 1:512 and ▲, 1:1,024 or higher.

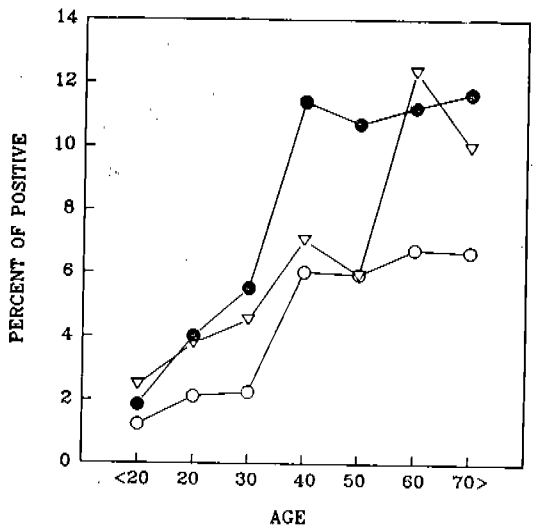


**Fig. 5.** Profile of antibody positive rates by age. ○, sera by ILA; ●, sera by ELISA and ▽, CSF by ELISA

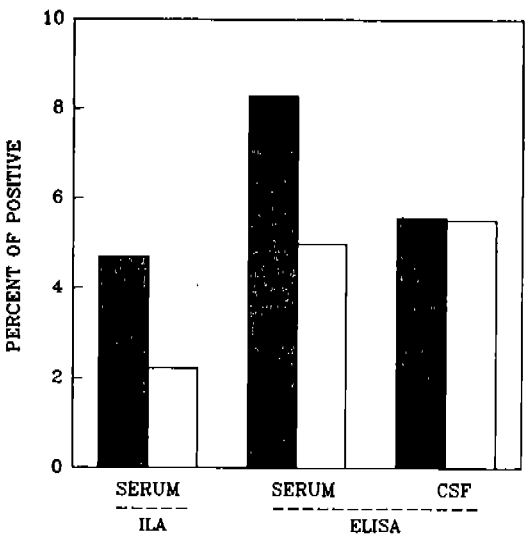


**Fig. 6.** Positive rates of anti-*Toxoplasma* antibodies by sex. ▪, male and □, female.

**Table 3.** Prevalence of ELISA positive cases by ILA titers in 2,016 neurologic patients

| ILA titers | No. of cases | No. of positive cases (absorbance*) by | | |
|---|---|---|---|---|
| | | serum | CSF | serum/CSF |
| <1 : 4 | (1,828) | 62(0.54±0.29) | 75(0.42±0.22) | 9(0.93±0.59/0.63±0.51) |
| 1 : 8 | ( 70) | 14(0.49±0.06) | 7(0.33±0.04) | 3(0.48±0.02/0.32±0.01) |
| 1 : 16 | ( 42) | 8(0.47±0.03) | 1(0.34 ) | 0 |
| subtotal | (1,940) | 84(4.33%) | 83(4.28%) | 12(0.62%) |
| 1 : 32 | ( 34) | 18(0.53±0.08) | 2(0.41±0.08) | 2(0.63±0.08/0.41±0.08) |
| 1 : 64 | ( 14) | 13(0.76±0.26) | 7(0.42±0.14) | 6(0.79±0.26/0.42±0.15) |
| 1 : 128 | ( 9) | 9(0.81±0.27) | 4(0.46±0.14) | 4(0.84±0.05/0.46±0.14) |
| 1 : 256 | ( 6) | 5(1.20±0.25) | 5(0.55±0.25) | 5(1.20±0.25/0.55±0.25) |
| 1 : 512 | ( 5) | 5(1.13±0.34) | 3(1.00±0.72) | 3(1.21±0.21/1.00±0.72) |
| 1 : 1,024 | ( 8) | 8(1.85±0.18) | 8(1.20±0.49) | 8(1.85±0.18/1.20±0.49) |
| subtotal | ( 76) | 58(76.3%) | 29(38.2%) | 28(36.8%) |

* Mean±Standard deviation of absorbance by ELISA

**Table 4.** Screening of sera by ILA for anti-*Toxoplasma* antibody

| Age | No. of positive*/examined cases | | |
|---|---|---|---|
| | Male | Female | Total |
| <20 | 4/195 | 0/128 | 4/323 |
| 20~29 | 8/253 | 1/170 | 9/423 |
| 30~39 | 9/249 | 0/149 | 9/398 |
| 40~49 | 15/210 | 3/ 88 | 18/298 |
| 50~59 | 13/204 | 7/132 | 20/336 |
| 60~69 | 8/106 | 4/ 72 | 12/178 |
| >70 | 2/ 38 | 2/ 22 | 4/ 60 |
| Total | 59/1255 | 17/761 | 76/2,016 |

* Titers of 1 : 32 or higher

76 cases of seropositive cases by ILA, 58 (76.3%) of sera and 29 (38.2%) of CSF were positive by ELISA (Table 3).

In Fig. 4, levels of anti-*Toxoplasma* antibody in serum and CSF were shown according to the antibody titers of ILA. The relations between absorbance in CSF and serum could be described by the following equation:

$$Absorbance_{CSF}=0.62\times Absorbance_{SERUM}-0.12.$$

And the 52.5% of cross points were explained by this equation.

### 3. Seroepidemiological findings of anti-*Toxoplasma* antibody in neurologic patients

**Table 5.** Screening of sera and CSF for anti-*Toxoplasma* antibodies by ELISA

| Age | No. of positive/examined cases | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Serum* | | | CSF** | | | Serum/CSF** | | |
| | Male | Female | Total | Male | Female | Total | Male | Female | Total |
| <20 | 6/ 195 | 0/128 | 6/ 323 | 3 | 5 | 8 | 0 | 0 | 0 |
| 20~29 | 11/ 253 | 6/170 | 17/ 423 | 10 | 6 | 16 | 1 | 0 | 1 |
| 30~39 | 18/ 249 | 4/149 | 22/ 398 | 12 | 6 | 18 | 3 | 0 | 3 |
| 40~49 | 28/ 210 | 6/ 88 | 34/ 298 | 18 | 3 | 21 | 11 | 1 | 12 |
| 50~59 | 24/ 204 | 12/132 | 36/ 336 | 12 | 8 | 20 | 6 | 6 | 12 |
| 60~69 | 13/ 106 | 7/ 72 | 20/ 178 | 12 | 10 | 22 | 7 | 2 | 9 |
| >70 | 4/ 38 | 3/ 22 | 7/ 60 | 2 | 4 | 6 | 1 | 3 | 4 |
| Total | 104/1,255 | 38/761 | 142/2,016 | 70 | 42 | 112 | 28 | 12 | 40 |

* $A_{490}$ of serum is 0.40 or higher ** $A_{490}$ of CSF is 0.27 or higher

*** $A_{490}$ of both serum and CSF is positive.

Table 4 showed the age and sex distribution of positive reactions of anti-*Toxoplasma* antibody as revealed by ILA. There were differences in the seropositive rates between male (4.7%) and female (2.2%). The positive rates increased with age from 1.2% in age group of under 20 years to 6.0~6.7% in age groups older than 40 years (Fig. 5).

The same patterns of age and sex distribution in positive reactions of the antibody were revealed as the serum was tested by ELISA. The only difference was twice higher rates of positive reactions. The similarly sensitive results were obtained in CSF tests by ELISA (Table 4 and Figs. 5 & 6). But the positive rates in CSF did not show sexual difference (5.6% in male, 5.3% in female).

## DISCUSSION

ELISA was sensitive enough to detect anti-*Toxoplasma* antibody in CSF, though ILA could not detect it. The antibody detection rate by ELISA was about twice higher than ILA when sera were tested. As shown in Fig. 5, however, the distribution patterns of positive reactions by age and sex were similar each other when the results by ILA and ELISA were compared. Most of ILA positive cases in sera were positive by ELISA (76.3%). But 4.3% of ILA negative sera were positive by ELISA. These discrepancies may be resulted from different antigenic epitopes recognized by ILA and ELISA (Lunde and Jacobs, 1983; Kasper *et al.*, 1984; Makioka *et al.*, 1991).

Many serological tests had been developed to test anti-*Toxoplasma* antibodies. Of these, ILA had been used for several years in this laboratory (CUMC) because of its easiness to perform and its comparable sensitivity and specificity after the improvement by Tsubota *et al.* (1977) and by Kobayashi *et al.* (1977) which resulted in the agreement of 94.4% with those of dye test (Sabin and Feldman, 1948). By ILA, Choi *et al.* (1982) obtained 4.3% positive among 412 patients of the St. Mary's Hospital, and Kim and Choi (1983) examined 7.2% positive among 874 patients of the Seoul Red Cross Hospital. Choi *et al.* (1983) screened 573 patients in the Seoul Mental Hospital that resulted in 1.9% of positive rates, with especially high percentage in hydrochondiacs of 7.4%. Later, Choi *et al.* (1984) examined 515 swine sera in outskirts of Seoul which resulted in 12.4% positive rate, and Choi *et al.* (1985) screened 377 pregnant women and 43 pelvic tumor patients of the Kangnam St. Mary's Hospital which resulted in 0.5% in the former and 7.0% in the latter. Choi *et al.* (1987) obtained 20 out of 131 (15.3%) cases in mammals, 2 out of 75 (2.7%) cases in birds, and none of 10 cases in reptiles from the Seoul Grand Park. And recently, Choi *et al.* (1989) reported 19 cases among 1,019 (1.86%) in general patient group, 11 out of 1,030 (1.07%) cases in asthma patient group of the Kangnam St. Mary's Hospital, and 45 cases out of 780 (5.57%) patient group at the Cheju island to be positive. In this study, the seropositive rate by ILA was 3.8% in the neurologic patients. By ELISA, the seropositive rate was 7.0% in the same patients and paired CSF showed the positive reactions in 5.6%.

The exact meaning of the positive reactions for anti-*Toxoplasma* antibody is difficult to evaluate in this study. The subjected cases were all neurologic patients mostly with CNS lesions by imaging diagnosis. But the etiologic relations of the positive antibody test should be evaluated carefully in these patients. Not all of antibody positive cases in serum were positive in CSF. Of 142 positive cases in serum antibody and of 112 positive cases of CSF antibody, only 40 cases showed positive antibody levels in both serum and CSF. Though toxoplasmosis is a systemic infection, CNS involvement is a relatively rare event unless infections occur in immunocompromised state. In this sense, antibody in CSF seems more significant in diagnosing CNS toxoplasmosis. The relationship between serum and CSF antibody levels has been known to be complex because of the blood-brain barrier. And antibody levels in CSF are influenced by normal

transudation, pathologic exudation of the serum antibody and intracranial synthesis of the antibody at the local lesion (Levy *et al.*, 1984; Cho *et al.*, 1988). To make the matters further complex, the correct pathological diagnosis of CNS toxoplasmosis is practically difficult. Therefore the relations between the protean image findings of CNS toxoplasmosis and positive antibody tests are hardly proven pathologically. Further studies are necessary in this aspect.

As repeatedly proven in antibody surveys for toxoplasmosis, the positive rates were not high in Korean population when compared with those reported in European and American countries. The results in this study were similar with the many previous reports. However, the rates in older people over 40 years were 6~7% by ILA and 10~11% by ELISA which were 2~3 times higher than the younger people. By sex, the rate in male was about twice higher than in female. Unlike the antibody positive rate in serum, that in CSF antibody did not show sex difference. Epidemiologically, these patterns of positive antibody rates by sex and age are similar with those of clonorchiasis in Korea. It is suggested that human *Toxoplasma* infection is related with drinking alcohol when undercooked pork is not uncommonly consumed.

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=국문초록=

## 간접 Latex 응집반응과 ELISA에 의한 중추신경계 질환 환자의 혈청 및 뇌척수액에서 *Toxoplasma gondii*에 대한 항체 검출

가톨릭의대 기생충학교실 및 중앙의대 기생충학교실*

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*Toxoplasma*증의 혈청학적 진단에 있어서 민감도를 증가시키기 위해 간접 latex 응집반응의 결과와 비교하면서 ELISA를 개발하였으며, 뇌척수액의 검사 시료로서의 가능성을 검토하였다. 아울러 중추신경계 질환환자로부터 기생충질환을 감별하기 위하여 1986년부터 1991년까지 전국 각 병원에서 채취한 혈청과 뇌척수액에 대하여 간접 latex 응집반응(ILA)과 ELISA를 실시하여 *Toxoplasma* 항체 보유 양상을 비교 검토하였다. 전체 2,016건의 혈청에 대해 ILA를 실시하여 76건(3.8%)의 양성(1 : 32 이상의 titer)을 얻었다. 그러나 양성 혈청환자에서 채취한 뇌척수액에서는 낮은 titer의 반응은 있었으나 양성은 나타나지 않았다. 이들 양성 혈청, 의양성 혈청 및 음성 혈청에 대하여 ELISA로 항체검사를 실시한바 ILA의 titer가 1 : 32인 군에서 통계적으로 유의한 차이를 나타내는 항체값을 얻었으며, 그 흡광도는 0.40이었다. 뇌척수액에 대한 ELISA로는 ILA의 1 : 64 titer군에서 통계적으로 유의한 차이가 나타났고 그때의 흡광도 0.27을 양성 판단의 기준으로 사용하였다. ELISA에 의한 항체 검사상 전체 혈청에서 7.0%의 양성을 검출하여 ILA보다 약 2배 정도의 높은 민감도를 보였으며, 뇌척수액에서는 5.6%의 양성률을 보여 ELISA는 뇌척수액에서의 항체 검출시 유용한 방법이라고 판단하였다. ILA에 비하여 ELISA는 약 2배 정도 높은 양성률을 내었고 양성률은 나이에 따라 40대 이후 급격한 증가를 보였으며, 여성보다는 남성에서 약 2배 정도 양성률이 높게 나타났다. ELISA에 의한 뇌척수액의 항체 검사에서는 양성률의 성별 차이를 나타내지 않았다. 이상의 결과로 판단할 때, ELISA가 ILA보다 *Toxoplasma* 항체 검출의 민감도가 높았으며, 뇌척수액은 ELISA의 좋은 검사시료가 되며, 특히 중추신경계 *Toxoplasma*증의 진단에 있어 뇌척수액에 대한 항체 검사에서 ELISA가 유용하다고 판단하였다. 〔기생충학잡지, 30(2):83-90, **June 1992**〕