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Toxoplasma antibody titers by ELISA and indirect latex agglutination test in pregnant women

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Abstract: The seroepidemiologic studies on anti-*Toxoplasma* antibody titers were carried out using ELISA and indirect latex agglutination test. Among 899 sera prepared from pregnant women, 39 cases (4.3%) revealed positive reaction and 218 sera from middle school students showed 4 positive reaction (1.8%) by ELISA. By LAT (newly established by National Veterinary Research Institute, Korea), the sera of 7 pregnant women (0.8%) showed positive reaction. When 80 sera showing ≥ 1:8 by LAT were used for comparing the results obtained from LAT and Toxotest-MT (Eiken Chemical Co., Japan), 7 cases and 8 sera were positive, respectively. All of 11 sera of proven toxoplasmosis patients showed positive reaction in both tests. Overall proportion of agreement between LAT kit and Toxotest-MT was 0.94 (κ-index = 0.632, $p < 0.01$), and LAT was considered to be useful for the screening of toxoplasmosis.

Key words: *Toxoplasma gondii*, indirect latex agglutination test, ELISA, pregnant women

INTRODUCTION

Congenital toxoplasmosis is associated with maternal parasitemia, infection of the placenta and subsequent infection of the fetus. The risk of maternal-fetal transfer of infection rises as the gestational age at the time of exposure progress, whereas the incidence of severe disease falls (Desmonts and Couvreur, 1974 a & b, 1979). Rapid diagnosis of congenital infection is required as there is some evidence that treatment given in the early post-natal period may reduce the incidence of ocular disease in later life (Wilson *et al.*, 1980; Chatterton, 1992).

To identify *T. gondii* tachyzoites from suspected patients, body fluids or ground tissues are inoculated into the mouse peritoneal cavity, and examine the smears of the lungs, spleen, and liver after 3-6 weeks. However, this method takes long time and it is difficult to find tachyzoites (Beaver *et al.*, 1984).

Many serologic tests, such as haemagglutination test, latex agglutination test, ELISA, indirect fluorescence antibody test, have been developed for detection of antibodies against *T. gondii*. Although Sabin and Feldman dye test is the accepted as a reference for toxoplasma serology, this test has a great disadvantage because it requires live *T. gondii* organisms.

This study was intended to reveal the prevalence rate of anti-*Toxoplasma* antibody by ELISA and indirect latex agglutination test

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on the pregnant women, and the usefulness of LAT kit made by Korea was evaluated.

MATERIALS AND METHODS

Collection of sera

Totally 899 sera were obtained from pregnant women who visited medical institution (public health center, local clinics for obstetrics and gynecology) in Yangpyong-gun and Kwangju-gun of Kyonggi-do for prenatal obstetric care from March 1993 to June 1994. The sera were frozen to -70°C until testing. Age range of the pregnant women was 15-42 years, and the average age was 31.9 ± 18.50 (Table 1).

Two hundred eighteen sera from middle school students in Yangpyong-gun were examined as negative control group. The age distribution of the students was 13-15 years. The number of schoolgirls and schoolboys was 112 and 106, respectively.

Enzyme-linked immunosorbent assay (ELISA)

Tachyzoites of *T. gondii* (RH strain) harvested from peritoneal exudates of experimentally infected mice, were disrupted by four cycles of freezing in liquid N_2 and thawing at 37°C. The homogenate was centrifuged at 4°C, 10,000 g for 1 hr and the supernatant was used as antigen. ELISA was performed according to the procedure of Voller *et al.* (1976). The protein concentration of *T. gondii* was 5 μg/ml. 1:1000 diluted peroxidase conjugated antihuman IgG and ortho-phenylene diamine were used. The optical density was read at 490 nm with ELISA reader (Dynatech Co., Swiss).

Table 1. Age distribution of pregnant women

Age Range	Number of women	percent
15~19	11	1.2
20~24	183	20.4
25~29	434	48.3
30~34	178	20.9
35~39	22	2.4
40~44	3	0.3
Unknown	58	6.5
Total	899	100

Indirect latex agglutination test

1) LAT kit

LAT kit was supplied from National Veterinary Research Institute of Korea.

1-1) Antigen preparation

The tachyzoites were seperated from mice peritoneal exudate cells by filtration with 2.0 μm polycarbonate membrane (Costar, USA) and disrupted by sonication. After centrifugation at 22,500 g for 30 min, the supernatant was stored at -80°C. The protein concentration was adjusted to 2-4 mg/ml.

1-2) Sensitization of latex particles

The antigen was adsorbed onto polystyrene latex particles of 1.0 μm in diameter (Polyscience Co, USA) by adding 2.5% latex suspension to equal volume of *T. gondii* antigen in 0.1 M Tris-HCl (pH 8.0) buffer and by incubating the mixture at 37°C for 30 min, and then 4°C for 8 hrs with slow agitation. After centrifugation at 6,000 rpm for 20 mins, sensitized particles were suspended in serum dilution buffer (0.2 M amino-methyl propanol, 0.2% BSA, 0.01% sodium azide). The beads were washed thrice. Final latex concentration was 0.1% and stored at 4°C until use.

1-3) Antibody titration of immune sera by LAT kit

The immune serum was diluted 2-folds serially from 1:4 to 1:2048 in a U-shaped 96 microtiter plates with serum dilution buffer, and reacted with sensitized latex particles for 16 hr at room temperature. The antibody titer was 1:256-1:512.

2) Toxotest-MT

Eighty sera with titers over 1:8 by LAT were tested by Toxotest-MT kit. The Toxotest-MT kit (Eiken Chemical Co (lot No. 5Y013), Japan) was used according to the manufacturer's instruction from the dilution of a 1/16.

RESULTS

Anti-*Toxoplasma* antibody titers by IgG-ELISA

The mean absorbance of 218 middle school students was 0.14 ± 0.052 (mean absorbance of schoolgirls: 0.142 ± 0.038, schoolboys: 0.147 ± 0.064). Two standard deviation above the mean value for control sera was selected

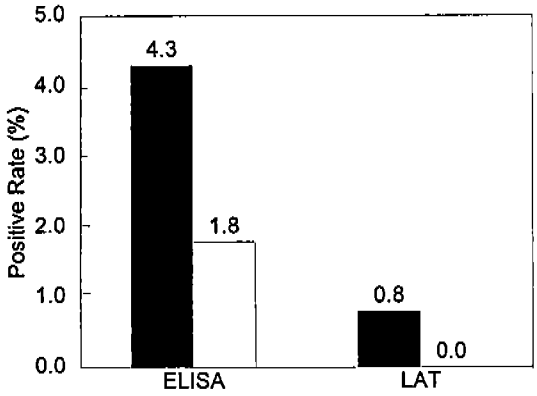

Fig. 1. Comparison of positive rate between ELISA and LAT.
■, 899 pregnant women; □, 218 middle school students
In ELISA, cut-off absorbance was 0.25.
In LAT, the positive border line was 1:64.

as the cut-off point and was determined to be 0.25. Thirty-nine (4.3%) out of 899 sera of pregnant women were found to be positive (Fig. 1). All of the 11 sera of proven toxoplasmosis patients showed positive reaction (0.43 ± 0.091) whereas only 4 cases (1.8%) showed positive reaction in the control group. The positive reaction rates increased with the age from 2.7% in age group of under 24 years and 5.6% in 30-34 years (Fig. 2).

Indirect latex agglutination test

The agglutination at dilution of 1:64 or higher by LAT kit was regarded as positive. Seven (0.8%) out of 899 pregnant women showed positive reaction at 1:64 or higher titers. All of the negative control group showed no positive reaction. On the other hand all of the toxoplasmosis patients showed positive reaction at higher titers (1:512-1:2048). When 80 sera with titers over 1:8 by LAT were tested by Toxotest-MT kit, 8 cases showed positive reaction (Table 2). In Toxotest-MT kit, the positive borderline was 1:32 according to the manufacturer's instruction. The sensitivity of LAT and Toxotest-MT was almost similar. All of the toxoplasmosis patients showed positive reaction, and 40 middle school students (control group) showed negative result by Toxotest-MT. Overall proportion of agreement between LAT kit and Toxotest-MT was 0.94 (κ-index = 0.632, $p < 0.01$, Table 3).

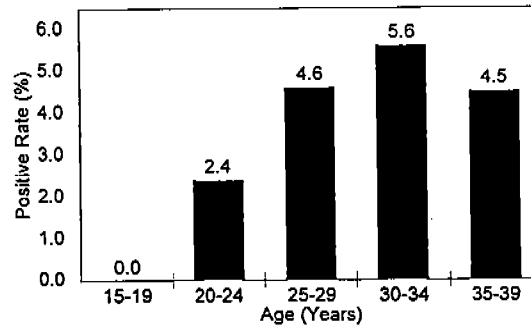

Fig. 2. Age-specific *Toxoplasma* antibody positive rate of 39 pregnant women showed positive reaction by ELISA.

Table 2. Comparison of frequencies of ILA titers between LAT and Toxotest-MT

Titer	LAT	Toxotest-MT
	No. of cases (%)	No. of cases (%)
1:16 <	6 (7.5)	58 (72.5)
1:16	53 (66.3)	14 (17.5)
1:32	14 (17.5)	3 (3.7)
1:64	3 (3.8)	2 (2.5)
1:128	1 (1.3)	0
1:256	0	1 (1.3)
1:512	2 (2.5)	1 (1.3)
1:2048	1 (1.3)	1 (1.3)
Total	80 (100)	80 (100)

In LAT and Toxotest-MT kit, the positive borderlines were 1:64 and 1:32, respectively.

Table 3. Overall proportion of agreement between LAT and Toxotest-MT in 80 pregnant sera with titers over 1:8 by LAT

		LAT		
		+	−	Total
Toxotest -MT	+	5 (6.2%)	3 (3.8%)	8 (10%)
	−	2 (2.5%)	70 (87.5%)	72 (90%)
	Total	7 (8.7%)	73 (91.3%)	80 (100%)

In LAT and Toxotest-MT kit, the positive borderlines were 1:64 and 1:32, respectively.
Overall proportion of agreement 75/80 = 0.94
κ-index = 0.632, p value < 0.01

Table 4. Overall proportion of agreement between IgG-ELISA and LAT in 899 sera of pregnant women

		IgG-ELISA		
		+	−	Total
LAT	+	3 (0.3%)	4 (0.5%)	7 (0.8)
	−	36 (4%)	856 (95.2%)	892 (99.2%)
	Total	39 (4.3%)	860 (95.7%)	899 (100%)

In IgG-ELISA, cut-off O.D. was 0.25.
Overall proportion of agreement 859/899 = 0.96
κ-index = 0.1089, $p < 0.01$

Agreement of IgG-ELISA and indirect latex agglutination test

The mean absorbance of 7 pregnant women who showed positive reaction by LAT kit was 0.37 ± 0.306. That of 8 women who had positive titers by Toxotest-MT kit, was 0.35 ± 0.296. Of 7 seropositive cases by LAT kit, three were positive by IgG-ELISA, and showed high absorbance values (0.72, 0.83 and 0.48). Overall proportion of agreement between IgG-ELISA and LAT was 0.96 (κ-index = 0.1089, $p < 0.01$, Table 4). The value of κ-index = 0.1089 means poor agreement between IgG-ELISA and LAT.

DISCUSSION

There are no obvious clinical characteristics which are unique or predominantly specific to the toxoplasmosis. Indeed, many infections are asymptomatic, or the symptom so mudane that they are overlooked (Remington, 1974). There are some reports on the prevalence of anti-*Toxoplasma* antibody titer in Korea. Choi *et al.* (1982, 1983, 1989, 1992) performed the latex agglutination test for diagnosis of toxoplasmosis in patients with mental disorder, general patients, residents in Seou,l patients with CNS disorder, and their antibody positive rate were 1.2%-7.2%. In this study, 0.8% of pregnant women showed positive reaction by LAT, and this value is similar to that of Choi *et al.* (1985), who reported 0.5% positive rate in pregnant women. Although positive rates were low by ILA, Im *et al.* (1991) reported 6.6% positive rate using IFA, 7.0% by ELISA in 618 pregnant women. In this study the seropositive rates by ILA and ELISA were 0.8%, 4.3% in pregnant women, respectively. his difference of positive rates may be resulted partly from different antigenic epitope recognized by ILA and ELISA (Choi *et al.*, 1992; Lunde and jacobs, 1983; Kasper *et al.*, 1984; Makioka *et al.*, 1991), partly from difference of limits of sensitivity of both tests. Channng Rodgers (1994) described approximate sensitivity of various immunologic methods and that of ELISA is < 1 μg/ml, agglutination 1 μg/ml.

Toxoplasma antibody prevalence in pregnant women varies with the age and geography. The antibody prevalence rate was 80-100% in Paris in France and around 10% in Oregon , USA according to the age (Ho-Yen and Chatterton, 1990). Comparing these values with our results, it seems to be that the variation in antibody rate of Korean women is lower than other countries.

In this study, the differenct of positive rate between pregnant women and students may be probably derived from difference of age (mean age of pregnant women 31.9, students 13.9) since it was known that the proportion of women who are immune increase with age (Desmonts and Couvreur, 1974; Viens *et al.*, 1977; Joss *et al.*, 1988).

ELISA is known to be a useful tool for serodiagnosis. However, ELISA is laborious and time-consuming, and also needs special instrument. In this respect, the ILA is simpler and easier method to perform than ELISA.

Toxotest-MT kit was developed in reaction conditions by Tsubota *et al.* (1977) and Kobayashi *et al.* (1977) and have been used in several countries for several years. In Korea, Suh and Lee (1993) and Suh *et al.* (1995) developed ILA kit and applied to bovine and pig sera. Since the agreement between Toxotest-MT and ILA kit is high, this ILA kit might be used in the diagnosis for animal toxoplasmosis.

The present study was designed to reveal the prevalence rate of anti-*Toxoplasma* antibody in the pregnant women by using LAT kit made by Korea. In the study reported here, fair agreement (κ-index = 0.632) was observed between LAT and Toxotest-MT although their

agreement of antibody titers under 1:64 was poor.

Although LAT was quite useful in this study, it might be required to develop an appropriate antigen for LAT to overcome the difference of antibody titer between LAT and Toxotest-MT, especially titers under 1:64, and to increase the sensitivity of LAT.

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=초록=

ELISA 및 간접 latex 응집반응검사에 의한 임산부의 항 톡소포자충 항체가

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이 연구에서는 경기도 양평군 및 광주군에 사는 임산부 899명을 대상으로 IgG-ELISA와 간접 latex 응집반응검사를 시행하여 톡소포자충에 대한 항체가를 측정하였다. IgG-ELISA에서는 0.25 이상을 양성기준으로 하였을 때 음성대조군 218명 중 4명이 양성(1.8%)인 반면 임산부에서는 39명이 양성으로 검출되어 4.3%의 양성율을 보였다. 간접 latex 응집반응검사는 수의과학연구소에서 만든 킷트(LAT)를 사용하였는데 1:64 희석배수 이상을 양성으로 하였을 때 음성대조군은 모두 음성반응을 보였고 임산부에서는 7명(0.8%)이 양성을 보였다. 임산부중에서 1:8 이상의 반응을 보인 80명을 대상으로 일본제품인 Toxotest-MT를 적용시키고 1:32 이상을 양성의 기준으로 하였을 때 임산부 8명에서 양성반응을 보였다. LAT와 Toxotest-MT의 두 반응간의 일치율은 0.94(κ-index = 0.632, $p < 0.01$)로 높은 일치율(fair to good agreement)을 보였으므로 LAT는 톡소포자충증의 예비진단에 이용될 수 있을 것으로 생각된다.

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