

DETECTION OF TOXOPLASMA GONDII ANTIGENS IN SERA FROM EXPERIMENTALLY INFECTED MICE

Saeedeh Shojaei¹, Keshavarz H², Rezaian M³, Mohebbali M⁴

ABSTRACT

Objective: Detection of *Toxoplasma* antigen in serum of mice by Immunoblotting.

Design: Serum samples isolated from Balb/C mice experimentally infected with *T. gondii*, RH strain. IgG isolated from rabbits that were immunized with *T. gondii* Immunoblotting was performed to detect *T. gondii* antigens in sera of mice.

Setting: School of Public Health. Tehran University of Medical Sciences.

Subjects: Serum samples from mice experimentally infected with *T. gondii* RH strain.

Main outcome measures: The value of Immunoblotting in diagnosis of toxoplasmosis in acute stage of infection.

Result: The antigen bands detected in serum sample of mice were experimentally infected with *T. gondii* tachyzoite in immunoblotting. Six bands demonstrated on seventh post infection day six bands were identified. Similarly on sixth day four bands, on day five three bands and on fourth post infection day two bands were identified. No band was detected in control group sera.

Conclusion: Immunoblotting is a sensitive method for diagnosis of acute stage of toxoplasmosis.

KEY WORDS: Toxoplasma Antigen, Immunoblotting, Infection.

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INTRODUCTION

Toxoplasmosis is a world –wide endemic disease caused by *Toxoplasma gondii*. The course of infection is benign.¹ However human infection is a serious disease in congenitally infected infants and in immunocompromised persons such as AIDS patients or recipients of organ transplantation.²

It has been shown that specific treatment is effective even for patients who present with

severe central nervous system lesions and it can be used as preventive therapy in asymptomatic newborns if it is initiated in the early phase of infection and continued for an adequate period of time.^{3,4} The diagnosis is routinely based on serological tests.⁵ Current serologic diagnosis is based upon demonstration of antibodies, which have been detected by a variety of methods.⁶

The high prevalence of Toxoplasma antibodies in normal individuals have complicated interpretation of serologic test results obtained in individuals suspected of having acute toxoplasmosis.⁷ Serological methods have poor efficacy, especially in neonates and in immunocompromised patients. Detection of parasite or its components might permit better definition of acute toxoplasmosis.^{8,9}

Some studies in laboratory animals and in human have suggested that antigenemia may be present during acute phase of infection with *T. gondii*.^{10,11} Some studies are concerned with the identification of circulating antigen in experimental *Toxoplasma* infections and detection of the antigens in serum by immunological methods.⁶

1. Dr. Saeedeh Shojaei,
Ph. D Candidate of Parasitology
2. Dr. Keshavarz H,
Professor of Parasitology
3. Dr. Rezaian M,
Professor of Parasitology
4. Dr. Mohebbali M,
Professor of Parasitology
- 1-4: Department of Parasitology,
School of Public Health
Tehran University of Medical Sciences,
P.O. Box: 6446-14155,
Tehran - Iran.

Correspondences:

Dr. Saeedeh Shojaei,
E-Mail: shojaei1980@yahoo.com

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This study was performed to determine whether *T. gondii* antigens could be detected in sera from mice infected with acute toxoplasmosis.

MATERIAL AND METHODS

Mice sera: One hundred Balb/C mice were infected by the intraperitoneal route with 5×10^4 tachyzoites of *T. gondii* RH strain. Serum sample of each five animal collected at 15, 18, 21 hours and 1,2,3,4,5,6,7 days post-infection. Sera were stored frozen at -20°C until use. Sera from five mice was collected after P.B.S. injection were used as controls.

Rabbit Immunization: Tachyzoites of RH strain was collected from peritoneal exudates of mice, filtered on polycarbonate membrane, washed with P.B.S and sonicated for 10 Seconds 15 times. After centrifugation at 10,000 rpm, in 4°C the supernatant was collected and protein content determined by the method of Bradford. Rabbits were injected intradermally with a mixture of soluble antigen of *T. gondii* and Freund's complete adjuvant. Two booster injections were given after 4 weeks and rabbits bled on the 20 day after the second booster immunization. Immune sera was stored at -20°C until use. Isolation of IgG from rabbit immunized sera was performed with 40% saturated ammonium sulfate precipitation and Ion-exchange column chromatography with DEAE-cellulose.

Immunoblotting: Mice serum was diluted 1:10 in Tris-Hcl buffer containing sodium dodecyl sulfate. SDS-PAGE performed using 10% separating gel and 3% stacking gel. After electrophoresis, protein bands were transferred on nitrocellulose membrane (45% im, porblote, macherey-nagel) over night. The membrane was saturated with skimmed milk 25% in P.B.S. Anti *T. gondii* rabbit IgG was applied followed by peroxidase conjugated anti-rabbit IgG(Dako) diluted 1:400. The reaction was then developed with the substrate, 50mg diaminobenzidine (sigma), 20ml H_2O_2 in 50ml Tris-Hcl, for 10 minutes. The molecular masses of proteins specific to *T. gondii* was determined by references to commercially available standards. (Sigma marker, High range).

RESULT

The titer of serum IgG antibody isolated from Immunized rabbit increased after each booster injection in ELISA. It showed that two booster injections resulted in high antibody production. Sera precipitated in 40% ammonium sulfate before chromatography, so after elution of precipitated sera with Ion-exchange column chromatography, the result was one specific band as IgG in SDS-PAGE. *Toxoplasma* antigens were identified on immunoblotting in sera from 4 days post infection. Five bands were identified on seventh post infection day with molecular weights of 106, 97, 38, 32, 30 and 22 kDa. Four bands with molecular weights of 106, 97, 38, 32, kDa were identified on seventh post infection day while on fifth day three bands were spotted with molecular weights of 106, 97 and 32 kDa whereas on fourth day two bands with molecular weights of 97 and 32 kDa were detected in serum samples. No band was detected in sera from control group (Fig-1).

DISCUSSION

The most commonly recognized clinical manifestation of acute acquired infection with *Toxoplasma gondii* in immunocompetent adults is lymphadenopathy.¹² Serologic diagnosis is preferable to having the patients undergo a

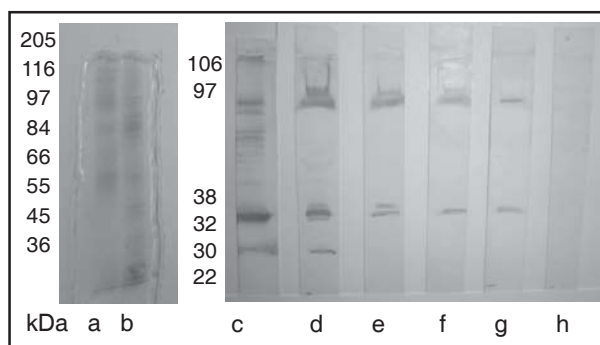


Fig-1: left, SDS-PAGE analysis of *T. gondii* antigen
a) Marker.
b) *T. gondii* antigen Right, Immunoblot analysis of serum sample from mice infected with *T. gondii*.
c) *T. gondii* antigen.
d) Serum seven days post infection (p.i.).
e) Serum six days (p.i.). Five days (p.i.).
f) Four days (p.i.). g) Control group.

biopsy.¹³ However, the high prevalence of *Toxoplasma* antibodies in otherwise normal individuals and the fact that titers may remain elevated for years following the acute infection have complicated interpretation of serologic test result obtained in individuals suspected of having acute toxoplasmosis.⁷

Raizman and Neva (1975) were the first to demonstrate circulating *Toxoplasma* antigens in sera from infected animals with use of counter-current electrophoresis and double-diffusion in agar.⁶ In our study antigen was detected in sera from experimentally infected mice and demonstrated from day four after infection. By increasing the days of infection, numbers of detected bands increased. In four days post infection two bands with molecular weight of 97 and 32 kDa were detected, that increased to three, four and six bands in days five, six and seven post infection respectively. Two bands with molecular weight of 97 and 32 kDa were common bands in these days.

Hafid et al., (1992) reported 2-7 bands with molecular weights of 110, 87, 75, 48, 30, 24, 22 kDa from first up to seven days of infection in serum samples of mice experimentally infected with *T. gondii*. In another study he detected 3-7 bands from the first up to seven days of infection with molecular weights of 110, 87, 75, 48, 30, 24 and 22 kDa. Huskinson demonstrated *Toxoplasma* antigens in sera of mice at 4 days after infection by ELISA.¹⁴ He also detected antigens in serum samples after 4 days of infection with *T. gondii* by immunoblotting. The results described above demonstrate that circulating antigens of *T. gondii* are present in sera of mice during acute stage of infection.

Detection of antigens for diagnostic purposes would be useful in those cases in which clinical findings are suggestive of acute toxoplasmosis and in those cases in which antibody response is suppressed because of serious underlying disease or immunosuppressive therapy.¹⁵ It is also important for congenitally infected children. Toxoplasmosis contracted during the first trimester of pregnancy induces the most serious symptoms and specific treatment could be effective if it is initiated in the early phase of infection.

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