Evaluation of *Toxoplasma gondii* soluble, whole and excretory/secretary antigens for diagnosis of toxoplasmosis by ELISA test

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Abstract The present study was performed to compare the soluble, whole and excretory/secretary antigens of *Toxoplasma gondii* (RH strain) in diagnosis of toxoplasmosis by ELISA method. Tachyzoites of *T. gondii*, RH strain were injected in intra-peritoneal cavity of BALB/c mice, after 4 days tachyzoites were harvested by peritoneal washing of the mice. For soluble antigen, exudates were centrifuged and sediment sonicated and then centrifuged at 4 °C, 1 h, supernatant collected and density of protein determined by Bradford method. For whole antigen after collecting, washing and centrifuging of peritoneal fluid the tachyzoites sediment was counted. In excretory/secretary antigen 1.5×10^8 tachyzoites were transferred in 1 ml tube of saline and incubated under mild agitation and after centrifuging, supernatant was collected and protein density determined by Bradford method. 176 human serum samples were evaluated for *T. gondii* IgG antibody with prepared antigens, and finally serum samples were evaluated by commercial ELISA kit (Trinity, USA) which was considered as gold standard method. In this study sensitivity and specificity of prepared antigens compared with commercial kit in ELISA method. Sensitivity and specificity of soluble antigen was 91.4 and 74.5 %, in whole antigen these parameters were 77.1 and 77.3 % and in excretory/secretary antigen were 28.5 and 74.5 % respectively. Soluble antigen had high levels of sensitivity and specificity in ELISA method and the results were rather resemble to commercial kit (Trinity, USA).

Keywords Toxoplasmosis · ELISA · *Toxoplasma gondii* · Soluble antigen · Whole antigen · Excretory/secretary antigen

Introduction

Toxoplasma gondii is the obligate intracellular parasite that invades the host cells and lysis them (Boyle and Radke 2009). Toxoplasmosis is associated with congenital infection and can cause severe problems in the fetus (Weiss and Dubey 2009). *T. gondii* can lead to serious diseases in immunocompromised patients such as HIV positive patients, transplant recipient and patients with cancer (Grant et al. 1990). Prevention and treatment is possible by quick and accurate diagnosis of toxoplasmosis (Hafid et al. 2001). Different methods can be used for *T. gondii* detection in human such as: histological, parasitological and serological methods (Dubey 2010). Diagnosis is routinely based on serological methods with detection of specific antibodies to *T. gondii* (Montoya and Liesenfeld 2004). These methods are used from years ago to evaluate appropriate antibodies such as IgG and IgM and contain several types such as hemagglutination, modified agglutination, latex agglutination, indirect immunofluorescence, enzyme-linked immunosorbent assay (ELISA) and avidity tests (Rahbari et al. 2012; Dubey 2010; Remington et al. 2004). It is notable that ELISA method with high levels of sensitivity and specificity (Dubey 2010) is commonly performed by soluble antigen (Chen et al. 2008). Also coating of ELISA plate with whole antigen is possible (Silva et al. 2002) and some studies have been used excretory/secretary antigen. To obtain an easy to use antigen with high levels of sensitivity and specificity, the present study was performed with soluble, whole, and excretory/secretary antigens of *T. gondii* in IgG-ELISA method.

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Materials and methods

Parasite

Tachyzoites of *T. gondii*, RH strain were injected in intra-peritoneal cavity of BALB/c mice. After 4 days, tachyzoites were harvested by peritoneal washing of infected mice.

Soluble antigen preparation

Tachyzoites that obtained from the peritoneal exudates were washed three times with phosphate buffered saline (PBS) at 3000 rpm for 10 min then sonicated (10–15 puls for 10 s with power of 1500–1700). Afterward the suspension was centrifuged for 1 h at 4 °C, 12,000 rpm, supernatant collected and the density of protein determined by Bradford method.

Whole antigen preparation

After collecting, washing and centrifuging of peritoneal fluid of *Toxoplasma* infected mice, the number of tachyzoites was counted to set the appropriate amounts.

Excretory/secretary antigen preparation

1.5×10^8 tachyzoites were transferred in 1 ml tube of saline and incubated at 37 °C for 1 h under mild agitation. After centrifuging for 5 min at 6000 rpm, the supernatant was collected and the protein density determined by Bradford method.

ELISA setup

To obtain appropriate dilution of serum, anti-human IgG conjugated with horse radish peroxidase (HRP) and the amount of antigen for each type of soluble, whole and excretory/secretary antigens the checker board method was used. Also serum samples were checked for anti-*T. gondii* IgG antibody by commercial Trinity kit (USA) as gold standard.

IgG ELISA by prepared antigens

A total of 176 serum samples that were referred for *T. gondii* IgG detection to different laboratories in Tehran, Iran were collected. These samples were evaluated for *T. gondii* IgG antibody with prepared antigens (soluble, whole and excretory/secretary). The ELISA procedure for all three antigens was the same and optimal amounts of

Table 1 The results of checker board for soluble, whole and excretory/secretary antigens

Prepared antigens	Optimum antigens	Optimum dilution of serum	Optimum dilution of anti-human IgG conjugated with HRP
Soluble	7.5 µg/ml	1:200	1:500
Whole	10^7 tachyzoite/well	1:200	1:750
Excretory/secretary	10 µg/ml	1:100	1:500

antigen, serum and anti-human IgG conjugated with HRP are shown at Table 1.

Briefly 96 well microtiter plates were coated with 100 µl of optimum antigen and kept at 20 °C until use (for soluble and excretory/secretary antigens 7.5 and 10 µg/ml respectively), for whole tachyzoites the microtiter plates were coated with 10^7 tachyzoites per well and incubated overnight, then washed with PBST (phosphate buffer saline, 5 % tween pH 7.2) and blocked with blocking buffer (skimmed milk 2.5 % in PBST) for 1 h at 37 °C. After incubation and washing, 100 µl of diluted sera in PBST were added to each well. In each time negative and positive control sera were tested too. After incubation for 1 h at 37 °C and washing, 100 µl of anti-human IgG conjugated with HRP (horse radish peroxidase) (DAKO, Denmark) in PBST was added to each well and afterward performed incubation for 1 h at 37 °C and washing. Then, chromogenic substrate OPD (ortho-phenylen-diamidine) (Merck, Germany) was added to each well. Enzymatic activity was obvious after 15 min. Reaction was terminated by adding of 50 µl of 20 % sulfuric acid. Optical density was recorded at 492 nm with automated ELISA reader (BIO-TEC, LX800, USA). For each of methods the amount of cut-off was calculated. In each procedure 30 negative sera was tested and the cutoff was determined as the mean plus two times of the standard deviation of the absorbance readings obtained for the negative samples ($X \pm 2$ SD). The optical density more and less than cut off were considered as positive and negative results respectively.

Finally, serum samples were evaluated by commercial kit (Trinity, USA) which was considered as gold standard method.

Results

In this study prepared antigens (soluble, whole, excretory/secretary) in ELISA method were compared with commercial kit (Trinity, USA). Sensitivity, specificity, validity and concordance parameters were calculated and shown at Table 2.

Table 2 Sensitivity, specificity, validity and concordance of soluble, whole and excretory/secretary antigens in ELISA method

Antigens	Sensitivity (%)	Specificity (%)	Validity (%)	Concordance (%)
Soluble	91.4	74.5	82.9	81.2
Whole	77.1	77.3	77.2	77.2
Excretory/secretary	28.5	74.5	51.5	56.2

Discussion

Accurate diagnosis of *Toxoplasma* infection in pregnant women, immunosuppressed patients and infants with congenital defects and chorioretinitis is very vital and significant (Candolfi et al. 2007). Different serological methods such as dye test, hemagglutination, modified agglutination, latex agglutination, indirect immunofluorescence and enzyme-linked immunosorbent assay (ELISA) had been used from years ago to evaluate appropriative antibodies such as IgG and IgM in *T. gondii* infection (Dubey 2010). Some new methods are improving the ability to diagnose recently acquired infection such as serum IgG avidity test and PCR (Remington et al. 2004).

Among different serological methods that have been used for detection of toxoplasmosis in human, ELISA assay have numerous modifications (Dubey 2010). This method is commonly performed by soluble antigen (Chen et al. 2008). Also coating of ELISA plate with whole antigen is possible (Silva et al. 2002). Some studies have been performed for use of excretory/secretary antigen in ELISA method (Son and Nam 2001). It is notable that, preparation of whole and excretory/secretary antigens are easier and faster than soluble antigen with no need to special equipments such as sonicator (Yamamoto et al. 1998; Son and Nam 2001).

Commercial ELISA kits use soluble or whole *T. gondii* antigens. The Trinity kit that was used in this study was performed with soluble *T. gondii* tachyzoites antigen with sensitivity of 95.3 % and specificity of 100 %. In the present study soluble antigen had good level of specificity (74.5 %) in comparison to the commercial kit, in addition sensitivity level was very close to the kit (91.4 %). The concordance of 81.2 and 77.2 % for soluble and whole antigen was estimated in comparison with commercial kit in this study. Although preparation of excretory/secretary antigen is easy, fast and low cost, but it has high levels of false positive and false negative results so it isn't suitable to use in ELISA method.

Conclusions

Soluble antigen has the most concordance with commercial kit but because of difficult and time consuming preparation, whole antigen could be used for ELISA method too.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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