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Original Article

A Comparative Study on Sensitivity and Specificity of Conventional and Unconventional IgG and IgM Assays for Diagnosis of Toxoplasmosis

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Abstract

Background: Toxoplasmosis is a zoonotic disease with widespread distribution throughout the world. It is caused by the protozoan parasite *Toxoplasma gondii*. As laboratory diagnosis of toxoplasmosis is not straightforward, this study was aimed to compare the sensitivity and specificity of conventional and unconventional methods of diagnosis based on the measurement of IgM and IgG for determination of the best method.

Methods: One hundred suspected cases of toxoplasmosis referred to two laboratories in Tehran and Karaj were entered into this comparative analytical study. The serum specimens of these cases were tested with ELISA, IFA, chemiluminescence (CLIA) and ELFA for presence of IgG and IgM.

Results: When compared with the ELFA IgG method, the CLIA IgG had the highest sensitivity, specificity, and positive and negative predictive values (100%). In comparison with ELFA IgM method, CLIA IgM and ELISA IgM had the same sensitivity (92%), but the sensitivity of ELISA IgM (100%) was higher than CLIA IgM (97.3%). The positive and negative predictive values of ELISA IgM were 100% and 97.4%, respectively and those of CLIA IgM were 96% and 98%, in that order.

Conclusion: Although, the reliability of the compared methods are close to each other, the automatic methods (CLIA, ELFA) are preferred because of high reproducibility, less personnel costs, shorter test time and etc. Therefore, we recommend application of these methods for diagnosing of toxoplasmosis and re-emphasize that these are the most suitable tests for measurement of toxoplasma IgM levels.

Keywords: *Toxoplasmosis, Sensitivity, Specificity, IgG, IgM*

Introduction

Toxoplasmosis is a zoonotic disease (1, 2). Serological studies have shown than this parasite infects humans and other endothermic vertebrates. It is estimated that about one third of the world population are infected with toxoplasmosis (2). Two forms of congenital and acquired infections have been described in humans of which, the impact of congenital toxoplasmosis on patient's health is higher. Toxoplasmosis has a variety of clinical manifestations and can be mistaken with other diseases. Therefore, confirmation of diagnosis of this parasitic infection is necessary by laboratory tests (2) including parasitical and serological methods. Although, the number of cases with toxoplasmosis is high, a sensitive and suitable method is not applied for detection of this infection in most diagnostic laboratories, which will lead to mismanagement of the involved cases.

Automated technique including CLIA (Chemiluminescence Immuno Assay) and ELFA (Enzyme Linked Fluorescent Assay) are partially new method for diagnosis of toxoplasmosis that have several advantages including reproducibility, cost effectiveness etc (3).

This study was aimed to compare the validity index of conventional and new methods of diagnosis based on the measurement of IgM and IgG for determination of the best method.

Materials and Methods

One hundred suspected cases of toxoplasmosis referred by clinicians to two laboratories in Tehran

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(Masoud laboratory) and Karaj (central laboratory of Fardis in west of Tehran Province) were entered into this comparative analytical study by convenience sampling. The cases were selected from a wide age range and nearly equal numbers of both genders. After preparation of serum samples, their IgG and IgM levels were measured by ELISA, CLIA (Chemiluminescence Immuno Assay), IFA (Indirect Immuno Fluorescent) and ELFA (Enzyme Linked Fluorescent Assay).

ELISA method

Purified antigens from the cell membrane of *Toxoplasma gondii* were used for coating of the solid phase (microplates) (*Toxoplasma* IgG and IgM kits, Genesis Diagnostics, UK). Diluted serum specimens (1:100) were added to the solid phase and incubated for 20 min. After washing, the plates were incubated for 20 min with rabbit anti-human antibody conjugated to horse radish peroxidase and washed. Then, the samples were developed by TMB enzyme substrate for 10 min. The absorbance (OD) was measured at 450 nm.

IFA method

This test was performed using *Toxoplasma* whole body antigen (Pasture Institute, Iran) for coating the solid phase and IgG-IFA and IgM-IFA kits (Baharafshan, Iran). Different diluted serum samples (1:20, 1:100, and 1:200) were added to the solid phase and the bound antibodies were detected with fluorescein-labeled anti-human antibody. Positive samples showed a green fluorescent color.

Chemiluminescence method

This method was performed using IgG and IgM CLIA kits (Diasorin, USA) and fully automated LIAISON system. For CLIA IgG, paramagnetic particles (solid phase) were coated with *Toxoplasma* antigen from a whole-cell lysate. For CLIA IgM, the magnetic particles were coated with a monoclonal IgG antibody to human IgM. Magnetic particles were incubated in serum for 10 min and washed three times. The samples were then incubated with a monoclonal anti-human antibody coupled with an isoluminol derivative for a further 10 min. After a final wash, the chemiluminescence signal was generated and measured by a photomultiplier.

ELFA method

This method was performed using IgG and IgM ELFA kits (bioMérieux, France) and VIDAS system. The assay principle combines an enzyme immunoassay method with a final fluorescent detection. In ELFA IgG, antibody was detected by membrane and cytoplasmic *Toxoplasma* antigens coated on the solid phase receptacle (SPR). In ELFA IgM method, the SPR was coated by anti-human M chain antibodies. The bound antibody was then incubated with alkaline phosphatase-conjugated monoclonal anti-human antibody and the fluorescent substrate 4-methyl umbelliferyl phosphate. The conjugated enzyme catalyzed this substrate into a fluorescent product (4-methyl umbelliferone), and the absorbance was measured at 450 nm.

Statistical methods

Data were analyzed using kappa test and Chi-square. ELFA test chooses as gold standard.

Results

Validity index (sensitivity, specificity, positive predictive value and negative predictive value) of mention diagnostic tests compared with statistical methods. Highest sensitivity and specificity between these tests belong to ELFA IgG test and CLIA IgG test and ELFA IgM with 100% (Table 1).

In ELISA IgG method that routinely used in laboratories, 73 specimens were positive, 23 were negative, and 2 positive and 2 negative specimens were not confirmed by this test. Sensitivity and specificity were 97.3%, and 92%, respectively. Agreement between this method and ELFA IgG was 96% (Table 1 and 2).

IFA method

In this method, 73 specimens were positive, 24 specimens were negative, and 2 positive and 1 negative specimens were not confirmed by ELFA IgG method. Sensitivity and specificity were 97.3%, and 96%, respectively, and positive and negative predictive values were 98.6% and 92%. Agreement between this method and ELFA IgG was 97% (Table 1 and 2).

Table l: Validity indexes in comparison with ELFA IgG method

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
CLIA IgG	100	100	100	100
ELISA IgG	97.3	92	97.3	92
IFA	97.3	96	98.6	92
CLIA IgM	92	97.3	92	97.3
ELISA IgM	92	100	100	97.4

Table 2: Agreement of comparative methods

	CLIA IgG	ELISA IgG	IFA	CLIA IgM	ELISA IgM
Agreement of methods in comparison with ELFA IgG (%)	100	96	97	96	98

Discussion

According to table 1 and 2 and in comparison with ELFA IgG method, CLIA IgG method was the most sensitive and specific method with highest positive and negative predictive values (100%). It was in complete agreement with ELFA IgG method. The results of IFA and ELISA IgG methods were close to each other. The results of CLIA and ELISA IgM were also close to each other. In a study performed in USA, four methods including ELFA-VIDAS system, OPUS-fluorescence system, ELISA-Abbot IMx system, and ELISA-Platelia system were compared with each other. All the systems were fully automated. The results showed that the most specific assay for IgM antibody detection was ELFA IgM method. This method also had the highest positive predictive value (80.8%). For IgG antibody, all comparative methods had similar sensitivities, specificities, and positive and negative predictive values (3). In another study, the ELFA method was used for equivocal specimens. The patients were evaluated between the first and the third month after symptom onset that ELFA IgG had %100 and ELFA IgM had 81.8% sensitivity respectively (4).

In current study, as the methods under investigation showed fairly similar reliabilities, other aspects of these assays were considered. In IFA and ELISA methods, the different stages of the tests are performed manually. As the result, the reproducibility of the tests were less, the personnel were exposed to more chemical hazards, and the probability of the effect of the environmental factors on the results was more when compared with automated methods. In addition, reduplication of the assays with a few specimens was very expensive and storage of specimens for next assay caused more intensive labor. But, in ELFA and CLIA methods, the stages for performing the test were automatic, therefore the time needed for performing the assays was shorter (5, 6), and operator and technical mistakes were omitted. In two latter methods, the reproducibility of tests was high and personnel costs were low. The stability of calibration (for 2 weeks) led not only to the faster performance of the tests with higher precisions, but also omission of the cost for multiple calibrations (6). Also, the higher limit of measurement enabled application of undiluted serum. In addition, the VIDAS system could electronically transfer the analysis results to the laboratory information system minimizing the operator's data entry errors. The ELFA method had the advantage of elimination of carryover due to application of specific SPR for each strip. Its other advantage is simple mechanical construction of VIDAS system, which keeps the maintenance costs of this system lower than other systems, and makes its operation easy with a very little training. ELFA method had the shortest incubation stage and overall run-time (7). Among

four techniques, ELFA method could be run straightforward at any time with no need to a preparatory stage (6). The availability of avidity kits for ELFA and CLIA assays makes precise determination of the time of infection possible (8). This can be important in the diagnosis of infection in pregnant women. Also, it can prevent unnecessary treatments that will lead to economical and psychological damages (5, 9).

It can be concluded that for Toxo IgG test, a diagnostic laboratory should choose an assay that suits its own circumstance in terms of costs, time, speed etc. But, considering the vital importance of Toxo IgM assay, it is recommended that this test is preferably performed by ELFA method, and when it is not available, by CLIA as an alternative.

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