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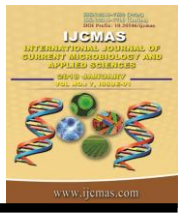

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Diagnosis of Human Toxoplasmosis Using Rapid Chromatographic Immunoassay and Enzyme-Linked Immuno-Sorbent Assay (ELISA) Compared to Molecular Technique (PCR) as Gold Standard Technique

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A B S T R A C T

Nowadays, there are many different procedures for the laboratory diagnosis of Toxoplasma infection in pregnant women, congenitally-infected fetuses and newborns, and they are mainly performed by serological testing, and PCR (for confirmatory purpose), beside many commercial diagnostic tests, which use Toxoplasma lysate antigen. These procedures differ from each other in many aspects; cost, time-consumption, and accuracy of the test, which should meet patient's needs. In this current study, serum samples were collected from one-hundred females suspected with toxoplasmosis and diagnosed by three different procedures for the *T. gondii* infection, rapid chromatographic (IgM or IgG immunoblot), ELISA (IgM or IgG) test and molecular technique. Molecular diagnoses were performed in peripheral blood by PCR using the *T. gondiiB1* gene as marker. The results were described as frequency and percentage of positivity; also, specificity and sensitivity were assessed. Of these 100 blood samples analyzed, 92% (IgG), and 55% (IgM) were positive when using PCR; the rapid chromatographic method for both IgM and IgG, has shown (83%) samples to be positive for *T. gondii*, while in ELISA test, 28% (IgM) and 72% (IgG) found to be positive. Sensitivity and specificity of ELISA (89% and 90% (IgM); 89% and 91% (IgG), respectively) have found to be relatively higher than immunoblot (88%and81% (IgM); 85% and 81% (IgG), respectively). While PCR technique has shown 100% and 98.8% (IgM); 99.3% and 96.1% (IgG), sensitivity and specificity, respectively. Since the higher sensitivity and specificity of ELISA, we concluded that ELISA, compared to the rapid chromatographic test, is more suitable for the detection of anti-*T. gondii* IgG and IgM antibodies in both acute and chronic infection, especially, the rapid chromatographic commercial kits can yield many false-positive results which in turn has many undesired consequences.

Introduction

Despite the advance techniques in diagnosis of parasitic disease, and protozoan disease; diagnostic methods must be renewed to be more rapid and specific. During the past few years, there has been an increased interest in the diagnosis of parasitic diseases using

techniques, which are rapid, simple and inexpensive as well as sensitive and specific (Dubey *et al.,* 2005). Old serological procedures such as indirect hem agglutination (Dubey and Su, 2009), complement fixation test (Linguissi, 2012) and immunofluorescence are tedious and difficult to standardize, conduct and interpret. Also, the reagents are consumptive and require highly trained technicians as well as expensive instruments (Joanna *et al.,* 2009). *T. gondii* is a coccidian parasite of the cat and its infection may lead to major public health problems (Evering and Weiss, 2006). The disease exhibits various clinical manifestations and therefore, poses difficulty in diagnosis (Rai *et al.,* 1995). Serological methods have been employed in aid of diagnosis of this disease. Detection of anti-toxoplasma IgG and IgM antibodies have been routinely used in many clinical laboratories to determine the probable immune status of individuals. The most used assay today is indirect enzyme linked immunosorbent assay (ELISA); require highly trained and expensive instruments (Sroka *et al.,* 2010). So, this research was planned, in parallel with rapid latex agglutination test and enzyme linked immunosorbent assay (ELISA), to standardize the commercially available Rapid chromatographic immunoassay (immunoblotting) technique, which is simple to perform and doesn't need expensive equipment to detect IgG and IgM specific antibodies against *Toxoplasma gondii*.

Materials and Methods

Samples

One hundred peripheral blood samples were collected, at the first visit, from females who presented at Obstetrics and Gynecology Department, at AL-Yarmoke Hospital and private outpatient clinics in Baghdad, Iraq, during September 2016- September 2017, and their clinical details at presentation were recorded. Sera were stored at -20 °C till analyzed.

Polymerase chain reaction

DNA was extracted from patients' blood using DNeas Kit (Qiagen, Hilden, Germany). The *Toxoplasma gondiiB1* gene (sequence of 592 bp) was amplified as described previously (8). Primer pair used had the nucleotide sequence as follows:

Forward primer: GCATTCCCGTCCAAACT

Reverse primer: AGACTGTACGGAAT GGAGACGAA

The PCR conditions consisted of 1 cycle of 5 min at 93°C, followed by 35 cycles of 1 min at 93°C, 30s at 55°C, 30s at 72°C, and a final cycle of 10 min at 72°C. Amplified products were visualized on 2% agarose gel under UV light. All assays were performed at least twice.

Rapid chromatography test

This test utilizing anti-*T. gondii* IgG and IgM antibodies rapid test, which is a qualitative, chromatographic immunoassay for detection of IgM, IgG antibodies against *T. gondii* antigen in patient's sera. It was performed according to the evolved instructions.

The procedure assay can be summary as follow: sample, controls and calibrator were diluted 1:40 by adding 5µ /200µl. Reaction was stoppedby100 µl stop solution and read at 450nm. All results above the cut-off value (10 IU/Ml) were considered as positive.

Enzyme Linked Immunosorbent Assay (ELISA)

The Biocheck® Kit was commercial obtained. Diluted patient's serum was added to the purified *T. gondii* antigen coated on the

surface of micro wells. The *T. gondii* IgG or IgM-specific antibody, if present, binds to its antigen, all unbound materials were washed away, then horse-radish peroxidase (HRP) conjugate was added, which binds to the antibody-antigen complex. After washing, the solution of tetra-methyl benzidine (TMB) reagent was added, the enzyme conjugate catalytic was stopped at specific time. The results were read by ELISA reader.

Statistical analysis

All data were presented as frequency and percentage of positive and negative results, and the cutoff value was determined. Sensitivity and specificity were calculated for each procedure as the following equations:

$Sensitivity = a/(a + c)$
$Specificity = d/(b + d)$

Whereas;

a = True positive
b = False positive
c = False negative
d = True negative

Results and Discussion

In the present study, 100patients suspected with *T. gondii* samples were tested between 2016 and 2017. Of these 100 blood samples analyzed, 92% (IgG) and 55% (IgM) were positive when using PCR; the rapid chromatographic method for both IgM and IgG, has shown 83% of samples to be positive for *T. gondii*, while in ELISA test, 72%of samples were IgG positive and 28% samples were IgM positive (Figure 1).

The evaluation of results obtained has shown that PCR test has 100% and 98.8% (IgM); 99.3% and 96.1% (IgG), sensitivity and specificity, respectively, ELISA has shown 89%and 90% (IgM); 89% and 91% (IgG), respectively, whereas in rapid chromatographic test it has shown 88%and81% (IgM); 85% and 81% (IgG), respectively (Table 1).

In developed countries, toxoplasmosis screening is part of the health tests included in the prenatal assessment. Toxoplasmosis contracted during the first trimester of pregnancy is responsible for spontaneous abortions, stillbirth or severe illness in more than 25% of pregnant women (Su *et al.*, 2010).

The aim of the current study was to determine the sero-prevalence of toxoplasmosis among pregnant women during the first trimester of pregnancy, in addition, to compare the common diagnosis tests in comparison to PCR technique.

In this study, of 100 sera of pregnant women, 83 samples (83%) showed positive for *T. gondii* by rapid chromatographic test for both IgM and IgG, while in ELISA test, 28% and 72% samples were IgM and IgG positive, respectively. The PCR technique has shown 92% (IgG) and 55% (IgM) positive cases. All cases have shown positivity for both IgM and IgG antibodies against *T. gondii* antigens.

A positive Toxoplasma Immunoglobulin M (IgM) result is regularly interpreted as an indicator of an acute infection. Though, IgM can persist for many years; however, Toxoplasma commercial IgM diagnostic test kits can yield a number of false-positive results. For these reasons, a chronic Toxoplasma infection can be erroneously classified as an acute infection, resulting in serious adverse consequences, especially in pregnant women, leading to emotional distress and unnecessary interventions, including termination of pregnancy (Dhakal *et al.*, 2015).

Fig.1 *T. gondii* cases diagnosed by PCR, ELISA, Chromatography

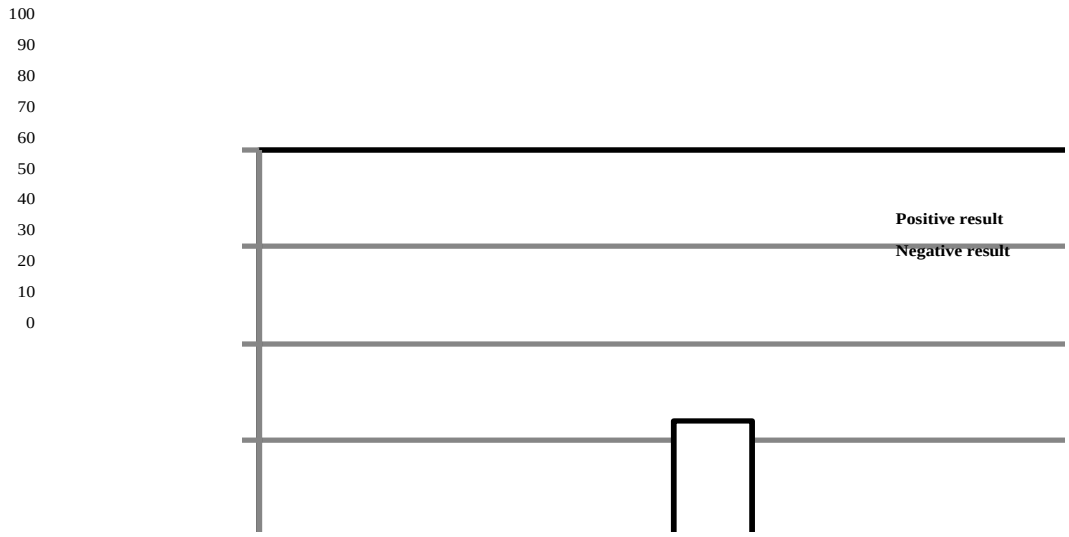

Table.1 Results of specificity and sensitivity ELISA of and Rapid test (IgG and IgM) according to PCR results

	PCR		ELISA		Chromatography	
	IgG	**IgM**	**IgM**	**IgG**	**IgG**	**IgM**
Specificity	96.1%	99.8%	90%	91%	86%	**81%**
Sensitivity	99.3%	100%	89%	89%	85%	**88%**

On the other hand, few women seek toxoplasmosis serologies during pregnancy in Baghdad; this could be due to the relatively high cost of these tests, especially, for predominantly low-income populations. In Baghdad, and due to the existence of a strong agro-pastoral activity especially in rural areas, which increases the spread of zoonotic diseases, Toxoplasmosis diagnosis should be systematic (Coulibaly and Yameogo, 2000). Indeed, previous studies have shown that the coexistence between humans and animals may be a contributing factor raising these zoonotic infections (Sroka *et al.*, 2010; Su *et al.*, 2010; Coulibaly and Yameogo, 2000; Rovamycine, 1994). Meat and milk are important dietary components for most of the population in Baghdad. However, contamination of water by oocytes could be the most likely source of infection with toxoplasmosis in Baghdad. The diagnosis of toxoplasmosis is necessary in pregnant women because of the low immunization coverage rate and the high level of exposure to these two infections which can be harmful to the newborn if contracted by women before the third trimester of pregnancy (Coulibaly and Yameogo, 2000).

Since the higher sensitivity and specificity of ELISA, we concluded that ELISA, compared to the rapid chromatographic test, is more suitable for the detection of anti-*T. gondii* IgG and IgM antibodies in both acute and chronic infection, especially, the rapid chromatographic commercial kits can yield many false-positive results which in turn has many undesired consequences.

Supplements

Instruction for the rapid chromatography test

Negative control

Only the control band (C band) shows color development. The two test bands (T1 and T2) show no color development.

Positive control

The C band and two T bands (T1and T2) show color development.

Interpretation of assay results; Negative result

If only the C band is present, the absence of color in both T bands (T1 and T2) indicates that no anti-*T. gondii* antibodies are detected (result is negative);

Positive result

In addition to the presence of Cband, if only T1 band color is developed indicate the IgM anti-Toxoplasma is presence in the specimen (IgM positive). While if only T2 band is developed indicate the (IgG positive) and if both T1 and T2 bands are developed in addition to the presence of Cb and that means both (IgM and IgG is positive), if noC band is developed, the assay is invalid regardless of any color in the T bands.

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