Comprative Study between LAT and Elisa in Detection of Toxoplasmosis in Groups of Women

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ABSTRACT:

BACKGROUND:

Toxoplasmosis is a zoonotic disease caused by the parasitic protozoan *Toxoplasma gondii* . This parasite is an obligate intracellular organism and is found in two forms in humans. widespread throughout the world, approximately half a billion humans have antibody to *T. gondii*.

OBJECTIVE:

This study aimed to compare the efficiency of two methods in diagnosis of toxoplasmposis in pregnant and non-pregnant women.

METHODS:

Blood samples were collected from ٣٥٠ (١٥-٤٦ year-old, ١٠٠ pregnant and ٢٥٠ non-pregnant) females. Two serological methods were used: Latex agglutination test (LAT) and Enzyme linked immune-sorbent assay for IgM detection (ELISA).

RESULTS:

The study showed that ٣٨,٨٦٪ of women had given positive results for LAT, of which ٣١ were pregnant and ١٠٥ were non-pregnant, while only ١٤,٢٨٪ of the total subjects had given positive results for ELISA.

CONCLUSION:

These results indicate that positive sera for LAT should be further investigate by more reliable method in order to confirm the infection with toxoplasmosis.

KEY WORDS: toxoplasmosis,elisa, latex.

INTRODUCTION:

Toxoplasma gondii is a protozoan parasite estimated to infect over one billion worldwide [١]. Although under normal immune conditions *toxoplasma* infection is largely asymptomatic [٢], it may provoke inflammation and inhibition to the apoptosis of infected cells which may lead to various pathological consequences. In this context, some recent reports have indicated an association between serum levels of anti-*toxoplasma* antibodies and brain cancer [٣].

As an effective vaccine has not yet been developed, continuous and detailed epidemiological surveillance is required to estimate the risk of infection, especially in pregnant women and the likelihood of reactivation in immunocompromised individuals.

The diagnosis of toxoplasmosis is commonly made by detecting the immunoglobulins (IgG and IgM) antibodies in serum samples of patients using a variety of methods such as enzyme linked immune-sorbent assay (ELISA) and latex agglutination test (LAT)[٤]. In Iraq,[٥] the seropositivity for *T. gondii* IgG anti-body by ELISA ٧٠ (٣٧,٦٣ %) and ١٠٨ (٥٨,٠٦ %) by LAT, The prevalence of toxoplasmosis was increased proportionally with the age of individuals, while gender has no effect on the prevalent rate.

The humoral immune response to *T. gondii* is rapid and intense, and forms the basis for useful diagnostic tests for the various forms of the disease. The high rate of infection revealed by LAT reflects the type of immunoglobulins detected by this technique. These immunoglobulins (IgG isotype) are usually present ١-٢ weeks after acquisition of the infection and persist for life [٦]. High titer of IgG anti-*Toxoplasma* antibodies in the absence of IgM antibodies are consistent with chronic latent infection acquired in the past [٣]. Furthermore, LAT an give rise to false positive results in many instances among which rheumatoid fever and cytomegalovirus infection [٧] which are supposed to be prevalent among the study population. Although it is simple, easy and rapidly achieved,

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LAT should only be used as a screening test and all sera that gave positive results, whatever the titers, should be sent to reference laboratory for confirmative diagnosis

As such, the prevalence of this parasite may vary according to the serological method used for detection, aside from other factors that influence the prevalence such as age, sex, and economic status. Thus, the present study aimed to compare the efficiency of two diagnostic methods (ELISA and LAT) in detection of IgM antibodies against *Toxoplasma gondii*[١٢،١٣].

MATERIALS AND METHODS:

١-Study population and samples:

A total of ٣٥٠ (١٥-٤٦ year-old, average ٢٦,٩±٢,١ years, ١٠٠ pregnant and ٢٥٠ non pregnant) females who attended Al-Zaafaraniya primary health center during the period from December ٢٠١٠ to March ٢٠١١ were used for this study. Five ML of venous blood were collected from each subject and put in eppendorf tubes. Serum was obtained from each sample, separated into two parts, and kept at -٢٠Ċ until used. A face-to-face interview was achieved with participants and information including age, pregnancy, and fate of the previous pregnancies were recorded.

٢-Serological methods:

A- Latex agglutination test: Antibody to T. gondii was determined in the first part of the serum sample using commercially marketed LAT kit (Toxocell latex, Biokit Sa, Barcelona, Spain). The detection procedure followed manufacturer's instructions.

B- Enzyme linked immunosorbent assay (ELISA): The other part of the serum sample was used to detect IgM antibodies to *Toxoplasma* using commercially marketed kit (Vidas toxo IgM, BioMerieux, Mercy-١ Etiole, France). The detection was done according to the manufacturer's instructions.

٣-Statistical analysis: SPSS ١١,٥software was used for analyzing the data. In order to check for differences,. A p-value of ٠,٠٥ was considered to be significant.

RESULTS:

١-Latex agglutination test: The agglutination at dilution of ١:١٦ or higher was regarded as positive according to manufacturer's instructions. One hundred and thirty six (٣٨,٨٦٪) of ٣٥٠ women showed positive reaction at ١:١٦ or higher titers. Of these positive cases, ٣١ (٨,٨٦٪) were pregnant, while ١٠٥ (٣٠٪) were non-pregnant (table ١).

Table ١: Detection of igm antibodies to *toxoplasma gondii* by latex agglutination test.

Groups	Titer of positive cases						Total
	١:٢	١:٤	١:٨	١:١٦	١:٣٢	١:٦٤	
Pregnant (n=٧٥)	١٠	١٤	٢٠	٢٥	٥	١	٧٥
Non-pregnant (n=٢١٥)	٢٠	٤٠	٥٠	٧٥	١٨	١٢	٢١٥
Total	٣٠	٥٤	٧٠	١٠٠	٢٣	١٣	٢٩٠

٢-Enzyme linked immune-sorbent assy; Table ٢ shows the results of anti-*Toxoplasma* IgM antibody by ELISA. Fifty (١٤,٢٨٪) of ٣٥٠ gave positive results for these antibodies. The infection was more prevalent in the age class ٢٠-٣٠ years (٥.٧٪) and ٣٠-٣٩ class (٤,٢٪), whereas the lest rate of infection was among the younger women ١٢-٢٠ years old (١,٤٪) with significant difference.

Table ٢: Detection of IgM antibodies *to Toxoplsma gondii* by ELISA technique.

Age lasses (years)	No. of positive (%)	No. of negative (%)
<٢٠	٥ (١,٤)[a]	٥٥ (١٥,٧)
٢٠-٣٠	٢٠ (٥,٧)[b]	١٨١ (٥١,٧)
٣٠-٣٩	١٥ (٤,٢)[b]	٣٥ (١٠)
٤٠-٤٦	١٠ (٢,٨)[a]	٢٩ (٨٢)
Total	٥٠ (١٤,٢٨)	٣٠٠ (٨٣,٧٢)

Note: different letters indicate significant differences

DISCUSSION:

Toxoplasmosis is a major public problem, with a high socioeconomic impact in terms of human suffering including the cost caring for sick, mentally retarded and blind children (٥). Because there is no fully effective treatment, early diagnosis of the infection in women can prevent the disastrous outcome upon her forthcoming child.

Toxoplasmosis is more prevalent in warm, moist areas of the world than in cold or hot dry areas [٦]. In Iraq many previous studies revealed different rates of infection according to different tests used , the percent of positive cases for IgM against *T.gondii* by ELISA test in the ١٢٠ cases of miscarriage was found to be ١٩,١٧٪, while the percent of positive cases of *Toxoplasma* antigen by immunohistochemical analysis in the ١٢٠ cases was found to be ٢١,٦٧٪.(٧).On the other hand, (٨) in a study conducted in Basra/Iraq , using LAT test he founded that the rate was ٤١,١ % in city center and ٥٢,١ % and ٤٤,٩ % in semi-rural and rural areas respectively.,and ٢٢,٠٦٪ of the total population is infected with *T.gondii* [٩].Toxoplasmosis in countries surrounding Iraq was studied , [١٠] reported the rate of ٤٩,٦ % in healthy persons in compared to ٧٢,٣ % in suspected patients by IFAT in Khoozestan province of Iran. , and in Saudi Arabia the incidence of human infection ranges between ٢١٪ and ٤٩,٣٪ [١١].

Detection of specific IgM ant-*Toxoplsam* antibodies is indicative for active infection [٨]. These antibodies may be detected for ١٨ months or more with sensitive assay [٩], and not surprisingly, prevalence rates were lower using ELISA (for specific detection of anti-*Toxoplsma* antibodies) compared with LAT. However, ELISA is laborious, time consuming and needs special instruments.

It is generally accepted that prevalence of anti-*Toxoplasma* antibodies increases with age [٧], may be due to cumulative risk of exposure with age. The highest prevalence among ٢٠-٣٠ years compared with older age classes pointed out the presence of active infection in this age class which is considered the most age where women become pregnant for the first time. The reason for this variation between the results of both tests may be due to the persistence of IgM for a long time after primary infection or presence IgM antibodies [١٤]. These antibodies not related to *Toxoplasma* infection, and cross reaction of *Toxoplasma* antibody with rheumatoid factor or other auto antibodies , which react with Fc portion of antibody give non- specific agglutination not related to *Toxoplasma* infection [١٥] by LAT.

The presence of IgG antibody only means exposure because asymptomatic human can develop high *T. gondii* antibody titers and remain elevated for several years or even whole life, if repeated exposure was encountered although an eight fold rise in antibody titer which took two weeks and it is indicative of a recent infection [١٦].

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