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Assessment of Screening Tests Used to Detect *Toxoplasma gondii* in Women in Sudan

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Abstract

Blood samples from 255 child bearing age women distributed into two villages named EL Nuba and EL Massoudia were collected according to their agreement and after obtaining their written consent form.

Plasma separated from 255 blood samples were tested at delivery for *Toxoplasma gondii* antibodies using three different screening tests. These tests were Latex Agglutination Test (LAT), Complement Fixation Test (CFT), and ELISA IgG. Evaluation of the screening test was done for the three tests. The sensitivity of LAT, CFT, and ELISA IgG were 82.5%, 96.8%, and 98.9% respectively. The specificity of these tests was 91.2%, 94.1%, and 97.1% respectively. The Positive predictive value was 96.2%, 97.8%, and 98.9% respectively. The negative predictive value was 66%, 91.4% and 97.8%, respectively. The positive likelihood ratio was 9.35, 16.4, and 33.6 respectively. The negative likelihood ratio was 0.192, 0.0345, and 0.11 respectively. According to these evaluations a proposed protocol was drawn to detect infection in women.

Keywords: Assessment; Screening tests; *Toxoplasma gondi*; Rural women

Introduction

Diagnosis of toxoplasmosis in humans is performed using different techniques. A few examples of these techniques are mouse inoculation, detection of anti-*Toxoplasma* antibodies, histological demonstration of tachyzoites in tissue sections or smears of body fluid, and detection of *Toxoplasma gondii* DNA by molecular methods [1-3].

Many serological methods for the diagnosis of toxoplasmosis have been established over the years. Most have been developed for detection of *Toxoplasma* infection in human and commercially available in kit form [4]. Detection of anti-*Toxoplasma* antibodies indicates that a person has been infected with *Toxoplasma gondii* some time in the past. There are many different serological techniques available, e.g. the dye test, the complement fixation test, the indirect immunofluorescent test, the latex agglutination test, the enzyme linked immunosorbent assay, and the immunosorbent agglutination test [5,1].

Latex Agglutination Test (LAT) is easy to perform and commercially available in kit form. LAT tests do not require species-specific anti-sera or conjugates and since it is available in kit form they have become popular for sero-diagnosis of toxoplasmosis. However, antibodies detected by LAT may appear later in infection than those detected by tests such as Dye test (DT) and IFAT and their sensitivity may therefore be low especially in acute infections [4].

The complement fixation test is the basic method in the diagnosis of toxoplasmosis [6]. Due to the latter and due to the availability of the antigen it has become the "golden standard" in the diagnosis of toxoplasmosis despite the fact that this attribute is usually associated with Sabin and Feldman dye test [7,8]. The results of both reactions correlate well and their standards and reproducibility are comparable. In addition to other facts the diagnostic advantage resides in the possibility of quantification of the detected levels of antibodies [6]. The significance of the CFT for the diagnosis and evaluation of *Toxoplasma* infection is sufficiently known particularly in Europe [9].

From the aspect of the determination of *Toxoplasma* infection from a single serologic examination, the CFT test yields more relevant prognostic values when compared with IgG antibodies when the same requirement is involved. Specific IgG antibodies detected by ELISA techniques are a reliable substitution of quantitative results that can be assessed by CFT. On the other hand despite the fact that the dynamics of CFT antibodies are more significantly associated with the course of the disease, the assessment of the phase of infection must be supported also by parallel examination of IgM [10] or IgA antibodies and in indicated cases also by that of the avidity of IgG antibodies [11].

The Enzyme Link Immunosorbent Assay (ELISA) for *Toxoplasma gondii* antibodies has been adapted for use in human and most domestic animals and modified methods have been developed for the detection of *Toxoplasma* antigen in body fluid. Because of its ease of use, cost-effectiveness and high sensitivity and specificity, it has replaced older tests in many laboratories [12]. These commercial ELISAs are usually based on antigen preparations derived from tachyzoites of *Toxoplasma gondii* [13].

The reaction of ELISA IgG is based on the principle of indirect enzymatic reaction. IgG antibodies usually appear within 1-2 weeks of acquisition of the infection, peak within 1-2 months, decline at various rates, and usually persist for life. Specific IgG antibodies present in the serum will bind to the antigen of *Toxoplasma gondii* coated the surface of wells. After replacement of residual of free antibodies, the

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Test Evaluation	LAT		CFT		ELISA IgG	
	Parameters	95%CI	Parameters	95%CI	Parameters	95%CI
Sensitivity	82.5%	76.2-87.7%	96.8%	93.1-98.8%	98.9%	96.1-99.9%
Specificity	91.2%	81.8-96.7%	94.1%	85.6-98.4%	97.1%	89.8-99.6%
Positive Likelihood Ratio	9.35	4.34-20.1	16.4	6.35-42.6	33.6	8.59-132
Negative Likelihood Ratio	0.192	0.139-0.265	0.0345	0.0157-0.0759	0.11	0.0028-0.0442
Positive Predictive Value	96.2%	91.9-98.6%	97.8%	94.5-99.4%	98.9%	96.1-99.9%
Negative Predictive Value	66.0%	55.5-75.4%	91.4%	82.3-96.8%	97.1%	89.8-99.6%
Odd Ratio	48.8	19.8-119	477	134-1695	3020	449-20307

Table 1: The Parameters, Quality, and Reliability of Screening Tests.

visualization of reaction takes place after the enzymatic hydrolysis of substrate. The main objective of this study was to evaluate the screening tests that used in women to detect the *Toxoplasma gondii* infection.

Materials and Methods

Study area and population

The study was performed in two villages; EL Massoudia and EL Nuba lay in the north of EL Geizera State (middle Sudan) located near Blue Nile, these villages belonging to EL Kamleen province about 50 kilometers south of the capital Khartoum. Most of population in these villages belongs to the same ethnic group. People in these areas presented low socio-economic status, thus people are farmers, animal breeders, or workers particularly after a big industry city was established near this area. Women live in simple life way and although most of them were not working but they lend a hand to improve the economic situation.

Study design

The study was cross-sectional study for toxoplasmosis in women at the child bearing age. The sample size was calculated as 243 on a prevalence of 20% obtained from first 10 samples collected, d = 0.05 at a confidence level of 95%. A total of 5% of the sample population was added to the sample size; so, the final study population size was 255 samples.

Samples collection

The blood samples were collected under direct medical supervision by medial venipuncture using 5 ml syringe into heparinized tubes, plasma was obtained by centrifugation of the blood at 5000 rpm for 10 minutes. Plasma and cells were kept in different labeled cryo tubes in -20°C till used.

Screening tests

Latex agglutination test (LAT): Latex agglutination test Toxo-Latex® (SPINRER EACT, S. A. Ctra. Santa Coloma, Spain) is a suspension of polystyrene latex particles coated with soluble *Toxoplasma gondii* antigen.

Complement fixation test (CFT): CFT (Test-line®) the reaction based on a bound between complement in reactive mixture and specific antigen-antibody complex.

ELISA IgG: ELISA IgG (Test-line®) is screening test used for detection of infection with *Toxoplasma gondii*.

Screening tests assessment

Assessments of the screening tests were done by calculating the sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratio, and ROC area. Sensitivity, specificity, and positive and negative predictive values were calculated as outlined by [14,15] according to the following formulas:

$$\text{Sensitivity}=\frac{\text{True positives}}{\text{True positives+false negatives}}$$

$$\text{Sensitivity}=\frac{\text{True positives}}{\text{True positives+false positives}}$$

$$\text{Positive predictive value}=\frac{\text{True positives}}{\text{True positives+false positives}}$$

$$\text{Negative predictive value}=\frac{\text{True negatives}}{\text{True positives+false negatives}}$$

Positive (LR+) and negative (LR-) likelihood ratios were calculated according to Akobeng, (2006 b) by these formulas:

$$\text{LR+}=\frac{\text{The probability of an individual with disease having a positive test}}{\text{The probability of an individual without disease having a positive test}}$$

$$\text{LR-}=\frac{\text{The probability of an individual with disease having a -ve test}}{\text{The probability of an individual without disease having a -ve test}}$$

Results

Screening tests evaluation

In this study three different screening tests were used to detect the *Toxoplasma gondii* antibodies in childbearing age women. Some of these tests detected whole antibodies against *Toxoplasma gondii* like Latex Agglutination test (LAT) and Complement Fixation test (CFT) and other test detect chronic infection by detecting IgG like ELISA IgG. Screening tests were evaluated through calculation of the sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, positive predictive value, negative predictive value, and odds ratio.

Latex agglutination test

Using IFT as standard method to evaluate LAT as shown in small table below:

	IFT		
LAT	Positive	Negative	Total
Positive	151	6	157
Negative	32	62	94
Total	183	68	251

From this table: 151 individuals were positive by LAT in accordance with IFT while 62 were negative in accordance by LAT and IFT. The remaining cells including 6 individuals were positive by LAT but negative by IFT (false positive) and 32 individuals were negative by LAT but were positive (false negative) by IFT.

Prevalence rate using LAT was 62.3%, the sensitivity of LAT was found to be 82.5% and the specificity was 91.2%. The positive Likelihood

ratio was 9.35 and the negative likelihood ratio was 0.19. The odd ratio was 48.8 while the positive predictive value was 96.2% and the negative predictive value was 66% as shown in (Table 1).

Complement fixation test

The CFT was evaluated using IFT and the result shown in (Table 1).

	IFT		
CFT	Positive	Negative	Total
Positive	179	4	183
Negative	6	64	70
Total	185	68	253

The sensitivity of CFT was 96.8% and the specificity was 94.1% while the positive and negative likelihood ratio was 16.4 and 0.0345, respectively. The odd ratio was 477 and the positive and negative predictive value was 97.8% and 91.4%, respectively (Table 1).

ELISA IgG

The evaluation was also done for ELISA IgG.

	IFT		
ELISA IgG	Positive	Negative	Total
Positive	183	2	185
Negative	2	66	68
Total	185	68	253

The results shown in (Table 1).

The sensitivity and specificity of ELISA IgG were 89.9% and 97.1% respectively. The positive and negative likelihood ratio was 33.6 and 0.01, respectively. The positive and negative predictive value was 98.9% and 97.1, respectively (Table 1).

Discussion

The immunodiagnostic of *Toxoplasma gondii* infection is widely used for screening pregnant women in order to prevent its congenital spread. Screening tests are ubiquitous in contemporary practice, yet the principles of screening are widely misunderstood [16]. Screening is the testing of apparently well people to find those at increased risk of having a disease or disorder. The usefulness of diagnostic tests, that is their ability to detect a person with disease or exclude a person without disease, is usually described by terms such as sensitivity, specificity, positive predictive value, and negative predictive value [17]. In this study, high sensitivity was found by ELISA IgG. This result was matched with evaluation done in samples at reference laboratory of toxoplasmosis in Czech Republic where more than 40% of laboratories diagnosing toxoplasmosis were using this kit [18]. The second test in sensitivity was CFT then LAT. High specificity was recorded by ELISA IgG and CFT. Results obtained in present study confirmed that ELISA IgG and CFT were the highest in both sensitivity and specificity. Sensitivity and specificity are important measures of the diagnostic accuracy of a test but cannot be used to estimate the probability of disease in an individual patient. The sensitivity of a test only tell us how good the test is for identifying people with disease when only looking at those with disease. Sensitivity tells us nothing about whether or not some people without the disease would also test positive and if so, in what proportion [19]. Specificity can only be calculated from those people who do not have the disease. Specificity tells us nothing about whether or not some people with the disease would also have negative result and if so, in what proportion [20]. A highly specific test is, therefore, most helpful to the clinician when the test result is positive. The significance of the classical complement fixation test for the diagnosis and evaluation of *Toxoplasma gondii* infection is sufficiently known [21,10,9].

Positive and negative predictive values provide estimates of probability of disease but both parameters vary according to disease prevalence [20].

In the present study, high positive and negative predictive values shown in ELISA IgG and CFT.

The sensitivity and specificity of a test cannot be used to estimate the probability of disease in a patient, but the parameters could be combined into one measure called the likelihood ratio which may be used in conjunction with disease prevalence to estimate an individual patient's probability of having disease [22,15].

Likelihood ratios are, clinically, more useful than sensitivity and specificity. They provide a summary of how many times more or less likely patients with the disease are to have that particular result than patients without the disease, and they can also be used to calculate the probability of disease for individual patients [23]. In current study, positive likelihood ratio of ELISA IgG was very high and women who were positive by the test for IgG were more than thirty three times to have latent toxoplasmosis while positive women by CFT may be more than sixteen times having infection by *Toxoplasma gondii* but this probability decreased in LAT to be less than ten times. Negative likelihood ratio clarify that negative women by CFT confirmed that women without infection. This confirmation being less in ELISA IgG. That means, CFT was better than IgG in negative cases.

Accurate treatment of toxoplasmosis is connected with good diagnosis of the disease. Therefore, accurate diagnosis is very important to manage the infection particularly each stage of the disease has different scheme of therapeutic. Clear protocol of examination and good interpretation of serological examination can lead to successful recovery and can control the disease particularly in childbearing age women. Once the disease is controllable in women, this can lead to prevent their children during pregnancy or lactation thus, decreases congenital toxoplasmosis.

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