Comparison between some serological methods used for detection of toxoplasmosis in women in Al Muthanna province

M. A. H. Al- Se´adawy
College of Sciences\ Al-Muthanna University

Abstract

The aim of this research is to study the prevalence of Toxoplasmosis in pregnant women in Al-Muthanna province during October, November, December 2010 and January 2011. Blood samples were collected from aborted women and were examined by the following serological methods: Latex agglutination, IgG ELISA and IgM ELISA tests which revealed the total rates infection 71%, 35.4% and 6.25% respectively there wasn't significant effect of age on proportion rate which is increased directly with age only IgG ELISA, highest rates infection were recorded in 31-35 age group when we used Latex agglutination and IgG ELISA, while it was at 15-20 age groups when we used IgM ELISA.

المقارنة بين بعض الطرق المصلية المستخدمة لتشخيص داء المقوسات في النساء في محافظة المثنى

مهند عبد الحسين حمزة السعداوي
كلية العلوم/ جامعة المثنى

الخلاصة

هدفت هذه الدراسة لمعرفة تفشي داء المقوسات في النساء الحوامل في محافظة المثنى خـــلال الأشـــهر (تشرين الأول, تشرين الثاني, كانون الأول للسنة 2010 م وكانون الثاني للسنة 2011). جمعت عينات دم من نساء مجهضات واستخدمت الفحوصات التالية: اختبار تراص اللاتكس والمعايرة المناعية الومضانية المرتبطة بالإنزيم للأضداد(الاليزا) IgG وIgM. بلغت نسب الإصابة 71%، 35.4% و6.25% على التوالي. لم تسجل فروق معنوية لتأثير العمر على نسب الإصابة وقد لوحظ ان نسبة تواجد الأضداد المناعية IgG تزداد بشـــكل طردي مع المجاميع العمرية. ظهرت أعلى نسبة إصابة للفئة العمرية (31 – 35) عند اســـتخدامنا لفحصـــي اللاتكس والاليزا للأضداد IgG بينما حازت الفئة العمرية (15–20) على أعلى نسبة إصابة عنـــد اســـتخدامنا لفحص الاليزا للأضداد IgM.

Introduction

Toxoplasmosis is a cosmopolitan disease arising from infection with the cat-borne Apicomplexan, coccidian protozoan *Toxoplasma gondii*, an obligate intracellular parasite that forms cysts in mammalian tissues throughout the body (1). Toxoplasmosis is a major public health problem, with a high socioeconomic impact in terms of human suffering including the cost of caring for sick, mentally retarded and blind children (2). The parasite is an extremely successful pathogen, responsible for significant morbidity and mortality, especially in congenitally infected and immuno-compromised individuals (3, 4, 5) although some subjects experience infection without overt disease or with mild symptoms (6, 7). *T. gondii* has a worldwide distribution in human populations infecting up to one third of the global population and a wide range of other mammalian and avian species (8, 9). On farms, *T. gondii* is a major cause of abortion and problems with fertility in livestock, especially among ewes (10) and therefore a significant cause of

lost profitability in livestock agriculture (11). The most important channels for transmission to humans are by ingestion of food or water contaminated with oocysts shed by cats, by eating undercooked or raw meat containing infective tissue cysts and via transplacental transfer, notably when the mother becomes infected for the first time during pregnancy (1, 5, 7, 12, 13, 14, 15). *T. gondii* infections in humans can only be detected by antibody levels and the current analysis is based on the prevalence of *T. gondii* specific IgG (peaking at 4 months after infection and persisting at low levels for life) and on *T. gondii* specific IgM (appearing within 1–2 weeks of infection and subsiding by 6–9 months) (8). A high risk is thus imposed on human communities that come into contact with cats (12). As in many cities throughout the world, Al-Muthana province in Iraq has a significant rodent problem for decades.

Materials and Methods

The study included 100 blood samples were collected from (18- 35) years old aborted women from delivery hospital in Al-Samawa province for 4 months. Clinical data about these samples included: Case history, name, age, gestation month and contact with animals.

- **Serological test:** Samples were collected in hospital laboratory by medically trained staff by using sterile syringe to collect blood was under taken from vein in plain tube and centrifuged to obtained serum and frozen at -20 C° for longer storage. We carried out a latex agglutination test (Toxocell Latex, Biokit, Barcelona) for detection of antibodies to *T. gondii* on 100 patients. The antibody Titer was also estimated by serial serum dilution. For 96 of the participants, specific anti-*toxoplasma* IgM and IgG antibodies testing were done using an enzyme-linked immunosorbent technique. A commercial ELISA kit (BioCheck, Inc. Foster City, CA 94404) was used for detection of anti-*Toxoplasma gondii* IgG and IgM antibodies. The technique was performed according to the manufacturer's instructions. The data of research were analysed by Chi square according to Rocco and James (16).

Results

One hundred women of 15-35 years age range participated in this study. The majority of the participants, 74 women (74%), were in their twenties; 15 were <20 years, 11 were 30-35 years. The *toxoplasma* agglutination test was positive in 71 women (71%) with titres ranging from 1:20 to 1:640 (Table 1,2) while enzyme-linked immunosorbent assay to detect specific anti-*toxoplasma* IgM and IgG were positive in 34 women (35.4) and 6 women (6.25) respectively.

Table (1) Number and proportion of *Toxoplasmal* infections

Type of test	No. of examined samples	No. of infected samples	Proportion of infected samples (%)
Latex agglutination	100	71	71
ELISA(IgG)	96	34	35.4
ELISA(IgM)	96	6	6.25

Table (2) Titres of *Toxoplasmal* infections

Titres	1:20	1:40	1:80	1:160	1:320	1:640
No. of infected samples	23	19	3	14	5	7

By using latex agglutination, significant effect of age wasn't observed on proportion rate, highest infection rates was in 31-35 age group, while lowest at 26-30 age groups (Table 3).

Table (3) Effect of age on *Toxoplasmal* infections by using Latex agglutination

Age groups	No. of examined samples	Infected samples	
		No.	%
15-20	15	13	86.6
21-25	31	26	83.8
26-30	43	22	51.1
31-35	11	10	90.9
Total	100	71	71

While in case of using ELISA to detect specific anti-toxoplasma IgG and IgM, the significant effect of age wasn't observed on rate of infection, highest rate was recorded in 31-35 and 15-20 age group respectively, while lowest at 15-20 and 21-25 age groups respectively (Table 4, 5).

Table (4) Effect of age on *Toxoplasmal* infections by using ELISA IgM

Age groups	No. of examined samples	positive samples (ELISA IgM)	
		No.	%
15-20	15	3	16.6
21-25	30	1	3.3
26-30	41	2	4.9
31-35	10	-	-
Total	96	6	6.25

Table (5) Effect of age on *Toxoplasmal* infections by using ELISA IgG

Age groups	No. of examined samples	positive samples (ELISA IgG)	
		No.	%
15-20	15	3	16.6
21-25	30	9	30
26-30	41	14	34.1
31-35	10	8	80
Total	96	34	35.4

Discussion

The data presented above revealed that there was poor agreement between results obtained with the Latex agglutination IgM test and the IgG and IgM ELISA. The presence of a positive Latex agglutination IgM test result and a negative IgM ELISA result was the most frequent discrepancy. In addition, whereas the IgM ELISA was negative in the 90 (93.75%) serum samples, the Latex agglutination IgM test was positive in 71 (71%) of these serum samples. Thus, in these sera the Latex agglutination IgM test result was clearly false positive. The reasons for the discrepancies between the two IgM tests are unclear but include differences in antigen preparation and differences in the method and selection of sera used to establish the cutoff between positive and negative sera (17, 18, 19, 20). Since there was not an accepted for detection of *toxoplasma* IgM antibodies, parameters of test accuracy for these reference tests such as specificity and positive predictive value are ill defined. Increase of the level of IgG with increasing of age groups reported in this study. This rising trend with age, reflects the continuing risk of infection throughout adult life and arises from the cumulative risk of exposure and infection with age in an environment where transmission is encouraged by the high density of feral cats (21). The relatively low percent of infection rate in this study(by using ELISA IgM) may be due to many factors including the sample size which was only 96 and the patients were selected from Al-Muthanna laboratories/ Iraq who had abortion and with suspension of toxoplasmosis during pregnancy (by history and physical examination). Therefore, this type of sample selection might reflect this low percent also ELISA test which is consider more specific technique than latex agglutination which was used in other studies (22).

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