

Toxoplasma gondii and cytomegalovirus seropositivity pathogens in high- risk patients in Iraq

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Abstract

Testing of patients who are deemed to be at high risk for pathogens, e.g., pregnant women, their fetuses, and neonates, are important so that specific treatment can be initiated. This study included women of age 20- 35 (789) during 2008 and 2010, which tested for *Toxoplasma gondii* and cytomegalovirus seropositivity s. Among 789 women of childbearing age, 22.9%, 55% and 8.7% tested positive for *T. gondii* IgG and IgM, for the years 2008, 2009 and 2010 respectively. Also 32.4%, 38.5, and 29.1% of patients were positive for cytomegalovirus (CMV) in the same periods receptivity. The decreasing prevalence of antibodies between 2008 and 2010 suggested that exposure to *T. gondii* and cytomegalovirus declined over this period in Iraq. Studies of newborn would be helpful to accurately estimate the incidence of congenital infection of toxoplasmosis and cytomegalovirus.

التشخيص المصلي لـ *Toxoplasma gondii* وفايروس Cytomegalo في المرضى الأكثر

عرضى في العراق

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الخلاصة

تعتبر الاختبارات التي تجري على الأشخاص والمرضى الأكثر تأثراً بهذين المرضين كالنساء الحوامل والأجنة وكذلك الأطفال حديثي الولادة من الأمور المهمة لغرض البدء بالعلاج الخاص بها بشكل مبكر. شملت الدراسة مجموعة كبيرة من النساء بلغ عددها (789) امرأة تراوحت أعمارهم بين 20- 35 سنة خلال الفترة ما بين 2008 و2010 وتضمنت الدراسة إجراء الفحوصات السيرولوجية (فحص الاليزا) للكشف عن حالات الإصابة بالمرض CMV ومرض داء القطط بما في ذلك الإصابات القديمة والحديثة، من بين 789 امرأة كانت نسبة 22.9% و56.8% و8.7% موجبة لفحص الأجسام المناعية لمرض داء القطط، خلال عام 2008 و2009 و2010 على التوالي وكذلك نسبة 32.4% و38.5% و29.1% موجبة خلال نفس الفترة الزمنية لـ CMV. لقد لوحظ انخفاض في مستوى الأجسام المناعية لكلا المرضين ما بين سنة 2008 و2010 مما يدل على انخفاض حالات الإصابة بالمرض خلال هذه الفترة في منطقة بغداد مكان إجراء الدراسة. الدراسات على الأطفال حديثي الولادة يساهم بشكل كبير في الحد من حالات نقل الإصابة من الأم إلى الجنين.

Introduction

Toxoplasma gondii is a ubiquitous parasite whose definitive hosts are members of the Felidae cat family. Cat shed millions of environmentally resistant oocysts in their feces after primary infection and are usually without clinical manifestations of disease(1,2). Intermediate hosts include almost all warm- blooded mammals and birds, including humans, who accumulate infectious, quiescent stages (bradyzoites) of the parasite in their tissues particularly in the skeletal muscle and the brain(3, 4).

Intermediate hosts may acquire infection by consuming raw or undercooked flesh from other intermediate hosts(5), or by ingesting of oocysts from the environment(6) sources of *T. gondii* (oocysts) include soil, water, shellfish, fruits, and vegetables(7, 8, 9).

Cytomegalovirus (CMV) is a leading cause of congenital infection in the United States, affecting between 0.2- 2.2% of all newborns(10). Each year in the United States, ~ 35.000 infants are born infected with CMV, with ~ 8.000 of these infants experiencing sequelae including vision loss, hearing loss, mental retardation, other neurologic abnormalities, and death (11, 12).

Risk of congenital infection is higher for seronegative women who have a primary CMV infection during pregnancy than it is for seropositive women who experience a reactivation or reinfection(13, 14). Adolescents and adults can be infected with CMV through sexual contact(15) and nonsexual, close contact with infected individuals, especially children(16). Infants can be infected with CMV in utero, during delivery, and through blood transfusions, breast feeding, and contact with other children who are excreting CMV(17, 18). (Studies done to diagnose cytomegalovirus and toxoplasma antibodies with other microorganisms as causative agent for abortion (19- 24)).

There fore this study was aimed to examine CMV and toxoplasmosis seroprevalence also it has been reported the prevalence of antibodies in the sera of a subset of the human population in Baghdad province and subjects referred for serological testing in hospital (Saint Raphael Hospital). Also it has been analyzed the overall prevalence titer of antibodies of IgG and IgM and the prevalence in relation to the year of samples collection (25).

Material and Methods

The serological response are analyzed for *Toxoplasma gondii* and cytomegalovirus subjects referred for routine hospital based serological tests in Baghdad, Iraq. Prevalence of current/ recent infection are assessed through an enzyme- linked immunosorbent assay (ELISA) for the presence of specific anti- *T. gondii* and cytomegalovirus IgM antibodies, and previous history of infection through IgG for the 789 women of age 20- 35 years, and 70% of the patients (550) had a history of abortion.

Blood samples (789) were collected in a disposable plastic tubes container, then the sera were separated and stored at -20°C till used.

ELISA kits: Imported from DIALAB company (Germany) and Bio check. Inc, used for the detection of cytomegalovirus and toxoplasma gondii antibodies in the sera. IgG and IgM antibody levels were quantified by using the commercially available kits, and by following the manufacturer's instructions. The positive result should be more than 0.9 IU/ml.

- Statistical analysis:

The data were analyzed by Chi- Square test, analyses of variance (ANOVA) and least significant difference (LSD) for differentiation among the means of groups (26, 27) p Value, <0.05 was considered as significant result.

Results

A total of 789 serum samples were collected from women of age ranging from 20- 35, 70%, 550 of them had abortion, 236 serum samples were positive for the presence of anti toxoplasma and CMV. The highest percentage of anti toxoplasma and CMV were (55%, 38.5%) in 2009 and the lowest percentage were (8.7%, 29.1%) in 2010. These results showed significant differences ($P<0.05$) among the positive samples of different years which are high in 2009 and low in 2010 (Table 1).

Table (1) The number and percentage of high- risk patients testing positive for toxoplasma and CMV during 2008- 2010

| Year of collection | Total samples | Positive samples | Percentage% | pathogen |
|--------------------|---------------|------------------|-------------|-------------------|
| 2008 | 287 | 66 | 22.9% | Toxoplasma gondii |
| | 71 | 23 | 32.4% | CMV |
| 2009 | 129 | 71 | 55% | Toxoplasma gondii |
| | 96 | 37 | 38.5% | CMV |
| 2010 | 103 | 9 | 8.7% | Toxoplasma gondii |
| | 103 | 30 | 29.1% | CMV |
| Total | 789 | 236 | | |

A total of 270 serum samples were collected from patients and for the level of IgG (135) and IgM (135) cytomegalovirus antibodies, they showed that the highest percentage of IgG was 17% in index (1.1- 1.4) IU/ml, and in IgM was 28.9% in index (1.0- 1.1) IU/ml and the lowest percentage was 3.7% in IgG with index (1.5- 2.0) IU/ml, and 17% in index (1.2- 1.4) IU/ml IgM. These results showed significant differences ($P<0.05$) among different antibodies and in different results which was significantly higher in IgM with 1.0- 1.1 then 1.2- 1.4. Also the highest IgG in the of 1.1- 1.4 and lowest in index 1.5- 2 (Table 2).

Table (2) Total results and percentage of cytomegalovirus IgG and IgM antibodies

| Type of Antibody | No. of samples tested | No. of positive samples | No. of Negative samples | Percentage of positive samples |
|------------------------|-----------------------|-------------------------|-------------------------|--------------------------------|
| IgG | 135 | 28 | 107 | 20.7% |
| IgM | 135 | 62 | 73 | 45.9% |
| Total of sample tested | 270 | 90 | 180 | 33% |

A total of 519 samples were collected from patients and tested for the level of IgG 260 and IgM 259 toxoplasmosis antibodies, they showed that the highest percentage of IgG was 17.7% in index 1.1- 1.4 IU/ml, and in IgM was 27.4% in index 1.0- 1.1 IU/ml and the lowest percentage was 3.5% in IgG with result 1.5- 2.0 IU/ml, and 7.7% in index 1.4- 1.4 IU/ml for IgM. These results showed significant differences ($P<0.05$) among different antibodies and in different results which was records higher in IgM with titer 1.0- 1.1 and lower in 1.2- 1.4. Also the highest IgG in the index of 1.1- 1.4 and the lowest in index 1.5- 2.0 (Table 3).

Table (3) Total results and percentage of Toxoplasma IgG and IgM antibodies

| Type of Antibody | No. of samples tested | No. of positive samples | No. of Negative samples | Percentage of positive samples |
|------------------------|-----------------------|-------------------------|-------------------------|--------------------------------|
| IgG | 260 | 55 | 205 | 21.2% |
| IgM | 259 | 91 | 168 | 35.1% |
| Total of sample tested | 519 | 146 | 373 | 26% |

Discussion

The results of antibodies testing suggested that approximately 78.8% of women of childbearing age in Baghdad have no IgG antibodies to *T. gondii* (21.2% were IgG positive) and hence are susceptible to infection with toxoplasmosis. Pregnant women

should take appropriate precautions to protect themselves against infection. Such precautions are including cooking meat, washing after handling raw meat or working in the soil; and avoiding contact with cats (1,3). Ninety one women of childbearing age 35.1% were tested positive for anti *T. gondii* IgM antibody. If they were pregnant, IgG avidity testing is the preferred method to confirm recent infection (20), because IgM antibodies can persist for months after initial infection in some individuals(21, 23). Alternate testing algorithms used higher IgM serologic titers as good indicators of acute infection when economic considerations preempt additional tests(25). Women with a history of 3 to 7 miscarriages were twice as likely to test seropositive for *T. gondii* as women with normal pregnancies. In the mean time women without high- risk pregnancies are also at risk for acquiring toxoplasmosis and transmitting it to their fetus(20, 25). *Toxoplasma gondii* Ab prevalence in high- risk patients declined significantly between 2008 and 2010. Declining seroprevalence suggests that knowledge about how to prevent *T. gondii* infection may have improved, the prevalence of *T. gondii* cysts in food animals in the region is declining, or that the fecal cat control program is reducing the quantity of oocysts entering the environment. The CMV is transmitted directly from person to person, particularly between children, in saliva, urine, and genital secretions. CMV Ab prevalence in high- risk patients increase significantly between 2008 and 2010, this may be due to exposure or to persistence of the virus in uterus of infected woman (14, 15). 33.3% percent of women had antibodies against CMV, this agree with other studies which explain why persons with positive CMV serostatus were more likely to be seropositive for *T. gondii* IgG antibodies. With unfortunately, vaccines for CMV have not yet been developed and cause an increase in the infection (14- 17).

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