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Seroprevalence of *Toxoplasma gondii* and *Cytomegalovirus* in Aborted Women in Baghdad-Iraq

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Abstract

Aim of this study was to assess the seroprevalence of Toxoplasmosis and *Cytomegalovirus* (CMV) infection among aborted women. This study was performed on 282 aborted women and 50 normal pregnant women (control group) collected from Al-Yarmouk Teaching Hospital and Fatima Al-Zahraa Hospital for Obstetrics and Children during October 2013 until the end of February 2014. The samples were tested with Latex Agglutination Test (LAT) and CMV Rapid Test Cassette as a primary screening for *T. gondii* and CMV antibodies in the women sera. The primary tests showed a percentage of positive antibodies of *T. gondii* in aborted women and normal pregnant women 99(35.1%), 13(26%) respectively, while for CMV, it was 101(95.3%), 36(72%) respectively for the same groups,. The second test used was Enzyme Linked Immuno-Sorbent Assay (ELISA) for the positive sera in (LAT) test of *T. gondii* antibodies and for the positive sera in cassette test of CMV antibodies. In this test, the women sera were divided into four groups: (IgG+ & IgM-), (IgG+ & IgM+), (IgG- & IgM+) and (IgG- & IgM-) for both aborted and normal pregnant women, the results of *T. gondii* were: 73(73.7%), 2(2%), 2(2%) and 22(22.3%) respectively compared with the control group 13(100%) for the first group only, while the results of CMV were: 98(71.5%), 0(0%), 2(2%) and 1(1%) respectively compared with the control group 35(97.2%) and 1(2.8%) for the first and second group respectively.

Keywords: *Toxoplasma gondii*, *Cytomegalovirus*, aborted women.

الإنتشار المصلي لطفيلي المقوسات الكوندية والفايروس المضخم للخلايا في النساء المجهضات في مدينة بغداد-العراق

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الخلاصة:

هدفت هذه الدراسة إلى تقييم الإنتشار المصلي لداء المقوسات الكوندية والإصابة بالفايروس المضخم للخلايا في النساء المجهضات. تم إجراء هذه الدراسة على 282 امرأة مجهضة و 50 عينة لنساء حوامل طبيعيات إعتبرن مجموعة سيطرة، وتم جمع هذه العينات من مستشفى اليرموك التعليمي ومستشفى فاطمة الزهراء للنسائية والأطفال خلال الفترة من شهر تشرين الأول 2013 وحتى نهاية شهر شباط 2014. تم إختبار هذه العينات في إختبار اللاتكس كتشخيص أولي لوجود الأجسام المضادة لطفيلي المقوسات الكوندية في مصل النساء، وكذلك بالنسبة للفايروس المضخم للخلايا فتم إستخدام شريط الفحص السريع (الكاسيت) كتشخيص أولي لوجود الأجسام المضادة للفايروس. أظهرت النتائج الأولية وجود نسبة إيجابية للأجسام المضادة للطفيلي في النساء المجهضات والحوامل الطبيعيات (مجموعة السيطرة): 99(35.1%) و

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13(26%) على التوالي، بينما كانت النسب للمجموعتين نفسها في التشخيص الأولي للفايروس: 101(95.3%) و 36(72%) على التوالي. تم استخدام إختبار الإيلازا للموصول الموجبة في إختباري اللاتكس والكاسيت، وكانت نتائج الطفيلي والفايروس مقسمة إلى أربعة مجاميع: (IgG+ & IgM-) ، (IgG+ & IgM+) ، (IgG- & IgM-) و (IgG- & IgM+) لكل من النساء المجهضات وذوات الحمل الطبيعي. نتائج الطفيلي كانت: 73(73.7%)، 2(2%)، 2(2%) و 22(22.3%) على التوالي مقارنة بنتائج مجموعة السيطرة (100%) 13 للمجموعة الأولى فقط. أما نتائج الفايروس فكانت: 98(71.5%)، 0(0%)، 2(2%) و 1(1%) على التوالي مقارنة بنتائج مجموعة السيطرة (97.2%) 35 و 1(2.8) للمجموعة الأولى والثانية على التوالي.

Introduction

Toxoplasmosis is an important zoonotic parasitic disease that is caused by protozoan parasite *Toxoplasma gondii* and distributed worldwide. Toxoplasmosis is among the global major zoonotic diseases [1]. Most persons infected after birth are asymptomatic, however, some develop a mild disease or in rare cases, a more severe systemic illness, but in individuals who are immunocompromised, such as in patients with AIDS, the parasites can become widely disseminated, causing severe toxoplasmosis and encephalitis [2]. Humans become infected post-natally by ingesting tissue cysts from undercooked meat, ingesting soil, consuming food or drink contaminated with mature oocysts (directly or indirectly), cleaning cat litter box, and contact with children sandpits or touching anything that has come in contact with cat feces [3]. Other infection pathways include transplacental transmission, blood transfusion, organ transplantation, accidental inoculation in the laboratory and mechanical transmission by flies, cockroaches and earthworm [4,5]. More than 90% of pregnant women who acquire a primary infection during gestation are asymptomatic [6]. Infection within the first two trimesters may result in death of the fetus in utero or spontaneous abortion while the infection in the last trimester usually results in newborns that are asymptomatic at birth, but may develop symptoms later in life [7].

Cytomegalovirus, a ubiquitous virus belonging to the herpes family, is known to cause abnormal fetal outcome. CMV is the most frequent cause of congenital infection in humans [8]. HCMV is an important viral cause of fetal infection which may lead to severe clinical complications in the newborn child, such as encephalitis, chorioretinitis, pneumonia, microcephaly and hearing loss, as well as impaired cognitive development [9]. The virus establishes a life-long infection with some cells being latently infected, a state where the virus has the ability to lie dormant within a cell [10]. Primary infection occurs by direct close personal contact via exposure to body fluids such as saliva, tears, urine, stool, semen, and breast milk. Infants may acquire CMV transplacentally because of maternal viremia or perinatally via breast milk. In later childhood, close physical contact facilitates transmission [11]. The rate of infection is also highly influenced by geographical location and socio-economic status [12]. The seroprevalence of CMV among women of childbearing age ranges from 30% to 90% in different countries especially in developing countries with lower socioeconomic conditions [13]. This study was aimed to determine the prevalence of anti *T. gondii* and CMV antibodies (IgG and IgM) among aborted women by using Latex Agglutination Test (LAT), CMV Rapid Test Cassette and Enzyme Linked Immuno-Sorbent Assay (ELISA) tests.

Materials and Methods

Blood samples were from aborted women with age range (15-49) years. Samples were collected during October 2013 till the end of February 2014, 282 blood samples were collected. Samples were collected from Al-Yarmouk Teaching Hospital and Fatima Al-Zahra'a hospital for Obstetrics and Children. Also 50 normal pregnant women were used as a control group. Five ml. of venous blood were drawn from each woman and the sera were separated by centrifugation at 3000 r.p.m for 10 minutes and stored at -20° C until used.

Serological technique: Detection of parasite and virus antibodies was achieved by using (LAT) kit (Spectrum-Germany), CMV rapid test cassette (CTK Biotech-U.S.A) and (ELISA) kits (Acon for sight-Germany) for IgG and IgM.

Statistical analysis: Chi-square test was used to significant compare between percentages in this study, $P \leq 0.05$ was considered as significant while $P \leq 0.01$ as highly significant. SAS [14]

Results and Discussion:

The results of LAT (Abs) test recorded a percentage of positive *T. gondii* antibodies in sera of aborted women and normal pregnant women (control group) 99 (35.1%) and 13 (26%) respectively, while the results of CMV rapid test cassette recorded a percentage of positive CMV antibodies in the same studied groups 101 (95.3%) and 36 (72%) respectively. The statistical analysis showed highly significant differences at ($P \leq 0.01$) between aborted and normal pregnant women, those showed in Table-1 and Table-2.

Table 1- The percentage distribution of anti *T. gondii* antibodies in 282 aborted women and 50 normal pregnant women (Control) measured by LAT (Abs) test

Women gestation	<i>T. gondii</i>							Chi-square- χ^2
	No.	+Ve		-Ve		Total		
		No.	Percentage (%)	No.	Percentage (%)	No.	Percentage (%)	
Aborted women	282	99	35.1	183	64.9	282	84.9 %	9.347 **
Normal pregnant women	50	13	26.0	37	74.0	50	15.1 %	11.081 **
Total	332	112	33.7	220	66.3	332	100 %	9.844 **
Chi-square- χ^2	--	---	4.219 *	---	4.219 *	---	13.7834 **	---

* ($P \leq 0.05$), ** ($P \leq 0.01$).

Table 2- The percentage distribution of anti CMV antibodies in 106 aborted women and 50 normal pregnant women (Control) measured by Cassette (Abs) test

Women gestation	No.	CMV						Chi-square- χ^2
		+Ve		-Ve		Total		
		No.	Percentage (%)	No.	Percentage (%)	No.	Percentage (%)	
Aborted women	106	101	95.3	5	4.7	106	67.9 %	14.289 **
Normal pregnant women	50	36	72.0	14	28.0	50	32.1 %	10.428 **
Total	156	137	87.8	19	12.2	156	100 %	13.527 **
Chi-square- χ^2	--	--	8.026 **	--	8.026 **	--	10.163 **	---

** ($P \leq 0.01$).

The ELISA test in this study recorded a percentage of positive *T. gondii* IgG and IgM antibodies in sera of aborted women: 73 (73.7%) for (IgG+ & IgM-) group, 2 (2%) for both (IgG+ & IgM+) and (IgG- & IgM+) groups and 22 (22.3%) for (IgG- & IgM-) group, while the results of normal pregnant women (control group) were all from (IgG+ & IgM-) group, 13 (100%). The statistical analysis showed no significant differences between aborted and normal pregnant women at ($P \leq 0.01$), those showed in Table-3.

Table 3- The percentage distribution of anti *T. gondii* antibodies in 99 aborted women and 13 normal pregnant women (Control) measured by ELISA IgG & IgM (Abs) test

<i>T. gondii</i>										
Test	ELISA									
	IgG ⁺ &IgM ⁻		IgG ⁺ &IgM ⁺		IgG ⁻ &IgM ⁺		IgG ⁻ &IgM ⁻		Total	
	No	%	No	%	No	%	No	%	No	%
Aborted women	73	73.7	2	2.00	2	2.00	22	22.30	99	88.4
Normal pregnant women	13	100	0	0.00	0	0.00	0	0.00	13	11.6
Total	86	76.8	2	1.80	2	1.8	22	19.6	112	100
Chi-square- χ^2	---	7.924**	---	0.063 NS	---	0.063 NS	---	8.255 **	---	13.937 **

** ($P \leq 0.01$), NS: Non-significant.

The ELISA test in this study also recorded a percentage of positive CMV IgG and IgM antibodies in sera of aborted women: 98 (71.5%) for (IgG+ & IgM-) group, 0 (0%) for (IgG+ & IgM+) group, 2

(2%) for (IgG- & IgM+) group and 1 (1%) for (IgG- & IgM-) group, while the results of normal pregnant women (control group) were 35 (97.2%) for (IgG+ & IgM-) group and 1 (2.8%) for (IgG+ & IgM+) group. The statistical analysis showed no significant differences between aborted and normal pregnant women at ($P \leq 0.01$), those showed in Table-4.

The 4th group of sera (IgG- & IgM-) in Tables-3 and 4 represents either the false positive result in LAT test or the sample have been spoiled before ELISA test has done and in both ways, it represents non infected women.

Table 4- The percentage distribution of anti CMV antibodies in 101 aborted women and 36 normal pregnant women (Control) measured by ELISA IgG & IgM (Abs) test

CMV										
Test Women gestation	ELISA									
	IgG ⁺ &IgM ⁻		IgG ⁺ &IgM ⁺		IgG-IgM ⁺		IgG&IgM ⁻		Total	
	No	%	No	%	No	%	No	%	No	%
Aborted women	98	71.5	0	0.0	2	2	1	1.0	101	73.7
Normal pregnant women	35	97.2	1	2.8	0	0	0	0.0	36	26.3
Total	133	97.1	1	0.7	2	1.5	1	0.7	137	100
Chi-square- χ^2	---	8.934 **	---	0.082 NS	---	0.060 NS	---	0.027 NS	---	11.426 **

** ($P \leq 0.01$), NS: Non-significant.

Toxoplasmosis is ubiquitous infection affecting 500 million person around the world, with range incidence 12% - 90%, increasing with age, low education, crowding, sanitary habits, socioeconomic, ethnic considerations, consumption of undercooked meat and animal contacts [15]. These results confirm the results of some Iraqi and Arabic studies on toxoplasmosis using LAT: Al-Dalawi [16] recorded 29.2% among aborted women in Baghdad, Ghazi *et al.* [17] recorded 31.6% among pregnant women in Saudi Arabia and AL-Qurashi [18] recorded 41.7% among women in rural areas in Saudi Arabia. In contrast, other researchers showed different results higher and lower than this study: AL-shikhly [19] recorded 51.5% among premarital females in Baghdad and Hasson [20] recorded 19.7% among pregnant women in Al-Najaf.

Using ELISA IgG and IgM, there were some Iraqi and foreign studies in the line with this study: Aziz and Druish [21] recorded 77.1% for IgG among pregnant women in Baghdad, Ayi *et al.* [22] recorded 73.6% for IgG among pregnant women in Ghana, Ribeiro *et al.* [23] recorded 2% for IgM among pregnant women in Brazil and Alvarado-Esquivel *et al.* [24] recorded 2.3% for IgM among pregnant women in Mexico. In contrast, other researchers showed different results higher and lower than this study: Mossa [25] recorded 94% for IgG among public women in Karbala'a, Majeed [26] recorded 21.2% for IgG among aborted women in Baghdad, Aziz and Druish [21] recorded 58.1% for IgM among pregnant women in Baghdad and Ayi *et al.* [22] recorded 76.1% for IgM among pregnant women in Ghana.

In Iran, Hoveyda *et al.* [27] used PCR detection technique and the seropositivity was 15.5% among aborted women, while it was higher in Egypt with also PCR technique, 26.5% by Hassanain *et al.* [28]. The seroprevalence of *T. gondii* infections ranges between 7.7% and 76.7% in different countries (United Kingdom, 7.7- 9.1%; Norway, 10.9%; India, 45%; Brazil, 50- 76% and Nigeria 75.4%) [9].

In the antibody response to *Toxoplasma* infection, IgM antibodies are detected within a few days to one week of infection and disappear generally after three to five months. The IgG antibodies are detected within one to two weeks of infection, reaching a peak after four months, then declining to lower levels and remaining positive for the remainder of the individual's life [7, 29]. It should be noted that, in general, default in serological methods like other diagnostic techniques is inevitable though concordance between serologic and molecular techniques (Real-time PCR) was confirmed in a recent assay by Pignanelli [30]. The associations of various risk factors were analyzed in relation to seropositive cases [31]. In developing countries, where standards of hygiene were low, many studies reported a higher and also variable results on the prevalence of anti- *T. gondii* in pregnant and aborted women, while in developed countries which enjoy a high standards of hygiene, the prevalence of anti- *T. gondii* is variable but lower than in the developing countries [32]. These different rates of

transmission are most likely due to placental blood flow, the virulence of the infecting strain, amount of *T. gondii* acquired, and the immunologic ability of the mother to restrict parasitemia [5].

CMV is known to have an intrauterine route of transmission with significant mortality and morbidity. These results confirm other results of CMV studies using rapid test cassette: Hannachi [33] recorded 96.3% among pregnant women in Tunisia and Yamamoto *et al.* [34] recorded 97% among pregnant women in Brazil. In contrast, other researchers showed different results lower than this study: Majeed [26] recorded 32.4%, 38.5%, 29.1% in the years 2008, 2009, 2010 respectively among aborted women in Baghdad, Barah [35] recorded 74.5% among female university students in Syria and Al-Jiffri *et al.* [36] recorded 63.3% among pregnant women in Saudi Arabia.

Using ELISA IgG and IgM, there were some Iraqi and foreign studies in the line with this study: Al-Azzawi [37] recorded 67.1% for IgG among premarital women in Baghdad, Hamdan *et al.* [38] recorded 72.2% for IgG and 2.5% for IgM among pregnant women in Sudan and Tamer *et al.* [39] recorded 2.6% for IgM among pregnant women in Turkey. In contrast, other researchers showed different results higher and lower than this study: Tamer *et al.* [39] recorded 97.1% for IgG among pregnant women in Turkey, Rajaii and Pourhasan [40] recorded 88.5% for IgG among female population in Azerbaijan, Falahi *et al.* [41] recorded 14.3% for IgG among aborted women in Iran, Sadik *et al.* [42] recorded 23.3% for IgG among women with Bad Obstetric History (BOH) in India, Majeed [26] recorded 45.9% for IgM among aborted women in Baghdad and Al-Marzoqi *et al.* [43] recorded 57.2% for IgM among pregnant women.

Previous immunization with CMV is not perfectly protective against either reinfection or vertical transmission of infection from mother to fetus [44]. It was suggested that pregnancy may reactivate the latent virus leading to further reproductive wastages [45]. These infections cause fetal and neonatal mortality and an important contributor to early and later childhood morbidity [46]. All viral pathogens usually cause a primary maternal viremia which may infect the placenta and thereby the fetus with the exception of HSV-I or II, which causes an ascending infection via the genital tract to fetal membranes and then to the fetus [10]. Primary infection with TORCH complex in pregnant women can lead to adverse outcome, which is initially in apparent or asymptomatic and thus difficult to diagnose on clinical ground [3]. The low rate of seroconversion and detection might be the reason in our study to the low yield of IgM seropositivity in women with spontaneous abortion. The seroconversion depends upon the prevalence of organism followed by the maternal immunity on the specific geography [47].

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