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Chlamydia trachomatous antibodies cross reaction with seropositive *Toxoplasma gondii* and *Cytomegalovirus* among women with abortion and outcomes of congenital abnormalities in Kirkuk City

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Abstract

From 1st Jan2009 to31th of Dec 2009 a total of 1406 women sera with abortions and outcomes of congenital abnormalities in Kirkuk city were screened for infections like *Chlamydia trachomatis*, *Toxoplasma gondii* and *cytomegalovirus* (CMV).These agents were determined in sera using commercial Elisa diagnostic kits.

Results of the study showed that CMV infection was more prevalent 39.74 % followed by *Toxoplasmosis* 16.37 %and *Chlamydial* infection 14.67 %.High rates of *Toxoplasmosis* and *CMV* were recorded among women aging from 15 to 25 year, while high of *Chlamydial* infection was recorded among women aging 26 to 35 years P<0.05. Sera of non-pregnant women reveal high rate of Chlamydial infection, while *toxoplasmosis* and *CMV* were exert in sera of pregnant women. High rates of IgM antibodies 8.33 %and 11.41 % were recorded with *CMV* and *Chlamydia* respectively compare to 3.84 % for *toxoplasmosis* ,P<0.05. Cross reactions rate among three microorganisms were detected in 29 sera with the rate 2.52 %, which divided in to 2.10 % for *Chlamydia* pulse *Toxoplasma* and 0.69 % for *Chlamydia* pulse *CMV*. Two serum samples show 0.54% of mixed IgM antibodies of *Chlamydia, toxoplasma, CMV* and *rubella*, the rate was 0.14 %.

Conclusion: The present study demonstrates a strong association between the infectious agents (*Chlamydia, Toxoplasma* and *CMV*) and women abortion and congenital outcomes. It is therefore recommended that all antenatal cases with such history should be routinely screened for these agents. *Chlamydia* IgM test should be added to preventive tests prior to marriage and parallel to *toxoplasma* test, *CMV* and *rubella* during screening for women abortion and congenital anomalies.

Key words: Abortion, *Toxoplasma, Chlamydia, Cytomegalovirus.*

Introduction

Toxoplasmosis is caused by infection with the protozoan parasite *Toxoplasma gondii.* Acute infections in pregnant women can transmit the parasite to the fetus and cause severe illness e.g., mental retardation, blindness, and epilepsy [1]. There are more infectious agents transmitted sexually from one partner to other such as *Treponema palladium, Neisseria gonorrhea, Trichomonas vaginalis*, Cytomegalovirus, Human herpes simplex type-2and Human immune-virus[2]. *Chlamydia trachomatis* is an obligate intracellular bacterial pathogen, sexually transmitted, that exhibit no symptoms but when symptoms do appear in women with this disease they may include a muco-purulent vaginal discharge [3], painful or frequent urination; lower abdominal pain; painful sex; and abnormal bleeding [4]. Chlamydia can influence a woman's ability to conceive in two ways: it can lead to Pelvic Inflammatory Disease (PID), which can cause infertility by damaging the fallopian tubes, and / or result in cervicitis, which can lead to temporary infertility by affecting the cervical mucus [5]. *Cytomegalovirus (CMV*) are part of Herpesviridae family, infection with *CMV* common rates are approximately 40% in developed areas, and approach 100%in undeveloped countries [6]. Infection may be a symptomatic, or may include: mononucleosis-like symptoms with prolonged fever and mild hepatitis. Infected individual may shed virus intermittently, without any detectable sign or symptoms, *CMV* can be shed in urine, saliva, blood, tears, semen breast milk[7].Women who are infected with *CMV* for the first time during pregnancy may transmit the virus to their unborn child, these infants may develop a generalized infection with symptoms ranging from enlargement of the liver, spleen to fatal illness & in untreated infants, it will for developing complications such as hearing loss, vision impairment and mental retardation [8] and[9]. Laboratory diagnosis based on antigen antibody combinations is more dominant for detecting the causative agents of women abortions and congenital abnormalities, so this study was planned to reveal antibody cross reaction between sera of women positive for *toxoplasmosis* with Chlamydia trachomatis, and cytomegaloviruses antibodies using Elisa techniques.

Materials and methods

This study was carried on 1406 women with abortions and outcomes of congenital abnormalities attending out patients of antenatal and private clinics in Kirkuk city from 1st January 2009 to 31th of December 2009. Women ages ranged from 15 to above than 46 years. Complete informations have been taken from each woman in special questionnaire. Five milliliters of venous blood has been drown from each woman and transferred into blood collecting tube. Processing of laboratory methods were done in Medical Researches

Laboratory in Kirkuk College of Medicine. Sera were separated after centrifugation and examined firstly for *Chlamydia trachomatis* IgM, and then to *Cytomegalovirus* and *Toxoplasma gondii* antibodies both IgM and IgG using Elisa kits purchased from local scientific markets .Kits has been manufactured by Bio-tic company-USA. Elisa procedures for toxoplasma, chlamydia and CMV were done according to Ismaiel[10],Ali[11]and Yasodhara [12] respectively .All obtained data has been organized in tables to assess statistical significances using Chi-square ant t-Student tests.

Results: From total of 1406 sera of women 300 samples (21.33%) were positive for infectious agents of women abortions and congenital out comes. This rate was divided in to 39.74 % for CMV antibodies followed by 16.37 % and 14.67 % for *Toxoplasma gondii* and *Chlamydia trachomatis* IgM antibody respectively, Table(1).

Table 1- Positive and negative rates of *Toxoplasma gondii, Chlamydia trachomatous and Cytomegalovirus* antibodies in sera of women.

Type of infection	No. Exam.	Percentages %	No. positive	Percentages % +ve	No. negative	Percentages % -ve
Toxoplasma gondii	910	64.72	149	16.37	761	83.62
Chlamydia trachomatis	184	13.08	27	14.67	157	85.33
Cytomegalovirus	312	22.20	124	39.74	188	60.26
Total	1406	100	300	21.33	1106	78.77

Relationship between frequencies of infectious agents' antibodies in regard of women ages was obvious in table (2), via which high rate 19.17 % of *toxoplasma* antibodies was recorded among women aging from 15 to 25 years. While high rate20.37 % of *Chlamydia* antibodies was recorded among women aging from 26 to 35 years compare to 6.89 % among women aging 46 years and above ,P<0.05 . Considering CMV antibodies the following high rates 47.72 % and 45.19 % among women aging from 15 to 26 and 26 to 35 years were recorded respectively ,P<0.05. Table (2) .

Table -2- Frequency of *Toxoplasma gondii, Chlamydia trachomatis* and *Cytomegalovirus* antibodies according to women age.

Age groups /years	*Toxoplasma*			*Chlamydia*			*Cytomegalovirus*		
	No. Exam.	No. +ve	% +ve	No. Exam	No +ve	% +ve	No. exam	No. +ve	% +ve
15 to 25	386	74	19.17 A	70	9	12.85	132	63	47.72 C
26 to 35	303	44	14.52	54	11	20.37 B	104	47	45.19
36 to 45	123	20	16.26	41	5	12.19	42	9	21.42
46 up to above	98	11	12.08	29	2	6.89	24	5	20.83
Total	910	149	16.37	184	27	14.67	312	124	39.74 D

A: d.f =3 F value at 0.05 =1.53, **B**: d.f. =3 t-value at 0.05 = 8.42
C: d.f =3 F value at 0.05 = 3.2 **D**: d.f=2 Chi 2 value at 0.05 =6.76

From total of 910 women enrolled the study 129 women were pregnant, their sera were exerted 48.64 % and 27.13 % positive rates for CMV and *Toxoplasma* antibodies respectively. Controversial to 781 non pregnant women their sera were revealing 38.46 % and 14.49 % for CMV and *Toxoplasma* antibodies respectively, P<0.05. Considering *Chlamydia trachomatous* IgM antibodies, sera of non-pregnant women reveal 10.61 % compare to 6.40 % in pregnant women, P<0.05. Statistical analysis of infectious agent distributions in regard to women gestations was significant, P<0.05(table3).

Table-3- Distribution of *Toxoplasma gondii, Chlamydia trachomatis* and *Cytomegalovirus* antibodies according to women gestation.

Women gestation	No. Exam.	*Toxoplasma gondii IgM antibody*		*Chlamydia trachomatous* IgM			*Cytomegalovirus* IgM		
		No. Positive	% Positive	No. Exam	No +ve	% Positive	No. Exam	No +ve	% Positive
Pregnant women	129	35 A	27.13	125	8	6.40 B	37	18	48.64 C
Non pregnant	781	114	14.59	187	19	10.16	275	106	38.46
Total	910	149	16.37	184	27	14.67	312	124	39.74

A: F 0.05(v1,v2) =22, B: F 0.05(v1,v2)=9.4 C: d.f. =1 t-value at 0.05 = 7.32

Table (4) was summarizing co-infections of infectious agent and type of antibodies as pure IgM, IgG and Mixed IgM+ IgG for each infectious agent. Total rate of single infection 19.27 % was high when it was compare with 2.52 % for more than one infectious agent, P<0.05.The more dominant antibody distribution sum for three infectious agents 31.70 % was with IgG, found in sera of single infections, this rate was involve high rates: 20.83 % and 10.87 % for *CMV* and *toxoplasma* antibodies respectively P<0.05. The same relationship was obtain with *CMV* and *Toxoplasma* IgM+ IgG antibodies, through which the following rates 10.57 % and 1.20 % were recorded with *CMV* and *Toxoplasma respectively*. Antibodies crossing among infectious agents were presented in double infections, via which total rate 2.52 % in 29 sera was recorded , this rate involve the following rates : 2.10 % with *Toxoplasma and Chlamydia*, followed by 0.69 % for *Toxoplasma* + *Chlamydia* IgM and 0.54 % for *Chlamydia* IgM and Rubella IgM P<0.05. While two sera with the rate 0.14 % exert antibody crosses among infectious agents *Toxoplasma* IgM+ *Chlamydia* IgM+ *CMV* IgM+ *Rubella* IgM. In general antibody crossing was high with IgM 0.99% followed by 0.78 % with IgG compare to 0.28 % of antibody crossing with IgM+IgG antibodies, P<0.05 .

Table-4-Frequency of co-infections of *Toxoplasma*, *Chlamydia* and *Cytomegalovirus* antibodies in sera of women with abortions and outcomes of congenital abnormalities

Types of infections	IgM		IgG		IgM +IgG		Total	
	No. +ve	% +ve	No. +ve	% +ve	No. +ve	% +ve	NO. +ve	% +
Single infectious agent								
Toxoploasma gondii	35	3.84	94	10.87	11	1.20	149	16.37
Chlamydia trachomatous	21 **B**	11.41	0	0	0	0	27 *	14.67
Cytomegalovirus (CMV)	26	8.33	65	20.83	33	10.57	124	39.74
Total	82	5.83	145	10.31	44	3.31	271	19.27 **A**
Double and triple infectious agents								
Chlamydia +Toxoplasma	9	0.82	10	0.91	4	0.36	23	2.10
Chlamydia + CMV	2	0.46	1	0.23	0	0.0	3	0.69
Chlamydia +Rubella virus	1	0.54	0	0.0	0	0.0	1	0.54
Chlamydia, Toxoplasma, CMV and *Rubella* virus	2	0.14	0	0.00	0	0.00	2	0.14
Total	14	0.99	11	0.78	4	0.28	29	2.52

B : Six samples with equivocal level (0.9 to 0.99 IU/ml) of chlamydia IgM antibodies were subtracted from total of 27 positive samples. **A:** ***d.f. =1 t-value at 0.05 = 36.5 .*** **Total** Number for *Toxoplasma*=910**,** ***CMV***=312 , ***Chlamydia***=184

Discussion

Toxoplasma, rubella and *CMV* are known to cause infection in utero and are often responsible for abortion, still birth, premature delivery and congenital malformation. There is considerable variation in the prevalence of these agents among the women of childbearing age in different geographical areas [12]. In the present study, the rate of women infections (21.33%)with *CMV, toxoplasma* and *chlamydia* in the present study was high and this may represent bad health conditions of women in Kirkuk province and also it was prognosis to pregnancy lost and outcomes of congenital abnormalities at women in this province .Especially toxoplasmosis and CMV, because they evoke miscarriages in early gestational period [13]. While *chlamydia*l infections during pregnancy may leads to conjunctival sequels post-delivery, on the other hand if the infection was persist it will affect women ability to conceive child (infertility) in future in addition to ectopic pregnancy [3].The rate of *chlamydial* infection is close to that reordered in the same province by Ali[11], while it was not agree with 24 % that recorded by Al-Shemitry [14] in Al-Najf city and with 28.1 % in India [5]. Variations in the rates may be attributed to type and mode of sampling, samples size and type of laboratory method especially the use of Elisa in the present study. Considering women ages and high rates of *CMV* and *Toxoplasma* antibodies among women aging from 15 to 25 years can be explained by more sexual activities during this age group than other ages. The association of pathogens distribution and women pregnancy was significant especially with *Toxoplasma* and *CMV* than in non-pregnant women, this reflects environmental contamination with oocysts of Toxoplasma parasite especially soil and meat. Moreover to non-protection against *CMV* during sexual intercourses at both partners. Highly prevalent of chlamydial infection among non-pregnant women in the present study predict adverse outcome of pelvic inflammatory disease (PID) and infertility. Since the infection is amenable to antibiotic therapy, screening and appropriate treatment may improve the outcome

[15]. From the immunological view, the interpretation of Elisa serological results is based on the fact that: IgM antibodies mostly means primary infection, while IgG antibodies regarding toxoplasmosis and *CMV* means long life immunity against previous infections, furthermore both IgM pluse IgG results require watching of rising antibodies to exclude primary or recent infection and to prove protective immunization against previous infection. In the present study women toxoplasmosis as IgM in 35 sera with 3.94% was indicating women exposure to habitual abortion and congenital abnormalities this rate is very low to 21.8 % recorded by Al-Jubori [16] in the same province .While 11.41% of *chlamydia* rate is lower than 14.8% recorded by Ali (11) in the same province and with 28.1 % in India [17] , This variations might be due to use of Elisa method in the present study compare to immune-chromatography test in Ali [11].In spite of *CMV* IgM rate was 8.33 % but real predictive rate might be higher because sera of 33 women reveal 10.57 % of *CMV* IgM pluse IgG antibodies that require retesting within 14 to 21 days to avoid sero-conversion and this may explain why *CMV* rate is still high among women in Kirkuk city. The result of CMV IgM was not agreeing 17.6 % recorded by Salman in the same province [13], 22% recorded in India[17], 7 % in Palestine [18]. High rate of co-infection record as *chlamydia* and *toxoplasma* 2.10% with high dominancy of IgM antibodies for both microorganisms exert bright light on the dangerous degree on the women health, fertility, abortion and outcomes of congenital anomalies. **Conclusion:** women in Kirkuk city were on risk of abortion, congenital outcomes due to toxoplasmosis and *CMV* infections in addition to chance of STD by Chlamydia trachomatis. *Chlamydia* IgM test should be added to preventive tests prior to marriage and parallel to *toxoplasma* test, *CMV* and rubella during screening for women abortion and congenital anomalies.

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التفاعل التصالبي للمستديمة الحثرية مع المصول الموجبة للمقوسة الكوندية وحمة المتضخم بين النساء اللواتي يعانين من الأجهاض ونواتج التشوهات الخلقية في مدينة كركوك

يحيى جرجيس سلمان

فرع الأحياء المجهرية الطبية ، كلية الطب ، جامعة كركوك ، كركوك ، العراق

الملخص

من الفترة الأول من كانون الثاني 2009 لغاية 31 من كانون الأول2009 مسحت مصول 1406 أمرأة يعانين من الأجهاض والتشوهات الخلقية للتحري عن الخمج بالمستديمة الحثرية, المقوسة الكوندية وحمة المتضخم في مدينة كركوك .هذه العوامل الممرضة قدرت في المصول بأستخدام عدة الأليزا التجارية.

النتائج الدراسة أظهرت نسبة أنتشار عالي 39.74 % لحمة المتضخم تبعها المقوسة الكوندية 16.37 % والمستديمة الحثرية 14.67 %. النسب العالية للمقوسات وحمة المتضخم سجلت في نساء اعمارهن بين 15 الى 25 سنة بينما أعلى نسبة للمستديمة الحثرية سجلت في نساء أعمارهن بين 26 الى 35 سنة. مصول النساء غير الحوامل أظهرت نسبة عالية للمستديمة الحثرية بينما مصول النساء الحوامل أظهرت نسب عالية من المقوسات وحمة المتضخم .سجلت النسب العالية التالية11.41 % و 8.33 % من الأجسام المضادة نمط (ام) مع المستديمة الحثرية و حمة المتضخم على التوالي مقارنة بالنسبة% 3.84 للمقوسة الكوندية. حددت التفاعلات التصالبية للعوامل الممرضة الثلاثة في 29 أنموذج و بنسبة 2.52 % والتي قسمت الى أعلى نسبة 2.10 % للمستديمة الحثرية +المقوسة الكوندية , و0.69%% للمستديمة الحثرية + حمة المتضخم. أنموذجين للمصول أظهرتا 0.14 % من الأجسام المضادة نمط (ام) للعوامل الممرضة التالية مشتركة (للمستديمة الحثرية +المقوسة الكوندية+ حمة المتضخم وحمة الحصبة الألمانية).

الأستنتاجات: أظهرت نتائج الدراسة الحالية علاقة قوية بين العوامل الثلاثة وحالات الأجهاض, التشوهات الخلقية والعقم: لذلك يوصى شمول اللواتي يعانين من مثل هذه الحالات بالفحص المسحي للعوامل الممرضة الثلاثة. فضلا عن أضافة فحص الأجسام المضادة نمط (ام) للمستديمة الحثرية للفحوصات الوقائية قبل الزواج وبموازاة مع فحوص المقوسة الكوندية, حمة المتضخم وحمة الحصبة الألمانية عند حالات الأجهاض وتوقع التشوهات الولادية .