

Seroprevalence of cytomegalovirus and toxoplasmosis in cases of miscarriages women in Al-Diwaniyah province

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الخلاصة

اجريت هذه الدراسة لغرض التعرف على مدى انتشار الإصابة الفيروسية لفيروس مضخم الخلايا وطفيلي المقوسات الكونيديه بين النساء المجهضات في مدينة الديوانيه . حيث تم استقصاء نسبة انتشار الاضداد المناعية المضادة لهما وذوات الحمل الطبيعي (كمجموعة سيطرة) باستخدام تقنية الامتزاز المناعي المرتبط بالانزيم ELISA و شملت الدراسة 125 عينة من المجهضات توزعوا على ثلاثة مجاميع: المجموعة الاولى تمثلت في النساء المجهضات خلال المرحلة الاولى من الحمل والثانية والثالثة التي تمثلت في النساء المجهضات خلال المرحلتين الثانية والثالثة من الحمل ، وكانت 25 عينة من النساء ذوات الحمل الطبيعي (كمجموعة سيطرة) انتهى حملهن بولادة طبيعية . الهدف من هذه الدراسة تحديد دور المقوسات الكونيدية (*T.gondii*) وفيروس تضخم الخلايا (CMV) في حالات الإجهاض وتحديد العلاقة المحتملة بين الإصابتين وتحديد دور الأجسام المضادة وعلاقتها مع الإصابة بالطفيلي والفيروس المذكورين لدى النساء المراجعات لمستشفى الولادة والأطفال والعيادات الخاصه في مدينة الديوانيه ، للفترة من تشرين الاول 2009 إلى اذار 2010 -جمعت المصل من النساء المجهضات والسيطرة لتخمين وجود الأجسام المضادة النوعية للمقوسات الكونيدية، وفحص الامتزاز المناعي لفيروس الخلايا المتضخمه. من مجموع 125 نموذج مصلي ظهر 65 (52%) موجباً للأجسام المضادة للمقوسات الكونيدية 43 - 22 اما النتائج للأجسام المضادة لفيروس تضخم الخلايا (CMV) من نوع گاما و ميو كانت 74 (%.59.2) أما نساء المجموعة الضابطة (السيطرة) فقد كانت النتائج سالبة لداء المقوسات وفيروس تضخم الخلايا. تم دراسة تأثير منطقة السكن والعمر على نسبة حدوث الإصابتين

Abstract

A total of 125 sera were collected from miscarriages women and healthy as control group. Latex agglutination test (LAT) and Enzyme linked Immunosorbent Assay (ELISA) were used to assess the presence of specific antibodies against *T.gondii* and CMV. 65(43&22) were positive for anti-*Toxoplasma* antibody. While 74 sera, (54 & 20) were positive for anti-CMV IgG and IgM antibodies respectively.

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Whereas all control group 25 have been found to be negative for both *T. gondii* and CMV. The occurrence of two pathogens in relation to the residence, age and occupation has been studied. This study was conducted to detect the role of *T. gondii* and CMV in cases of miscarriage and detect the possible association between the two infections at the Children Maternity Hospital and private laboratories in Al-Diwaniyah Province during the period from November 2009 to march 2010.

The study has found that the highest prevalence of Toxoplasmosis recorded for women in the second trimester of pregnancy, whereas in the first trimester for CMV infection and in women with multiple abortions more than with single abortion for both pathogens.

Introduction

Toxoplasmosis is a disease caused by *Toxoplasma gondii* a protozoan parasite which infect human when eat the uncooked meat of infected cattle and transmitted from infected mother to the fetus resulting miscarriage⁽¹⁾. *Toxoplasma gondii* is a coccidian obligate intracellular parasite which responsible for zoonotic infection among human and other mammals⁽²⁾. It was found in cats as well as birds and other domesticated animals including sheep, pigs, and horses⁽³⁾. The parasite causes stillbirth or fetal abnormality, it is of one the common infections associated with food⁽⁴⁾. There are three stages of the infection; acute, sub acute, and chronic⁽⁵⁾. The parasite is transmitted by three-four routes; contaminated soil with oocyst, Ingestion of raw or uncooked infected meat, congenital rout (in which maternal infection is passed translucently), and through blood transfusion transmission.

Human Cytomegalovirus (HCMV) is a member of herpes virus family, this virus associated with persistent, latent and recurrent infection⁽⁶⁾. The infection with this virus is relatively common 60-90% of community, and usually asymptomatic in healthy immunocompetent individuals the virus persist in a latent state throughout the life of the host, under the control of cell-mediated immune surveillance⁽⁷⁾.

Cytomegalovirus (CMV) is the most common cause of congenital malformation resulting from viral intrauterine infection⁽⁸⁾. Up to 15% of intrauterine CMV infections result in asymptomatic congenital disease at birth and 10 to 15 % of those born with a symptomatic congenital CMV will develop significant clinical sequel in infancy⁽⁹⁾

To shed light on the relationship between the occurrence of toxoplasmosis or CMV infection and the frequent miscarriages in Al-Diwaniyha Province , this study has been designed.

The measurement of anti toxoplasmosis and anti HCMV in the sera of recurrent miscarriages women using appropriate conventional serological assay. Residence of the incidences of these two diseases among pregnant women in Al- Diwaniyah. To reveal an association between the two diseases and miscarriages.

Materials and Methods

Patients

In this study 125 aborted women were selected to study the role of *T. gondii* and CMV in their abortions and the possible association between these two infections, relation with anti-*Toxoplasma* and anti-CMV antibodies in aborted women with these antibodies. Patients were from visitors and in hospital patients of Maternity and Children Hospital. The age of patients ranged between (15- 40) years old. The mean age was 27 years old. A blood sample was taken from each patient. The study was conducted from November 2009 to April 2010. The serum samples were separated from blood samples and stored at -20°C in the laboratory of General Educational hospital.

Control group

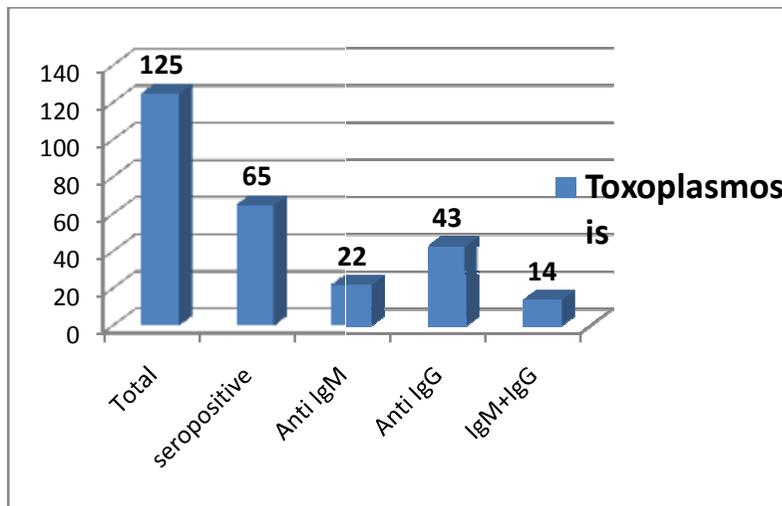
Twenty five apparently healthy women were selected as a control group. They were pregnant women in different periods of pregnancy. They were almost similar with patients regarding age ranges, occupation, socio-economic status and their residence.

Collection of Blood Samples

Five ml blood samples were obtained by vein puncture from all studied women after cleaning the skin with 70% alcohol. Blood samples were stored in plastic tubes and left to clot undisturbed for about 1/2 hr at room temperature. Then they were centrifuged for 5 min at 3000 r.p.m. and then the serum was transferred into other tubes⁽¹⁰⁾. After the serum samples were collected, they were stored at -20°C until they were tested.

Results and Discussion

A total of 125 sera obtained from aborted pregnant women admitted to a gynecological emergency in Maternity and Children Hospital of Al-Diwaniyah Hospital and 25 sera from healthy women (control) were examined for anti-*Toxoplasma* and CMV antibodies by using ELISA test. From aborted women, 65 (52%) sera out of 125 were positive for anti- *Toxoplasma* antibody with titer ≥ 20 IU/ml that considered as a positive titer according to Griffiths⁽¹¹⁾. While 60(58%) sera from 125 aborted women as well as all control sera were negative.



Figure(1) Distribution of Toxoplasmosis antibodies according to age groups in Al-Diwaniyha province by ELISA.

Table(6) Anti *Toxoplasma* Antibodies and Percentage in Miscarriages Women According to The Age Groups

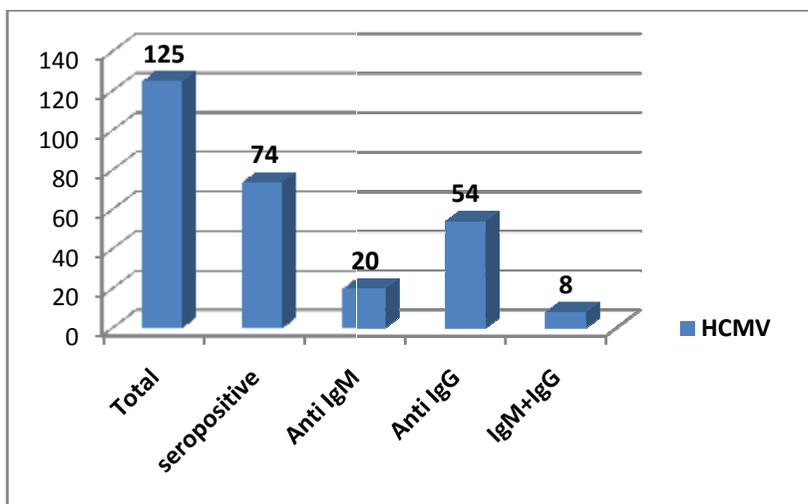
Age Groups	Control Groups (Negative)	Total Tested Samples	Seropositive					
			IgM	%	IgG	%	IgM& IgG	(%) From Seropositive
15-20	4	23	4	17.39	2	8.69	4	66.66
>20-25	8	32	5	15.62	6	18.75	3	27.27
>25-30	7	29	7	24.13	15	51.72	4	18.18
>30-35	3	21	4	19.04	8	38.09	2	16.66
>35-40	3	20	2	10	12	60	1	7.14
Total	25	125	22	17.6	43	34.4	14	21.53

The highest percent of acute infection was occurred at the age group (>25-30) years, whereas the lowest one was at the age of (>35-40)years . The highest occurrence of chronic infection was at the age groups(>35-40). Table(4) showed the different IgG and IgM Anti-toxoplasmosis among patients distributed over different age groups, such finding may be due to difference in the immunological status of women understudy at the period of samples collection and this agreed with ⁽¹²⁾. These results were similar to what mentioned by Al-Adlaan (2007) in Thi-qaar in his study, Who reported 18.42 % for IgM and (32.46 %) for IgG antibodies whereas the percent of both antibodies were (12%) by AL-Rubyii, and Al-Fakahany⁽¹³⁾in Egypt ; the percent of IgM as (27.3 %) and (36.4 %) for IgG antibody. These results are accordance with Austria (30%) and Canada(38%).

It has been observed that significantly greater incidence of abortion occurred in patients with high antibody titers of IgM antibody, this result is supported by Gray ⁽¹⁴⁾. The presence of anti-*T.gondii* (IgG) antibodies in the sample of serum is sufficient to establish the fact that the patient has been infected ⁽¹⁵⁾, while the absence of IgG early or before pregnancy allows identification of women at risk for acquiring the infection ⁽¹⁶⁾. However, IgM class of antibodies is generally not helpful in the diagnosis of acutely

presenting illnesses because these antibodies take 1-9 weeks to develop⁽¹⁷⁾, and persists for months or years.

Human Cytomegalovirus(HCMV)



Figure(2) Distribution of HCMV infection among 125 miscarriages women in Al-Diwaniya province

Table(2) Distribution of HCMV antibodies according to age groups in Al-Diwaniya province by ELISA.

Age Groups	Control Groups	Total Tested Number	Seropositive Samples					
			IgM	%	IgG	%	IgM & IgG	(%) From Seropositive
15-20	4	23	9	39.13	8	34.78	0	0
>20-25	8	32	3	14.28	18	56.25	4	19.4
>25-30	7	29	3	10.34	10	34.48	2	15.39
>30-35	3	21	4	19.04	7	33.33	2	18.18
>35-40	3	20	1	8.33	11	55	0	0
Total	25	125	20	16.0	54	43.2	8	10.81

The highest percent of acute infection was occurred at the age group (15-20) years, whereas the lowest one was at the age of (> 35-40)years. The highest occurrence of chronic infection was at the age groups(>20-25), and (IgM and IgG) at ages of(>20-25)also.

Table(2) showed the different IgG and IgM Anti-HCMV among patients distributed over different age groups, such finding may be due to difference in the immunological status of women under study at the period of samples collection and this agreed with ¹⁸⁾The presence of anti-CMV IgM antibody represents the acute state of CMV (primary infection) or reactivations of latent infection, where those patients were also positive for anti-CMV (IgG) antibody in the same time which represents previous infection and this is found to be in accordance with ⁽¹⁹⁾ who revealed that primary infection with CMV .

Table(4) Prevalence of HCMV and toxoplasmosis in miscarriages women and the association infection in Al-Diwaniya Province.

Disease	(IgM(%)	I (IgG (%)	IgG &IgM(%)
CMV	20 (16.0)	54(43.2)	8 (10.81)
Toxoplasmosis	22 (17.6)	43 (34.4)	14 (21.52)
CMV and Toxoplasmosis	6 (14.28%)	8 (8.24%)	4(18.18)

Anti- T. gondii (IgG) antibodies were found in 43(34.4%) cases of miscarriages women which are not found in healthy women (control) and this may explain the role of T. gondii infection in most recurrent miscarriage cases whereas anti-CMV IgG and IgM antibodies were positive in, 54 and 20 sera respectively of 74 miscarriage women, whereas all control were negative and this refers to the role of CMV infection in some miscarriage cases, and anti T.gondii (IgM) antibodies were found in 22 (16 %)case from miscarriages women while for CMV(IgM) founded in 20 case. The highest prevalence of T.gondii and CMV infections have been found in the age groups (35-40) and (20-25) years old respectively.

The prevalence rate of *T.gondii* and CMV infections were higher in miscarriages women from rural areas than from urban areas. The highest prevalence of toxoplasmosis has been found in the second trimester of pregnancy, whereas the CMV infection was found in the first trimester in women with multiple miscarriage more than those with signal miscarriage for both pathogens.

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