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EVALUATION OF IGG AND IGM ANTIBODY AND MOLECULAR STUDY OF CMV ABORTED WOMEN IN IRAQ

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Abstract

Cytomegalovirus (CMV) is a major herpes virus and a significant human pathogen. Infection is common with seroprevalence rates increasing steadily from 65% among 40 to 49 year olds to 91% in those aged 80 years or over. This research aimed to compare between the results of immunological assays by ELISA test and molecular methods by PCR technique in the diagnosis of Cytomegalovirus as a direct cause of spontaneous abortion in Iraqi pregnant women especially in the first trimester. The study included ninety four aborted patients and twenty five apparently normal females as a control group who attended to Al Alweiya teaching hospital and Al Furat hospital for the period from beginning of November 2018 to October 2019. Five ml of blood was collected from each patient for serological examination, and PAP smear specimen was taken for molecular detection. The results explained that among 94 aborted women 68 (72.3%) of them showed the presence of CMV IgG whereas the CMV IgM was detected in 66 (70.2%). A total of 35 patient samples exhibited both IgG by ELISA and DNA by PCR. Likewise, IgM was detectable by ELISA in plasma samples from 35 patients with DNA concomitantly demonstrable by PCR.

Key words : IgG, IgM, antibody, CMV.

Introduction

Abortion in medicine is defined as the expelling of the contents of the pregnancy before the completion of twenty weeks, including types of abortions, abortions, miscarriages and repeated miscarriages known as pregnancy loss 20 weeks ago and 15-20% of abortion rates in general (Gao., 2013). It is caused by chromosomal abnormalities occurring in women over the age of 35, uterine deformities, ovarian malformation, coagulation, as well as the use of vaginal detergents containing chemicals and bacterial vaginal infections (Gaboon *et al.,* 2015). Human cytomegalovirus (HCMV) is a member of the family Herpesviridae, the virus contains a core with double-stranded DNA, an icosahedral capsid, and a phospholipid-rich envelope. Although most HCMV infections are asymptomatic, certain patients groups are at risk to develop serious illness and long-term effects from an HCMV infection. The HCMV can infect any cell of the body (Brooks *et al.,* 2010). Cytomegalovirus (CMV) is a common virus in the herpes virus family. Fifty per cent people have been infected by young adulthood and up to 85 per cent by 40 years of age. Peaks of infection occur in children less than 2 years age, and during adolescence. Once a person becomes infected, the virus remains alive but usually inactive (dormant) within that person's body for life (Enders *et al.,* 2011, Griffiths *et al.,* 2015). Transmission of CMV can occur due to a maternal primary infection in previously seronegative women or after a non-primary infection (reactivation of an endogenous strain or reinfection with a new CMV strain) in women with preconceptional immunity (Britt, 2015). Maternal primary infections early in pregnancy occur less frequently but are thought to carry the highest risk of fetal abnormalities as seen by ultrasound (US) and/or symptomatic disease at birth. This has not been established for non-primary infections. CMV-associated fetal US abnormalities are broadly defined as cerebral or extracerebral and can be transient, non-specific, and may occur in any trimester (Picone *et al.,* 2013, CDC, 2019). According to the Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO), CMV can infect people of all ages;

over 50% of adults are infected with CMV by the age of 40 (Maysara *et al.,* 2012 Wills *et al.,* 2015). Primary infections are recognized by CMV-immunoglobulin G (IgG) seroconversion or positive CMV-immunoglobulin M (IgM) with a low IgG avidity index (AI). When looking at CMV-IgM kinetics following primary infection, peak levels are seen in the first 1–3 months, after which the titers begin to decrease. Occasionally, persistent (low) levels of CMV-IgM can be detected >3 months or up to a year (Brodin *et al.,* 2015). Non-primary infections are difficult to diagnose but may be recognized by positive CMV-IgG prior to conception/early gestation in combination with a positive CMV-IgM and CMV-IgG with a high IgG AI and/or a significant increase in CMV-IgG titer during gestation (Spyridopoulos., 2016). The first-line drug therapy for CMV infection is ganciclovir (GCV) or its prodrug valganciclovir (VGCV). GCV or VGCV must be activated by phosphorylation before they act on human CMV. This phosphorylation is carried out by the viral kinase UL97, and activated GCV subsequently inhibits the viral DNA polymerase UL54. Clinically, GCV resistance usually arises first from a UL97 mutation resulting in decreased accumulation of the activated drug. Subsequent UL54 mutations can confer high levels of GCV resistance and various degrees of cross-resistance to the second-line drugs cidofovir (CDV) and foscarnet (FOS) (Gilbert *et al.,* 2011, Fisher *et al.,* 2017). The aim of this study was to evaluate the frequency of CMV-IgG and IgM antibodies with UL54 gene among aborted patients in Baghdad city.

Materials and Methods

The current research included ninety four aborted patients and twenty five apparently normal females as a control group who attended to Al Alweiya teaching hospital and Al Furat hospital for the period from beginning of November 2018 to October 2019. Five ml of blood was collected from each patient by vein puncture using disposable syringes. The blood was placed in plastic disposable tubes; it was left to stand at room temperature (20- 25°C) to allow it to clot, then the sera was separated by centrifugation 10000 rpm for 5 minutes and stored at -20°C until the time of test. Serological investigation included detection of CMV-IgG antibodies and CMV-IgM antibodies by using enzyme-linked immunosorbent assay (ELISA) (Abbott kits, Abbott Laboratories, USA). The procedure was carried out according to the manufacturer's instructions.

PAP specimen add to a plane tube containing phosphate buffer saline preparing for molecular detection.

Statistical Analysis

The statistical analysis was performed using chi-square test. P values less than 0.05 were considered statistically significant.

Results and Discussion

Cytomegaloviruses are ubiquitous herpesviruses that are common causes of human disease. It is present throughout the year, with no seasonal variation seen in infection rates. Most people become infected with this virus at some time during their life; in the United States, as many as 80% of individuals older than 35 years have been exposed to this virus and carry a lifelong infection. Cytomegalovirus is endemic in most areas of the world. The seroprevalence of CMV varies in different geographical areas and it ranges from 30-100% (Mohammed and Hadeel., 2011, Qinge *et al.,* 2016).

Characteristics of patients

A total of ninety four aborted female with significant difference ($Pd \leq 0.05$) in addition to 25 apparently healthy female used as control (Table 1).

All 119 subjects were at age ranged from 20 – 44 year (< 20), (20 - 29) (30 - 39) and (>40).

Patients were located into four categories depending on their age. The results exposed that the high percentage of patients (35.1%) found in the second and third group, followed by the patients of first group (21.3%) and the lowest percentage (8.5%) was for the latest group. Statically there was high significant difference ($P \leq 0.05$) between age groups.

In current study we found that the higher rate of aborted women in age group between 20- 39 and 30 -39 years old (35.1 %), while lowest rate of aborted women was in age group over 40. These results agree with many previous Iraqi studies which showed that the higher rate in abortion in ages group between 20- 40 years, they are highly relationship between abortion and the age, (Hussein *et al.,* 2014). Maysara *et al.,* 2012 revealed that the age of patients were range from (20- 30) years in the pregnant women with abortion also many studies reported that the increased risk of abortion and infertility with women age, also showed that the older women have fertility problems because declining egg quality, and other problem which that more current in older women (Ganatra *et al.,* 2014).

Risk factors for CMV infection have been correlated with the socioeconomic status within a community. Other studies showed that elderly persons seem to be well protected against symptomatic CMV disease due to accumulation of CD28 effector cytotoxic T lymphocytes. This is a characteristic feature of all age groups but is

Table 1: Distribution of patients and control according to age.

Age group (Year)	Group				Total	
	Patient		Control			
< 20	N	%	N	%	N	%
20-29	33	(21.3)	6	(24.0)	26	(21.8)
30-39	33	(35.1)	2	(8.0)	35	29.4)
eH 40	8	(8.5)	1	(4.0)	9	(7.9)
Total	94	(100)	25	(100)	119	(100)
Chi square (P-value)	0.023 *					

Table 2: Results of CMV IgG test among aborted women using ELISA technique.

IgG		Group				P-value
		Patient		Control		
		N	(%)	N	(%)	
IgG	-ve	26	(27.7%)	18	(72.0%)	0.001**
	+ve	68	(72.3%)	7	(28.0%)	
Total		94	(100%)	25	(100%)	

Table 3: Results of CMV IgM test among aborted women using ELISA technique.

IgG		Group				P-value
		Patient		Control		
		N	(%)	N	(%)	
IgM	-ve	28	(29.8%)	25	(100.0%)	0.001**
	+ve	66	(70.2%)	0	(0.0%)	
Total		94	(100%)	25	(100%)	

most pronounced in elderly persons (Fowler *et al.,* 2003, Munier *et al.,* 2007).

ELISA technique was chosen for the detection of IgM, and IgG anti-CMV and is usually diagnosed on the basis of IgM antibody in acute infections, and the IgG, IgM antibodies levels increase within 1-2 weeks of infection in general. The aim of detection of specific IgM and IgG for cmv has been used to determination the time of infection, and the negative result of IgM test with a positive result of IgG are usually indicate that the infection occurred at least six months ago, (Prince and Lape., 2014) (Table 2, 3).

The results explained that among 94 aborted women 68 (72.3%) of them showed the presence of CMV IgG compared with 25 apparently healthy group that showed only 8 (28.0%) of them detected with IgG antibody whereas the CMV IgM was detected in 66 (70.2%) aborted women with no detection for IgM antibody in control group as shown in (Table 2, 3).

The detection of CMV IgG indicates that the women were previously infected with CMV, while the presence of IgM indicates recent infection or re infection and the IgM was formed immediately after infection and disappeared of short period.

Higher percentage of CMV IgM antibodies were reported in studies by Khalf *et al.,* (2012) that disagree with recent study where (15.7%) detected and Lone *et al* (2004) who reported the presence of CMV specific IgM antibody in (15.98%) and even more higher results were reported by Hassan *et al* (2014) in which (32.6%) anti CMV (IgM) positive were found. The reasons for the presence of CMV IgM antibodies in these studies and the absence of them in this study could be justified by the variation in sample size and duration of the study.

Majeed in 2011 reported a study involving 270 women of 20-35 years. The result revealed 90 cases positive for CMV, of which 62 cases were positive for CMV IgM and 28 cases were positive for CMV IgG.

Anti- CMV IgM, IgG were found in 13.3% and 4.1% respectively in the study of Sheelan and Nuha., 2017 that agreed with our study.

The seroprevalence of CMV is varying in the worldwide, in developed countries the seropositivity of HCMV ranges between 30-90% of human population, and the increasing of prevalence is parallel with the age (Adler., 2011). In Iraq, many studies conducted to detect the seropositivity of CMV in aborted women, and the seropositivity of CMV in AL-Anbar province was 6.1% (Nada and Fawzia., 2015) in waste province was 60.2% (13), in Baghdad was 10%., 15.7% (14), 9.3%, and in sulimania county was 9.18% (Salih and Kazhal, 2013).

Risk factors for CMV infection have been correlated with the socioeconomic status within a community Primary CMV infection in an individual can be detected by demonstration of CMV specific IgM antibody, also primary infection in pregnancy has a higher incidence of symptomatic congenital infections and fetal loss (Divya *et al.,* 2017).

The molecular detection of this study, also involved detection of UL54 gene in all patients and control. The results showed the presence of DNA band (550bp), which referred to this gene, whereas the biopsy of control group have no band (Fig. 1).

HCMV DNA was detected in 35/94 (37.2%) of the samples tested, the cross-tabulation between PCR and ELISA (IgM and IgG) is shown in (Table 4, 5). A total of

Table 4: Correlation between IgG level and the presence of CMV UL54 gene.

Anti-body test result		550 bp UL54				Total	
		-ve		+ve			
		N	(%)¥	N	(%)	N	(%)
IgG	-ve	26	(27.7)	0	(0.0)	26	(27.7)
	+ve	33	(35.1)	35	(37.2)	68	(72.3)
Total		59	(62.8)	35	(37.2)	94	(100)
Chi square (P-value)		0.001***					

Table 5: Correlation between IgG level and the presence of CMV UL54 gene.

Anti-body test result		550 bp UL54				Total	
		-ve		+ve			
		N	(%)¥	N	(%)	N	(%)
IgM	-ve	28	(29.8)	0	(0.0)	28	(29.8)
	+ve	31	(33.0)	35	(37.2)	66	(70.2)
Total		59	(62.8)	35	(37.2)	94	(100)
Chi square (P-value)		0.001***					

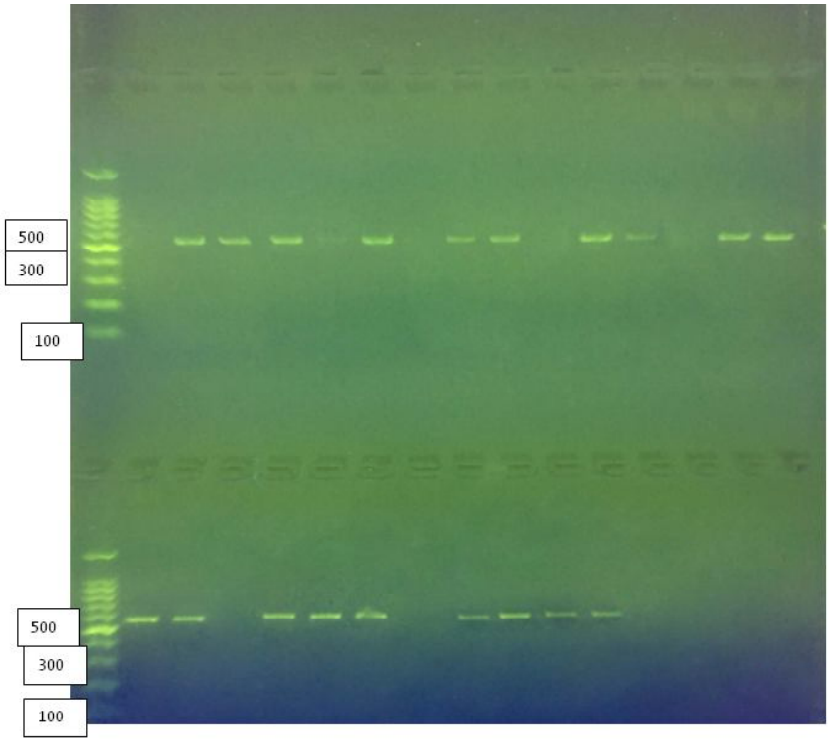

Fig. 1: PCR product, the band size 550 bp. The product was electrophoresis on 2% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours. N: DNA ladder (100).

35 patient samples exhibited both IgG by ELISA and DNA by PCR. Likewise, IgM was detectable by ELISA in plasma samples from 35 patients with DNA concomitantly demonstrable by PCR. By comparison, IgG was detected in samples from 68 patients, with DNA detectable by PCR in 35 sample. Similarly, IgM was present in 66 serum samples tested by ELISA, but DNA was detected by PCR in only 35 samples.

PCR method was used in the present study as a highly sensitive and specific method to predict which patients will develop HCMV disease. This might be attributed to the fact that quantification of HCMV DNA is considered both more sensitive and more specific and the concentration of viral DNA is not enough to detected (Heli., 2004).

However, the discrepancy between the obtained negative results using IgM ELISA with the corresponding positive results by PCR technique may be partially attributable to the time lag between primary infection and IgM antibody production since IgM antibodies may remain undetectable because of delayed seroconversion due to patient treatment with immunosuppressive agents, HCMV DNA was not detected in 31patients who were IgM positive, this might be due to the persistence of IgM antibodies for an extended period of time after primary infection (Revello, and Gerna., 2002).

In conclusion, the results of our study confirm a high prevalence of CMV infection among women with abortion. CMV infection increased in age group between 30-30 years. Serological screening program for early detection of CMV infection in pregnant and aborted women in Iraq is necessary but should be confirmed with molecular technique.

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