

## The Reactivity of Anti-HCMV IgM to Various Specific HCMV Antigens Among Pregnant Women in Kirkuk.

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### Abstract

*Human Cytomegalovirus* (HCMV) is a DNA virus of the Betaherpesvirinae subfamily and Herpesviridae family. HCMV is a leading cause of congenital infections throughout the world. This study was carried out in Kirkuk governorate from July 2017 to January 2018, aimed at studying there activity of anti-HCMV IgM to various specific HCMV antigens among 180 pregnant women attending hospitals, primary health care centers and some private medical laboratories. The pregnant women were examined for HCMV-IgM seroprevalence using Electro-chemo-luminescence(ECLIA) technique then examined their reactivity for specific HCMV antigens using line immune assay. The rates of HCMV-IgM seropositive were 22 (12.22 %). Regarding the reactivity of determined HCMV-IgM against various HCMV antigens, the rates 14 (63.63%), 13(59.09%), 21(95.45%), 15(68.18%), 21(95.45%) and 16(72.72%) were seropositive for HCMV IE1, CM2, p150, p65, gB1 and gB2 antigens, respectively. Concerning the band intensity of HCMV-IgM reactions with HCMV antigens, the rates of (+++) were higher than other band intensity for all antigens with highest rate for gB1 antigen (85.71%). So, the highest rate of HCMV-IgM reaction with the number of antigens was 40.90% for three antigens at the same time. In conclusion, the highest rate of HCMV-IgM in pregnant women was for gB1and p150 antigens; while the highest rate of intensity reaction was (+++)and the highest rate of HCMV-IgM ability for reaction was for three antigens at the same time.

**Keywords:** HCMV; ECLIA; CM2; p150; gB.

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## فاعلية الأجسام المضادة نوع (ام) للفيروس المضخم للخلايا البشرية لمختلف المستضدات الخاصة بالفيروس بين النساء الحوامل في كركوك.

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### الملخص

الفيروس المضخم للخلايا البشرية هو من الفيروسات ذات الحمض النووي دي والتي هي من العائلة الفرعية بيتا التابعة لعائلة فايروسات الهيريس (الحلأ) وتسبب تشوهات خلقية ولادية في جميع أنحاء العالم. استهدفت الدراسة التي أجريت في محافظة كركوك في الفترة من تموز 2017 إلى كانون الثاني 2018 إلى معرفة مدى تفاعل الأجسام المضادة نوع (ام) مع مختلف مستضدات الفيروس المضخم للخلايا البشرية من بين 180 امرأة حامل راجعن مستشفيات ومراكز الرعاية الصحية الأولية وبعض المختبرات الأهلية في كركوك. حيث تم فحص مصل النساء الحوامل للتحري من الأجسام المضادة نوع (ام) مع مختلف مستضدات الفيروس المضخم للخلايا البشرية ومن ثم معرفة تفاعلاتهم مع مختلف مستضدات الفيروس المضخم للخلايا البشرية باستخدام اختبار المناعة الخطي. حيث كانت معدل الانتشار المصلي 22(12,22)%. فيما يتعلق تلك الأجسام المضادة المحددة ضد المستضدات المختلفة؛ حيث أظهرت أن 14(63,63)٪، 13(59,09)٪، 21(95,45)٪، 15(68,18)٪، 21(95,45)٪ و 16(72,72)٪ تفاعلت مع IE1، CM2، p150، p65، gB1 و gB2 على التوالي. أما بالنسبة إلى شدة نطاق التفاعلات مع المستضدات؛ حيث كانت معدل شدة التفاعل (+++) أعلى من شدة التفاعلات الأخرى لجميع المستضدات وبأعلى معدل لمستضد gB1 حيث كانت 85.71٪، وكذلك فإن أعلى معدلات التفاعل لتلك الأجسام المضادة مع عدد المستضدات حيث كانت 40,90 ٪ لثلاث مستضدات في نفس الوقت. واستنتجت الدراسة أن أعلى نسبة من النساء الحوامل لديهن أجسام مضادة نوع (ام) للفيروس المضخم للخلايا البشرية لمستضدات gB1 و p150، في حين كان أعلى معدل كانت لشدة التفاعل (+++) وأعلى معدل من قابلية الأجسام المضادة للتفاعل مع ثلاث مستضدات في نفس الوقت.

الكلمات الدالة: gB.؛ p150؛ CM2؛ ECLIA؛ HCMV.

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## 1. Introduction:

*Human cytomegalovirus* (HCMV) is one of eight human herpesviruses [1-3]. HCMV is formally called HHV-5. It is related to the Betaherpesvirinae subfamily. Its characteristics are slow replication and clinically asymptomatic infections in healthy individuals. It has the largest genome than any of the HHVs—230 kb with an estimated 160–200 protein encoding genes. Consequently, it has various mechanisms to manipulate the infected cell and interfere with host immune responses while ensuring its own replication [4]. HCMV can remain latent within body for long periods. HCMV is also the most frequently transmitted virus to a developing fetus [5].

Rates of congenital HCMV transmission are as high as 50% in women who acquired primary HCMV infection during pregnancy [6]. Intrauterine HCMV transmission rates for primary infection are about 30% [7]. HCMV can also be transmitted from mother to child intrapartum. Primary HCMV infection is mostly asymptomatic. Congenital HCMV infection represents a relevant cause of deafness and neurological damage in newborns. In addition, congenital HCMV infection may lead to intrauterine growth retardation, microcephalia, petechiae, jaundice, hepato-splenomegaly, and ophthalmological disorders [8].

The humoral immunity plays a crucial role in restricting viral dissemination response mediates substantial protection against HCMV transmission, contributing to minimizing the clinical manifestations of the disease [9]. The spectrum of humoral response against HCMV includes: structural tegument proteins (e.g., pp65 and pp150), envelope glycoproteins (predominantly gB and gH and gH/gL, pentameric complex), and non-structural proteins (such as the IE1 protein) [10].

The cellular immune response to HCMV has different components originating from both the adaptive and innate immune systems. The importance of the functional capacity of the HCMV-specific T cells in response to the virus directly (CD8<sup>+</sup> and CD4<sup>+</sup> T cells) lies in demonstrating the extreme complexity and breadth of the T cell responses that are elicited by the human host in response to natural infection [11-13].

HCMV is sometimes considered the master of immune evasion [14] and encoding numerous viral immune evasion proteins [15]. A substantial part of its genome is dedicated to the expression of proteins that subvert the immunological responses of the host. The host is

eliciting very potent immunological responses, both humoral and cellular. The virus is clearly able to evade both responses sufficiently to persist [16].

HCMV has evolved many ways to avoid host humoral responses. One of the most important mechanisms is the route of transmission and systemic spread of the virus. The preventing exposure to the antibody response provides a mechanism of evasion, which is another way of evading humoral immunity [17, 18]. Additionally, HCMV has evolved a number of mechanisms for controlling the host immune response, including the down regulation of MHC-I and MHC-II molecules, the expression of MHC-I homologues, and NK evasion [19].

## 2. Materials and Methods:

A cross sectional study was carried out in Kirkuk governorate from July 2017 to January 2018 for studying the reactivity of anti-HCMV IgM to various specific HCMV antigens in 180 pregnant women whose age ranges between 18-42 years, attending Azadi General Teaching Hospital, Kirkuk General Hospital, primary health care centers, Dubiz primary health care sector and some private medical laboratories. The pregnant women were examined for HCMV-IgM seroprevalence by using Electro-chemo-luminescence (ECLIA) technique; then the reactivity of determined HCMV-IgM with specific HCMV antigens (IE1, CM2, p150, p65, gB1 and gB2) was examined by using line immune (*Recom Line; Mikrogen, GmbH, Germany*) assay. Computerized statistically analysis was performed using SPSS (Statistical Package for Science Services) version 17, SPSS Inc. USA. Comparison was carried out using methods of Chi-square ( $X^2$ ) and Probability (P value). The P value  $\leq 0.05$  was categorized as statistically significant (S), and less than 0.01 was considered as highly significant (HS) and greater than 0.05 was considered as non-significant.

## 3. Results:

A total of 180 pregnant women with ages ranged between (18-42 years old) were examined for seroprevalence of specific HCMV-IgM using ECLIA technique. The seroprevalence of HCMV- IgM was 22 (12.22%), while the seroprevalence of HCMV-IgM in 120 control (non-pregnant) was 4 (3.33 %), as shown in [Table 1](#).

**Table 1:** The seroprevalence of specific HCMV- IgM among pregnant women and control by using ECLIA technique.

Results	HCMV- IgM Antibody			
	Pregnant women		Control	
	No .	%	No .	%
Positive	22	12.22	4	3.33
Negative	158	87.78	116	96.67
Total	180	100	120	100
$X^2 = 92.004$ $P = 0.0001$ $P < 0.01$ Highly Significant				

Regarding the specificity of the determined HCMV-IgM to various specific recombined HCMV antigens using line immune assay technique, the rates of HCMV-IgM against antigens among the total 22 HCMV-IgM seropositive pregnant women was 14(63.63%), 13(59.09%), 21(95.45%), 15(68.18%), 21(95.45%) and 16(72.72%) seropositive for HCMV IE1, CM2, p150, p65, gB1 and gB2 antigens, respectively as shown in Table 2.

**Table 2:** The rates of specific of HCMV IgM seropositive pregnant women against various HCMV antigens using Line immunoassay.

HCMV antigens	HCMV-IgM seroprevalence					
	Positive		Negative		Total	
	No .	%	No .	%	No .	%
IE1	14	63.63	8	36.37	22	100
CM2	13	59.09	9	39.91	22	100
p150	21	95.45	1	4.55	22	100
p65	15	68.18	7	31.82	22	100
gB 1	21	95.45	1	4.55	22	100
gB 2	16	72.72	6	27.28	22	100
$X^2 = 15.80$ $P = 0.0096$ $P < 0.01HS$						

Regarding the assessment of the band intensity, the highest rates of (+/-) was 7.69% and (++) was 30.76% for CM2 antigen, (+) was 15.38% for IE1 antigen; while (+++) was 85.72% for gB1 antigen, as shown in Table 3.

**Table 3:** The band intensity of the examined HCMV-IgM against various HCMV antigens.

HCMV antigens	HCMV IgM										
	Band intensity in relation to the cut-off band										
	+ / -		+		++		+++		Total		
	No .	%	No .	%	No .	%	No .	%	No .	%	
IE1	1	7.14	2	15.38	3	21.42	8	56.06	14	100	
CM2	1	7.69	1	7.69	4	30.76	7	53.86	13	100	
p150	1	4.76	1	4.76	2	9.52	17	80.96	21	100	
p65	1	6.66	2	13.32	2	13.32	10	66.70	15	100	
gB 1	1	4.76	1	4.76	1	4.76	18	85.72	21	100	
gB 2	1	6.25	2	12.50	2	12.50	11	68.75	16	100	
		$X^2 = 8.717$		$P = 0.89$		$P > 0.05NS$					

Regarding the determined specific HCMV-IgM against the 6 examined HCMV antigens in relation to their specificity reactivity to numbers of antigens, the highest rate 40.90% of HCMV-IgM was for three antigens at the same time during the HCMV infection, as shown in Table 4.

**Table 4:** Relation of specific HCMV-IgM among seropositive pregnant women and their reactivity with the numbers of HCMV antigens.

Reactivity with the number of HCMV antigens	HCMV- IgM seropositive	
	No .	%
One Ag	0	0
Two Ag	1	4.54
Three Ag	9	40.90
Four Ag	6	27.27
Five Ag	4	18.18
Six Ag	2	9.09
Total	22	100

#### 4. Discussion:

In the present study, the rate of HCMV- IgM seropositive was 12.22% by using ECLIA technique in comparison to the rate of control groups, which was (3.33%), which is highly significant ( $P < 0.01$ ). These results of HCMV- IgM were close to world HCMV- IgM prevalence 0-10 %, but still higher than recorded ones. Other studies in Iraq revealed that it was 2.5%; while in Turkey, it was 1.2%, in Australia 1.2%, in Finland 4 %, in India 4 %, in Iran 4.35% and in Malaysia 7.2% [20-32]. The variation of results may be attributed to the



kinetics of HCMV-IgM response during primary infection which may vary greatly among individuals depending substantially on the type of technique that these studies mainly used ELISA technique. Also, this variation may be due to variability of viral accessibility and its circulation rate in the community.

Usually, the development of HCMV-specific IgM antibodies occurs within 1-2 weeks after initial infection. These antibodies represent a sensitive indicator of a current or continuing infection of HCMV. Particularly, a test of HCMV IgM is useful in diagnosing infections in pregnant women. In addition, such test guide the appropriate treatment options. The treatment options aim at decreasing the congenital infection risk. Within several months after infection, IgM antibodies typically decrease to below detectable levels [33].

Regarding the specificity of the determined HCMV-IgM to various specific HCMV, the highest rates of HCMV-IgM against p150 and gB1 antigens was 21(95.45%) seropositive; while lower rate was for other antigens with highly significant relation  $P < 0.01$ . This result may be due to the immune-modulatory properties of HCMV that alter the host immune response to infection specially induces a Th1 biased profile in clinically evident primary infection of renal transplant recipients, HCMV-induced dysregulation of normal cytokine profiles during pregnancy, and exposure of antigens to the human immune system during pathogenesis and its replication cycle [34].

Concerning the assessment of the band intensity of the examined HCMV-IgM against various HCMV antigens, the highest rates of (++++) were 85.72% for gB1 antigen; while lower rates of band intensity for the remaining HCMV antigens with non-significant relation  $P > 0.05$ . This shows that the gB1 is highly immunogenic. Five antigenic domains (AD) have been described on HCMV gB: AD-1 to AD-5. AD-1 is highly immunogenic with a seropositivity rate of 100%. Antibodies binding to AD-1 have virus neutralizing activity as indicated by a number of neutralizing AD-1-specific human monoclonal antibodies[35].

Depending on the functions of gB, it was supposed that any alteration in the gB gene might predispose to HCMV disease either by facilitating viral replication or eliciting a severe immunopathological response [36, 37]. The HCMV gB is considered as the major target for neutralizing antibodies [38]. So, the low rate of pp65 reactivity may be due to attenuation of interferon response. Thus, the role that pp65 has in immune evasion in HCMV infections is to prevent infected cells from being destroyed by the immune system [39].

Regarding the specific HCMV-IgM in relation to their specific reactivity to numbers of antigens, the highest rate was (28.23%) of HCMV-IgM for three antigens at the same time during the HCMV infection in pregnant women with HCMV-IgM seropositive. This result may be due to the different rates of HCMV-IgM with the various HCMV antigens; as well as the stimulation of immune response to the HCMV which depends on the process of HCMV exposure and expression of its antigens to the human immune system and the strategy of HCMV replication cycle.

## 5. Conclusion:

The highest rate of HCMV-IgM in pregnant women was for gB1 and p150 antigens; while the highest rate of intensity reaction was (+++) and the highest rate of HCMV-IgM ability for reaction was for three antigens at the same time.

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