

The role of IFN-gamma in Humoral immune response for HCMV antigens among pregnant women

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ABSTRACT

Background: Human cytomegalovirus (HCMV) is a highly host-specific virus belongs to the β -herpesvirus subfamily, which is a leading cause of congenital infections. The immune cytokines such as Interferons-gama (IFN- γ) may have direct inhibitory effects on HCMV replication and control of viral infections.

Aim of the study: This research aims at determining the specificity of anti-HCMV antibodies for different HCMV antigens in relation to serum INF- γ levels.

Materials & Methods: A cross sectional study was carried out in Kirkuk governorate from April 2018 to June 2019. The number of pregnant women understudy was **400** women presented to some private medical laboratories. The pregnant women were examined for the seroprevalence of HCMV-IgM and IgG by using ECLIA technique then their specificities determined for different HCMV antigens by using line immune assay, In addition to estimation the level of serum INF- γ levels by using ELISA technique.

Results: The rate of HCMV-IgG , HCMV-IgM and both HCMV-IgG and IgM at the same time were 288(72 %), 32(8%) and 18(4.5%) respectively. Regarding the specificity of the determined HCMV-IgM to various **HCMV antigens (IE1, CM2, p150, p65, gB1 and gB2)**, the highest rate of HCMV-IgG was **96.25%** specific for gB1 antigen, while highest rate of HCMV-IgM were **96.87%** specific gB1 and p150 antigen. Considering the specificity of these antibodies for the examined antigens in relation to serum INF- γ levels, the highest of pregnant women with increased INF- γ level had antibodies for IE1 antigen.

Conclusions: There was significant relation of the serum INF- γ level with specificity of anti-HCMV antibodies to divers HCMV antigens.

Keywords: HCMV; INF- γ ; CM2; IE1; gB

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دور الانترفيرون - كما في الاستجابة المناعية الخلطية لمختلف المستضدات

الخاصة بفيروس المضخم للخلايا البشري بين النساء الحوامل

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المستخلص

الفيروس المضخم للخلايا البشرية هي من الفيروسات ذات الخصوصية العالية للمضيف والتي هي من العائلة الفرعية بيتا التابعة لعائلة فايروسات الهيربس وتسبب إصابات ولادية. السايوتوكينات المناعية منها الانترفيرون- كما ربما لها دور مباشر في تثبيط تكاثر الفيروس والسيطرة على الإصابة الفيروسية. يهدف البحث الحالي الى تحديد خصوصية الأجسام المضادة لمختلف مستضدات الفيروس المضخم للخلايا البشري وعلاقتها بمستوى الانترفيرون- كما بين النساء الحوامل.

أجريت دراسة عرضية في محافظة كركوك في الفترة من نيسان 2018 ولغاية حزيران 2019 على 400 امرأة حامل راجعن عدد من المختبرات الأهلية في كركوك لمعرفة نسبة انتشار الاجسام المضادة نوع (ام و جي) الخاصة بفيروس المضخم للخلايا البشري باستخدام تقنية التألق المناعي الكيمويميائي ومن ثم معرفة خصوصيتها لعدد من المستضدات الخاصة بالفيروس باستخدام اختبار المناعة الخطي. إضافة الى تقدير مستوى الانترفيرون- كما في المصل باستخدام تقنية الاليزا.

أظهرت الدراسة معدل الانتشار المصلي للأجسام المضادة نوع (ام) و (جي) والاثنين (ام و جي) معا في نفس الوقت هي 288(72%) ، 32 (8%) ، 18(4.5%) على التوالي. فيما يتعلق بخصوصية تلك الأجسام المضادة للمستضدات المختلفة (IE1 ، CM2 ، p150 ، p65 ، gB1 و gB2)؛ حيث أظهرت الدراسة ان اعلى النسب للأجسام المضادة نوع (جي) 96.25% لها خصوصية للمستضد gB1، بينما اعلى النسب للأجسام المضادة نوع (ام) 96.87% لها خصوصية للمستضد gB1 و p150. أما بالنسبة لعلاقة مستوى الانترفيرون- كما بخصوصية الاجسام المضادة لتلك المستضدات ؛ حيث كانت النسبة الأعلى من النساء اللواتي لديهن زيادة في مستوى الانترفيرون- كما كانت لديهن اجسام مضادة خاصة بالمستضد IE1 .

كما أظهرت الدراسة علاقة معنوية بمستوى الانترفيرون- كما في المصل بخصوصية الأجسام المضادة لمختلف مستضدات الفيروس المضخم للخلايا البشري.

الكلمات الدالة: HCMV ; INF- γ ; CM2 ; IE1 ; gB.

1. Introduction

Human-CMV is one of eight human herpesviruses belongs to the family of Herpesviridae, prototype of the subfamily of β -herpesvirinae, described as an important etiological agent of intrauterine infection in pregnant women which may lead to miscarriage, stillbirth and fetus developmental retardation . Virions of HCMV consist of the four major components that are characteristic for herpesvirus particles: the liner double-stranded DNA (dsDNA) genome that is packaged in an icosahedral capsid, the tegument, and the viral envelope[1-6].

The spectrum of humoral responses against HCMV includes: structural tegument proteins like (pp65 and pp150), envelope glycoproteins (predominantly gB) and non-structural proteins as(IE1 and CM2) [7,8] . HCMV expresses viral proteins to modulate the host immune responses at every step of its life cycle, which play a crucial role in viral pathogenesis [9]. Interferon gamma (IFN- γ) is a longest soluble cytokine that is the only member of the type II class of interferons. It is a critical for innate and adaptive immunity against viral infection and is an important activator of macrophages and inducer of MHC II molecule expression. IFN- γ is produced predominantly by NK and natural killer T (NKT) cells as part of the innate immune response, and by CD4 Th1 and CD8 cytotoxic T lymphocyte (CTL) effector T cells once antigen-specific immunity develops[10].

IFN- γ are part of the innate immune response to viral infections and confer potent antiviral effects. IFN- γ , binds to a cell-surface receptor, which is known as the type II IFN receptor. Upon viral infection, IFN- γ activates cellular signaling networks. Because of the vital role of in IFN- γ response, it is reasonable that a virus may encode a protein to modulate immunity response by IFN- γ -induced antiviral responses[9].

2. Methodology

A cross sectional study was carried out in Kirkuk governorate from April 2018 to June 2019. The number of pregnant women understudy was 400 women presented to some primary health care centers and some private medical laboratories. The pregnant women were examined for the seroprevalence of specific HCMV-IgG and HCMV -IgM antibodies, then their specificity determined for specific HCMV antigens. Five to Seven ml of blood was collected by vein puncture using 10 ml disposable syringe from each women enrolled in this study. Blood samples were placed into sterile test tubes, lefted for 30 minutes at 37 °C then

were centrifuged at 3000 rpm for 15 minutes then the clot removed and re-centrifuged the remain for 10 minutes twice time and the obtained sera aspirated and transferred into clean test tube. Label was fixed on each test tube which then stored in deep freeze at -20°C for late serological testing for detecting specific HCMV-IgG and HCMV -IgM by using Electro-chemo-luminescence (ECLIA) technique, then their specificity and reactivity determined for specific HCMV antigens (IE1, CM2, p150, p65, gB1 and gB2) by using line immune assay line immune (RecomLine; Mikrogen, GmbH, Germany) . In addition to estimation, the level of serum IFN- γ levels by using ELISA technique. A computerized statistically analysis was performed using Statistical Package for Science Services (SPSS) version 17, Inc.USA. The comparison was carried out using of Chi-square (X^2) and Probability value (P). The $P \leq 0.05$ was categorized as statistically significant (S), and less than 0.01 was considered as highly significant (H.S.) and greater than 0.05 was considered as non-significant(N.S.).

3. Results

A total 400 pregnant women were examined ,their ages ranged between (18-42 years old) , for detection of specific HCMV antibodies HCMV-IgG (+), HCMV-IgM (+) and both HCMV-IgG(+) and IgM(+) at the same time were 288(72%), 32(8%) and 18(4.5%) respectively as shows in Table 1

In the present study, form the total 288 pregnant women with HCMV-IgG (by ECLIA) were 80 pregnant investigated by using recomline technique for the determination their reactivity to various HCMV antigens (IE1, CM2, p150, p65, gB1 gB2) separately, the highest rate of HCMV-IgG among the total 80 examined HCMV-IgG (+) seropositive were 77 (96.25%) positive for gB1 antigen and with different rates for other used antigens as shows in Table 2 .

Regarding the specificity of the determined HCMV-IgM to various specific HCMV antigens, the rates of HCMV-IgM among the total 32 examined HCMV-IgM seropositive were 31(96.87%) specific for each p150 and gB1 antigens as shown in Table 3.

The rates of HCMV-IgG against HCMV-antigens among the total 21 pregnant women with both HCMV-IgM and IgG at the same time, the highest rate of HCMV-IgG were 17(94.44%) for gB1 antigen as shown in Table 4 .

Rates of HCMV-IgM against various HCMV antigens among the total 18 pregnant women with both HCMV- IgM and IgG at the same time by line immunoassay, the highest rate were 17(94.44%) for gB1 as shown in Table 5 .

Regarding the relation of serum IFN- γ level with specific anti-HCMV antibodies in pregnant women. The rates of increased serum IFN- γ level higher than decreased levels, so the highest rate (61.12%) of increased serum IFN- γ level was seen in pregnant women with both HCMV- IgM and IgG at the same time, while the highest rate of normal serum IFN- γ level were seen among pregnant women with HCMV- IgG as shown in Table 6.

Considering the relation between the specificity of anti-HCMV antibodies against various HCMV antigens and level of serum IFN- γ level among seropositive pregnant women, the highest rate of increased serum IFN- γ level was 36.36% seen in women with HCMV-IgG for IE1 antigenas it is shown in Table 7 .

Regarding the relation between the specificity of anti-HCMV antibodies against various HCMV antigens and level of serum IL- IFN- γ level among seropositive pregnant women, the highest rate of increased serum IL- IFN- γ level was 65.0% seen in women with HCMV-IgG for IE1 antigenas it is shown in Table 8.

Table 1: Seroprevalence of HCMV-IgG and HCMV-IgM among pregnant women by using ECLIA technique.

Antibodies state	Seroprevalence rate	
	No.	%
HCMV- IgM (-) / IgG (+)	288	72.0
HCMV- IgM (+) / IgG (-)	32	8.0
HCMV- IgM (+) / IgG (+)	18	4.5
HCMV- IgM (-) / IgG (-)	62	15.5
Total	400	100

Table 2: Rates of specific HCMV-IgM (-) / IgG (+) for various HCMV antigens by using line immunoassay.

HCMV antigens	HCMV-IgG of HCMV-IgG (+) /HCMV-IgM(-) (n=80)			
	Positive		Negative	
	No.	%	No.	%
IE1	44	55.00	36	45.00
CM2	34	42.50	46	57.50
p150	75	93.75	5	6.25
p65	42	52.50	38	47.50
gB 1	77	96.25	3	3.75
gB 2	56	70.00	24	30.00
X ² = 93.31 P = 0.00001 H.S.				

Table 3: Rates of specific HCMV-IgM (+) / IgG (-) for various HCMV antigens by using line immunoassay.

HCMV antigens	HCMV-IgM of HCMV-IgG (-) /HCMV- IgM (+) (n=32)			
	Positive		Negative	
	No.	%	No.	%
IE1	20	62.50	12	37.50
CM2	21	65.62	11	34.38
p150	31	96.87	1	3.13
p65	20	62.50	12	37.50
gB 1	31	96.87	1	3.13
gB 2	23	71.78	9	28.22
X ² = 23.9 P = 0.0002 H.S.				

Table 4 : Rates of specific HCMV- IgG in pregnant women with HCMV-IgM and IgG at the same time for various HCMV antigens by using line immunoassay.

HCMV antigens	HCMV-IgG of HCMV-IgG (+) /HCMV- IgM (+) (n=18)			
	Positive		Negative	
	No.	%	No.	%
IE1	9	50.00	9	50.00
CM2	7	38.88	11	61.12
p150	16	88.88	2	11.12
p65	10	55.56	8	44.44
gB 1	17	94.44	1	5.56
gB 2	12	66.67	6	33.33
X ² = 23.39 P = 0.0003 H.S.				

Table 5: Rates of specific HCMV- IgM in pregnant women with HCMV-IgM and IgG at the same time for various HCMV antigens by using line immunoassay.

HCMV antigens	HCMV-IgM of HCMV-IgG (+) /HCMV- IgM (+) (n=18)			
	Positive		Negative	
	No.	%	No.	%
IE1	9	50.00	9	50.00
CM2	7	38.88	11	61.12
p150	15	83.33	3	16.67
p65	9	50.00	9	50.00
gB 1	17	94.44	1	5.56
gB 2	11	61.12	7	38.88
X ² = 12.33 P = 0.03 Significant				

Table 6: Relation of HCMV- antibodies with serum IFN- γ Level.

HCMV antibodies	Serum IFN- γ Level							
	Normal		Increased		Decreased		Total	
	No.	%	No.	%	No.	%	No.	%
HCMV-IgM (-) / IgG (+)	52	65.00	25	31.25	3	3.75	80	100
HCMV-IgM (+) / IgG (-)	12	37.50	19	59.37	1	3.13	32	100
HCMV-IgM (+) / IgG (+)	7	38.88	11	61.12	0	0	18	100
X ² =10.80 P = 0.02 Significant								

Table 7: Correlation between serum IFN- γ level and specificity of HCMV-IgG to various HCMV antigens.

HCMV antigens	Serum IFN- γ level							
	Normal		Increased		Decreased		Total	
	No.	%	No.	%	No.	%	No.	%
IE1	26	59.09	16	36.36	2	4.55	44	100
CM2	21	61.76	11	32.35	2	5.89	34	100
p150	51	68.00	21	28.00	3	4.00	75	100
p65	26	61.90	14	33.37	2	4.77	42	100
gB 1	51	66.23	23	29.87	3	3.90	77	100
gB 2	35	62.56	18	32.14	3	5.35	56	100
X ² =2.76 P =0.036 Significant								

Table 8: Correlation between serum IFN- γ level and specificity of HCMV-IgM to various HCMV antigens.

HCMV antigens	Serum IFN- γ level							
	Normal		Increased		Decreased		Total	
	No.	%	No.	%	No.	%	No.	%
IE1	7	35.00	13	65.00	0	0	20	100
CM2	9	42.85	11	52.38	1	4.76	21	100
p150	11	35.48	19	61.29	1	3.22	31	100
p65	8	40.00	12	60.00	0	0	20	100
gB 1	11	34.48	19	61.29	1	3.22	31	100
gB 2	9	39.13	13	56.52	1	4.35	23	100
X ² =4.12 P =0.045 Significant								

4. Discussion

Human-CMV is the most common worldwide congenitally transmitted pathogen and is a major global contributor to long-term neurologic deficits, including deafness, microcephaly, neuro-developmental delay, as well as fetal loss and occasional infant mortality [5]. The present study revealed that the HCMV infection was relatively common among pregnant women, that the HCMV-IgM(+)/IgG(-) was found in 72.0% of pregnant women , while the HCMV-IgM(+)/IgG(-) and HCMV-IgM(+)/IgG(+) were 8.0% and 4.5% respectively among them as they are shown in .. Table 1.

There was wide ranges of rates of HCMV-IgG and HCMV-IgM in the present study and Most studies in the different places and countries recorded this variation rates, this may be attribute to many factors including the difference in the kinetics of anti-HCMV-IgG and anti-HCMV-IgM responses during the infection and the violation of systemic and local intercellular interrelations between B and T-cells, leads to the imbalance in the antibodies production [5,11].

The importance of antibody response for diagnostic purposes led to significant insights into the kinetics of the antibody response against human cytomegalovirus-specific proteins and thus a better understanding of the function of the humeral immune mechanisms. These explorations showed that the synthesis and catabolism of HCMV-specific antibodies is a highly dynamic and complex process, with major differences between primary and

secondary infections[12] . Therefore this study conducted to determine the specificity of these HCMV antibodies to various specific HCMV antigens, (IE1, CM2, p150, p65, gB1 and gB2) and revealed that the highest rates of HCMV antibodies were reactive with gB1 antigen which were 96.25% for HCMV-IgG and 96.87% for HCMV-IgM , while 94.44% for each of IgG and IgM of pregnant women with IgM and IgG at the same time with significant relation ($P < 0.05$)..Tables 2 and highly significant relation ($P < 0.01$) .. Tables 3-5. This finding may be due to the ability of HCMV gB1 antigen to trigger and stimulating the humeral immune response depending on its characters and structure.

The gB structure provides a starting point for elucidation of its antigenic and immunogenic properties. So the humeral immune response directed against gB is of particular interest [13]. The gB plays an important role in virus infectivity, cell to cell spread and is a major target for antibody mediated immunity and is the major antigen for the induction of neutralizing antibodies against HCMV. The gB considered to be a multifunctional envelope component responsible for virion entry, cell to cell spread, syncytium formation and is the major target for neutralizing antibodies[5,14].

The entry of HCMV into cells requires the conserved gB, thought to function as a fusogen and reported to bind signaling receptors. gB also elicits a strong immune response in humans and induces the production of neutralizing antibodies although most anti-gB Abs are non-neutralizing [13].

The second most common HCMV antigens have high reactivity rates with HCMV antibodies in the present study was p150 antigen which were; 93.75% ,96.87%,88.88% and 83.33% for HCMV-IgG, HCMV-IgM, HCMV-IgG of both seropositive and HCMV-IgM of both seropositive at the same time respectively with highly and significant relations ..Tables 2-5. The immunological reactivity for HCMV-encoded protein antigens is characterized by a high frequency of HCMV-specific CD4⁺ and CD8⁺ T lymphocytes and stable levels of antiviral antibodies. The predominant CD8⁺ T lymphocyte response following HCMV infection was initially proposed to be directed against a limited set of virus-encoded antigens and the dominant targets of HCMV-specific CD8⁺ T lymphocytes were pp150 and one of the most immune-reactive antigens with HCMV-IgG and HCMV-IgM [5]. Although T-cell reactivity against pp150 seems to form a substantial part of CMV-specific cytotoxic T

lymphocyte (CTL) response, with T-cell reactivity is directed towards pp150, unique short (US) proteins[12]. The viral tegument proteins including pp150 elicit powerful and long-lasting antibody responses [15].

On the other hand the rates of the reactivity of IE1, CM2, p65 and gB2 with HCMV antibodies are convergent and similar were ranged from 38.88% to 71.78%, but still these rates lower than gB1 and p150 antigens. Tables 2-5. This finding may be due to the correlation properties of these antigens, exposures to the human immune system, steps of HCMV replication cycle and contacts of these antigens to host tissues and cells especially IE1 and p65, that pp65 mediates the phosphorylation of viral immediate-early proteins, which blocks their presentation to the major histocompatibility complex class I (MHC I) molecules^(84,86). In addition to that not all parts or sequences of CM2 antigen trigger or stimulate the immune system and mainly humoral immune response. It is known that the central part of pUL57 is a major reactive protein during acute HCMV infection [16,17].

The present study revealed that the levels of serum IFN- γ were increased among the most HCMV seropositive pregnant women with significant relation ($P < 0.05$). Tables 6, this finding may be due to the immune response act to overcome the HCMV infection and its mortality to mother and the fetus. Regarding the relation of serum IFN- γ levels with the specificity of anti-HCMV antibodies to various HCMV antigens, the present study revealed that the increased levels of serum IFN- γ varies with different HCMV antigens so, the highest rate among seropositive for IE1 antigen with significant relation ($P < 0.05$). Tables 7-8.

This protection is likely through humoral and cell-mediated immune responses with secretion of high IFN- γ . Increase level of IFN- γ is prerequisite for a Th1-dependent protective immune response. There is a significant correlation between IFN- γ level and degree of protection conferred, suggesting that IFN- γ -dependent Th1-predominant immunity is critical for protection against HCMV infection [18-20]. Many studies described the induction of protective Th1-cell mediated immunity against HCMV infection, that the cellular immunity is mediated by both CD4⁺ and CD8⁺ cells through cytokine secretion especially IFN- γ [10] so, the non-structural IE-1 protein expressed in the earliest stage of the HCMV replication cycle in infected cells and known to be major targets for T cells [21].

5. Conclusion

The HCMV- antibodies were varies in the specificity for different HCMV antigens. There was significant relation of HCMV antibodies with various HCMV antigens. The highest rate of HCMV-IgG and HCMV-IgM were specific for gB1 and p150 antigens. There was significant relation of the serum IFN- γ level with anti-HCMV antibodies and specificities to various HCMV antigens.

Notes

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