Relation of Human Cytomegalovirus Antibodies with Some Serum Proteins Levels among Pregnant Women in Kirkuk Province/ Iraq

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Abstract:

Human cytomegalovirus (HCMV) is a beta-herpesvirus that causes lifelong infection in humans especially during pregnancy can cause of congenital abnormalities. The process of viral entry and replication mainly depending on the interactions between virus proteins and host cell proteins.

Objectives: The present study aimed to detect the relation of HCMV antibodies with some serum proteins levels among pregnant women in Kirkuk province.

Methods: The current study was done in Kirkuk city from 15 January to 15 July 2017. It was included a total of 260 pregnant and 110 non-pregnant women (control group) aged (18-24 years old); who showed up at some private main laboratories in Kirkuk City (Mainly at Saya Main Lab. Which is Located at Dibis district). They were examined for the presence of HCMV antibodies (HCMV-IgG and HCMV-IgM) by using of Enzyme Linked Immunosorbent Assay (ELISA) technique, and they were also examined for the total serum protein, serum albumin, and serum globulin levels using spectrophotometry technique.

Results: The present study revealed that IgG antibodies rate (71.53 %) was the highest among the seropositive antibodies in pregnant women. The highest rate (47.05 %) of decreased level of serum albumin was found in those pregnant women who showed seropositivity for both (IgM and IgG) to HCMV at that same time. The study also showed non-significant relation between seropositive HCMV antibodies and total serum protein and globulin levels in pregnant women.

Conclusions: High rate of pregnant women infected by Human Cytomegalovirus in Kirkuk province. The study also concluded that the HCMV infection may affect the rate of the serum albumin levels in pregnant women.

Keywords: Human Cytomegalovirus, total Serum Protein, globulin, HCMV-IgM, IgG, Pregnant Women.

Introduction:

The Human Cytomegalovirus (HCMV) is one of the eight human Herpesviruses. Herpesviruses are viruses widely distributed in the animal kingdom, can cause serious illness in neonates as well as in adults such as cold sores (Herpes Simplex Virus type 1, HSV-1), sexually transmitted diseases (HSV-2) and tumors (Epstein-Barr Virus, EBV, and Kaposi's Sarcoma associated Herpesvirus, KSHV)[1,2].

The HCMV is capable of producing a permissive or latent infection in the human host. The HCMV is the most common pathogen in uterus during pregnancy, which may lead to some serious results such as miscarriage,

stillbirth, and cerebellar malformation [3,4].

Human is the only known reservoir. Cytomegaloviruses found in many animal species are not infectious to humans. Infection occurs throughout the world[5]. The measurement of seropositive antibodies to specific HCMV proteins is a beneficial way for the evaluation of the host immune responses to infection and the production of vaccines to HCMV [6].

Many studies showed that the concentration of single type of protein or several types changed greatly during pregnancy. Most of these changes occur as a result of increasing in hormonal activities. During pregnancy both progesterone and estrogen are required for maintenance and initiation of pregnancy. Changes in metabolic, structure, & endocrine functions of the mother will be happened during pregnancy [7,8].

Some conducted studies showed that the seroprevalence rate of seropositive HCMV antibodies was 88% in Jordan and 77.8% in Babylon, Iraq among pregnant women. While in Jordan the seroprevalence rate of seropositive HCMV antibodies was 95%, in comparing to 4.8% in Baghdad, Iraq, among women who showed bad obstetric history [9].

The aim of present study was to detect the relation of Human Cytomegalovirus antibodies with some serum total proteins, albumin and globulin levels among pregnant women in Kirkuk province/Iraq.

Materials and Methods:

A cross sectional study was conducted in Kirkuk city from 15 January to 15 July 2017. It was included a total of 260 pregnant women their ages were (18-24 years old) and 110 non-pregnant women (control group) aged (18-24 years old); who showed up at some private main laboratories in Kirkuk city (Mainly at Saya main lab. which is located at Dibis district/Kirkuk).

Patients:

A peripheral venous blood (10 ml) was withdrawn from 260 pregnant women aged (18-24 years old) and 110 non-pregnant women (control group) aged (18-24 years old) who attended at some private main laboratories in Kirkuk city (Mainly at Saya main lab). Both the pregnant women and control group were tested for having seropositive antibodies (Both IgG, IgM) to HCMV through utilizing of ELISA technique, and they were also examined for total serum protein, albumin, and globulin concentration using a colorimetric method. Both the pregnant women and non-pregnant women were unknown to have HCMV infections during the time of blood withdrawal.

Procedures:

Centrifugation of clotted blood was done to get a clear serum for detection of the presence of antibodies (IgG, IgM) to HCMV through using of ELISA technique for Human Cytomegalovirus HCMV–IgM and HCMV–IgG (From BioCheck, Inc 323 Vintage Park Dr.Foster City, CA 94404). Which was done through following the instructions of the manufacturer strictly. Diluted patient serum was added to the wells where the purified

HCMV antigen was coated on the surface of microwells. Both CMV-IgM, and CMV-IgG antibodies, if found, were conjugated to the antigen. All unconjugated materials were washed away. The conjugate reagent (Horse Radish Peroxidase=HRP-conjugate) was added. A complex of (antibody-antigen-conjugate) formed. Excessive HRP-Conjugate particles was washed off and a substrate reagent (Tetramethyl Benzidine =TMB) added to the wells. The enzymatic reaction was stopped at a specific time. The intensity of the color generated was proportional to the amount of CMV-IgM or CMV-IgG-antibodies in the sample. The results were interpreted on the basis of seropositive or seronegative samples. The remains of serum used for each patient to detect the concentration of total serum protein and serum albumin (From Biolabo SA, 02160, Maizy, France. For Invtro diagnostic use) through using spectrophotometric method.

Statistical Analysis:

Computerized statistically analysis was performed using SPSS (Statistical Package for Science Services). Comparison carried out using; Chi-square (X^2) and Probability (P value). The P value $\leq$ 0.05 was considered statistically significant (S), and less than 0.01 considered highly significant (HS), while greater than 0.05 considered non-significant results.

Results:

Seroprevalence of HCMV antibodies in pregnant women and control group using ELISA technique.

The highest rate (71.53 %) of seropositive HCMV-IgG antibodies was found in pregnant women, and the result was highly significant as shown in table 1.

Relation of seropositive HCMV antibodies with total serum protein level.

The highest rate (35.29 %) of increased total serum protein was found in pregnant women who had both HCMV-IgM and IgG antibodies in their blood at the same time, and the result was non-significant $P > 0.05$ as shown in table 2.

Relation of seropositive HCMV antibodies with serum albumin level.

The highest rate (47.05 %) of decreased serum albumin level was seen in pregnant women who showed seropositivity for both HCMV-IgG, and IgM and the result was highly significant $P < 0.01$ as shown in table 3.

Relation of seropositive HCMV antibodies with serum globulins level.

The highest rate (41.18%) of increased serum globulins level was detected in pregnant women who showed seropositivity for both HCMV-IgG and IgG at the same time, and the result was non-significant $P > 0.05$ as shown in table 4.

Relation of seropositive HCMV antibodies with serum albumin to globulins (A/G) ratio.

The highest rate 47.05 % of decreased serum albumin to globulins ratio was seen in seropositive pregnant women who have had both IgM and IgG antibodies to HCMV at the same time, and the result was non-significant P > 0.05 as shown in table 5.

The biochemical parameters levels in control group (seronegative pregnant women).

The result was non-significant P > 0.05 as shown in table 6 in pregnant women who showed no sero-reactivity to HCMV.

Table (1): Seroprevalence of HCMV Antibodies in Pregnant Women and Control Group by ELISA .

HCMV-Antibodies Type	Pregnant		Control	
	No.	%	No.	%
IgM (-) and IgG (+)	186	71.53	36	32.72
IgM(+) and IgG (-)	24	9.25	4	3.63
IgM(+) and IgG (+)	17	6.53	3	2.75
IgM (-) and IgG (-)	33	12.69	67	60.90
Total	260	100	110	100
X^2 =91.17 P = 0.0001 P < 0.01 Highly Significant				

Table 2: Relation of Seropositive HCMV Antibodies with total serum protein level.

Total serum protein level	Seropositive of HCMV antibodies					
	HCMV- IgG		HCMV- IgM		HCMV(IgG & IgM)	
	No.	%	No.	%	No.	%
Normal *	138	74.19	16	66.66	10	58.83
Increased**	48	25.81	8	33.34	6	35.29
Decreased***	0	0	0	0	1	5.88
Total	186	100	24	100	17	100
X^2 = 7.84 P = 0.16 P > 0.05 Non Significant						

* Normal Range = 6.0 –8.0 g/dl

** Increased = More than 8.0 g/dl

*** Decreased = Less than 6.0 g/dl

Table (3): Relation of Seropositive HCMV Antibodies with serum Albumin level.

Serum Albumin level	Seropositive of HCMV antibodies					
	HCMV- IgG		HCMV- IgM		HCMV(IgG & IgM)	
	No.	%	No.	%	No.	%
Normal *	158	84.94	15	62.5	9	52.95
Increased**	0	0	0	0	0	0
Decreased***	28	15.06	9	37.5	8	47.05
Total	186	100	24	100	17	100
$X^2 = 19.6$ P = 0.00001 P < 0.01 Highly Significant						

* Normal Range = 3.5 –5.0 g/dl
** Increased = More than 5.0 g/dl
*** Decreased = Less than 3.5 g/dl

Table (4): Relation of Seropositive HCMV Antibodies with serum Globulins level.

Serum globuline level	Seropositive of HCMV antibodies					
	HCMV- IgG		HCMV- IgM		HCMV(IgG & IgM)	
	No.	%	No.	%	No.	%
Normal *	124	73.80	16	66.66	11	58.82
Increased**	62	26.20	8	33.34	6	41.18
Decreased***	0	0	0	0	0	0
Total	186	100	24	100	17	100
$X^2 = 5.81$ P = 0.268 P > 0.05 Non Significant						

* Normal Range = 2.5 –3.5 g/dl
** Increased = More than 3.5 g/dl
*** Decreased = Less than 2.5 g/dl

Table (5): Relation of Seropositive HCMV Antibodies with serum Albumin to Globulins (A/G) ratio.

Serum (A/G) ratio	Seropositive of HCMV antibodies					
	HCMV- IgG		HCMV- IgM		HCMV(IgG & IgM)	
	No.	%	No.	%	No.	%
Normal *	121	65.05	13	54.16	9	52.95
Increased**	0	0	0	0	0	0
Decreased***	65	34.95	11	45.84	8	47.05
Total	186	100	24	100	17	100
$X^2 = 7.55$ P =0.109 P > 0.05 Non Significant						

* Normal Range = 1.2:1 –1.5:1
** Increased = More than 1.5:1
*** Decreased = Less than 1.2:1

Table 6: The Biochemical parameters levels in Control Group (Seronegative pregnant women).

Results	Biochemical parameters							
	Total serum protein		Serum albumins		Serum globulins		A/G ratio	
	No.	%	No.	%	No.	%	No.	%
Normal *	31	93.93	30	90.90	31	93.94	30	90.90
Increased**	2	6.07	2	6.07	1	3.03	2	6.07
Decreased***	0	0	1	3.03	1	3.03	1	3.03
Total	33	100	33	100	33	100	33	100
X^2 =0.453 P = 0.998 P >0.5 Non significant								

Discussion:

Cytomegalovirus is present worldwide, mostly found in sexually active women ranging 45% to more than 90%. The seroprevalence of Cytomegalovirus varies and tends to be lower in developed countries (ex; In Australia about 60-70%, Western Europe about 40-70%, and United States of America 50-60%, while it shows to be higher in developing countries (In India and Turkey more than 90%, and in South Africa ranged from 80 to 90%[11,12].

The current study revealed the frequency of anti-HCMV-IgG antibodies was 71.53% and 32.72 % among pregnant and non-pregnant women respectively as shown in table 1. The result was highly significant for the frequency of seroprevalence of HCMV antibodies in pregnant women and control group in which the probability was P< 0.01. This was closed to the results of some studies conducted in Iran (69.6% IgG), in Poland (76.7% IgG), while it was lower than what was recorded in Babylon, Iraq (77.8%) [12], in Turkey (97.3% IgG) [13], in Malaysia (84% IgG) [14], In Nigeria (97.2% IgG)[15], (97.8% IgG) [16], in Korea (98.1% IgG) [17], these different results recorded may be due to the differences in the sensitivity and specificity of the ELISA kits used in the other studies for detection of HCMV-IgG antibodies in comparing with the present study, in which the sensitivity was 95.0% and the specificity was 96.7% respectively[18]. Also the technique, steps, reagent numbers and device of the used tests may have a great role for obtaining different results. Noticeably the result was higher than what was recorded in Germany (42.3% IgG) [19] and in France (46.3% IgG) [20]. These different results may be due to differences in hygienic, socioeconomic, and cultural factors, also most of these countries considered developed countries. Studies from different countries showed that the prevalence of antibody to HCMV among women of childbearing age in developing countries and in populations with low socioeconomic status is generally

higher than that in developed countries [21].

The present study also detected the seroprevalence of HCMV-IgM antibodies among pregnant and non-pregnant women was 9.25 % and 3.63% respectively. This was close to the results of a study conducted in Kirkuk city in 2013 by Aljumaili *et al*[22] in which the seroprevalence of HCMV-IgM antibodies among pregnant women was 8.3%, but it was higher than what was recorded in Hodeidah city, Yemen in which the seroprevalence of HCMV-IgM among pregnant women was 1.8%, This is may be attributed to the fact that different technique used in this study instead of ELISA technique, which was Electro-Chemiluminescence Immunoassay (ECLIA) for screening of HCMV antibodies, in which their sensitivity and specificity different from ELISA technique[23]. This was also higher than what was recorded in Iran (2.5% IgM) [24], in Taiwan 3.5 % IgM [25], in Bangladesh 5 % IgM[26].

The variation of results may be attributed to the kinetics of anti-HCMV IgM responses during primary infection which may vary greatly among individuals depending substantially on the test or commercial kit specially ELISA, also this variation may be due to variability of viral accessibility and its circulation rate in the community.

The current study showed non-significant relation of the seropositive HCMV antibodies with total serum protein levels as shown in table 2, this was also true for the relation of seropositive HCMV antibodies and serum globulin levels as shown in table 4. These results may be due to the physiological and hormonal changes occur normally during pregnancy; furthermore many studies investigated a single protein or several proteins have revealed that concentrations during pregnancy varies significantly from expected average values observed in non-pregnant female [7], plus there was no study of similarity has been published among pregnant women in Iraq to compare the results of the present study with.

The present study revealed highly significant results regarding the relation of serum albumin levels with the seropositive HCMV antibodies, in which the highest rate (47.05%) of decreased serum albumin levels found in patient who showed reactivation of HCMV infection as shown in table 3, this was agreed with Gautam B. *et al*; who detected low serum albumin level was associated with CMV reactivation in patients [27]. Human Cytomegalovirus may affect the hormonal activities in pregnant women specially estrogen and progesterone hormones; which have impact on serum albumin level during pregnancy leading to lower their levels [28].

Although the highest rate (47.05%) of decreased albumin to globulin ratio was found in pregnant women who showed seropositivity for both HCMV-IgM, and IgG antibodies, but the result was non-significant as shown in table 5. This was may be associated to the normal physiological changes happening during pregnancy, or may related to

low protein intake, this was may be true because most of the patients were from poor neighborhoods, meaning it was hard for them to get protein-based meals mostly. Furthermore hormonal changes related to pregnancy may also stimulate reactivation of CMV, since viral secretion in urine and cervical–vaginal fluids increases during pregnancy with increasing gestational age [29].

The present study showed no significant relation of seronegative pregnant women with biochemical parameters as shown in table 6, this was probably happened because this study was included seronegative pregnant women who did not affected by HCMV, thus their albumin, globulin, and total serum protein levels didn't changed that much, this was a good evidence on the influence of HCMV on serum protein levels in seropositive pregnant women in comparing with seronegative pregnant women. From this point we may say the HCMV infection affect the serum protein levels in pregnant women.

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