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The Correlation between CMV Infection and Hypertension in Iraqi Patients

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Abstract:
In order to study the correlation between CMV infection and increase in blood pressure among Iraqi patients, 201 blood samples were collected including; 88 male, 113 female hypertensive patients and 40 control samples. Patients sera were grouped into patients who are under medication (T), and patients who do not take any treatment for hypertension(NT). Samples were tested for CMV-IgM/IgG by ELISA, renin enzyme and interleukin-6(IL-6) levels were determined by ELISA too. The optical density readings difference for CMV-IgG were significant between (T) and (NT) groups as well as when each group compared to control, level of renin enzyme showed significant difference between patients group(T and NT) and between each group compared to the control, (135 ± 98), (201 ± 102) and (130 ± 90) respectively, while IL-6 level was not significant either between the groups nor within the group. Furthermore, there was a positive correlation between the optical density readings of (NT) group for CMV-IgG and renin enzyme level with the systolic and diastolic blood pressure with r-value ranged (0.4-0.6). This study may high light on the role of previous infection or re-infection by CMV and increase in blood pressure.

Keywords: Cytomegalovirus (CMV), Hypertension, Renin-enzyme, renin–angiotensin-system (RAS), angiotensin-II.

العلاقة بين الاصابة الفيروسية لفيروس المضخم للخلايا وارتفاع ضغط الدم عند المرضى العراقيين

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الخلاصة:
لهدف دراسة العلاقة بين الاصابة بفيروس المضخم للخلايا(CMV) وضغط الدم عند المرضى العراقيين تم جمع(201) عينة دم وكان عدد الذكور(88) وعدد الاناث (113) من المرضى المصابين بارتفاع ضغط الدم و(40) عينة سيطرة. وقد قسمت مصول المرضى الى مجموعتين:مجموعة المرضى الخاضعين للعلاج(T) ومجموعة المرضى غير الخاضعين للعلاج(NT), وتم اختبار العينات والتحري عن وجود فيروس(CMV) للاجسام المضادة IgG, IgM من خلال اختبار (ELISA) وكذلك تم قياس مستوى كل من انزيم الرنين وانترلوكين (IL-6) بواسطة (ELISA) ايضا, وقد اعطت قراءات الكثافة الضوئية (optical density) فرقا معنويا بين المجموعتين (T) و(NT) مقارنة مع مجموعة السيطرة بالنسبة CMV-IgG حيث كانت القراءات كالاتي: (98±135), (102±201) , (90± 130) على التوالي, اما بالنسبة للانترلوكين (IL-6) كانت القراءات غير معنوية. بالاضافة الى ذلك تم ايجاد علاقة موجبة بين قراءات الكثافة الضوئية لمجموعة(NT) بالنسبة CMV-IgG وانزيم الرنين مع كل من الضغط الانقباضي والانبساطي بقيمة r بين (0.4-0.6). هذه

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الدراسة سلطت الضوء على تأثير الاصابة المسبقة او تكرار الاصابة بالفيروس المضخم للخلايا(CMV) واحتمالية علاقته بارتفاع ضغط الدم.

Introduction:

Cytomegalovirus (CMV) is an enveloped DNA virus that is like other members of the herpesvirus family, establishes lifelong latency following primary infection. Subsequent viral reactivation or re-infection with a different CMV strain sometimes occurs, and is referred to as recurrent infection.
CMV spreads through contact with infected body fluids, such as urine or saliva. Infection among pregnant women most frequently occurs through close contact with young children or through sexual transmission. CMV infection tends to cause few symptoms in immunocompetent individuals, but can cause serious diseases among HIV-infected persons, organ transplant recipients on immunosuppressive therapy, and fetuses. Among congenitally infected infants, CMV can cause permanent disabilities such as hearing loss, vision loss, and mental retardation [1]. HCMV is also responsible for approximately 8% of infectious mononucleosis cases and is the main viral cause of birth defects often causing deafness and mental retardation in the fetus, if a woman is infected during pregnancy[2]. Cytomegalovirus (CMV) has been implicated in playing a role in inflammatory and proliferative diseases, including certain cardiovascular diseases and cancer. Epidemiological and pathological studies have espoused a strong link between CMV and atherosclerosis. Several mechanisms have been proposed in which CMV could influence the development of atherssclerotic vascular abnormalities[3].Hypertension Is a major risk factor for ischaemic and haemorrhagic stroke, myocardial infarction, heart failure, chronic kidney disease, cognitive decline and premature death. Untreated hypertension is usually associated with a progressive rise in blood pressure.

In any individual person, systolic and/or diastolic blood pressures may be elevated. Diastolic pressure is more commonly elevated in people younger than 50. With ageing, systolic hypertension becomes a more significant problem, as a result of progressive stiffening and loss of compliance of larger arteries. At least one quarter of adults (and more than half of those older than 60) have high blood pressure [4].

It was indicated that CMV infection alone leads to an increase in blood pressure, whereas CMV acts as a co-factor, along with high cholesterol diet to induce atherosclerosis in the mouse aorta. A persistent CMV infection of endothelial cells and an increased pro-inflammatory cytokine expression, including renin and angiotensin II, may underlie the molecular mechanism by which CMV infection induce an increase of blood pressure [5]. Viruses have the ability to turn on human genes, and in this case, the CMV virus is enhancing expression of renin, an enzyme directly involved in causing high blood pressure. When the scientists inactivated the virus using ultraviolet light, renin expression did not increase, suggesting that the actively replicating virus may cause the increase in renin. Increased expression of both renin and angiotensin II are important factors in hypertension in humans. A persistent viral infection in the vessels and endothelial cells is leading to increased expression of inflammatory cytokines, rennin, and angiotensin II, which are leading to increased blood pressure [6].

This research was planned to study the correlation between CMV infection and increase in blood pressure among hypertension Iraqi patients by testing for CMV immunoglobulins IgM, IgG by ELISA and measuring renin and IL-6 levels in patients groups.

Materials and methods:

Total number of blood samples was (201); (88) males, (113) females from hypertensive patients and (40) controls. The patients' specimens were classified into (3)groups according to patients' treatment: treated (T), non-treated (NT) and control groups(C), samples were collected for the period from October/2013 till February/2014 from Ibn-Al nafess hospital in Baghdad. Patients' sera were separated immediately after clot formation by centrifugation at 1000 rpm for 15 minutes, and stored at (-18 to -20°C) until analyzed.

Sera were tested to detect the presence of HCMV (IgG and IgM) by using ELISA kits which were supplied from (Human Wiesbaden/ Germany, lot No. 51203, 51103 respectively). The principle of the assay based on the direct ELISA. The assay procedure was carried out according to the descrition of the manufacture [7].Sera were tested for the concentration of Renin enzyme by ELISA kit supplied from (DEMEDITEC Diagnostics GmbH/Germany). The DEMEDITEC Renin ELISA is an enzyme immunoassay for the quantitative in *vitro* diagnostic measurement of active Renin in human serum and plasma based on the sandwich principle [8]. The assay procedure was carried out ac-

cording to the description of the manufacture. (standard curve of renin enzyme for determination the concentration of enzyme in humans' sera was determined, figure1.
Sera were tested to determine the concentration of Interleukin-6 by ELISA kit supplied from CUSABIO BIOTECH CO., LTD. This assay employs the quantitative sandwich enzyme immunoassay technique. The assay procedure was carried out according to the description of the manufacture.(standard curve of interleukin-6 for determination the concentration of IL-6 humans' sera was determined, figure 2. The ELISA microtiter plate reader was used to measure the optical density at 450 nm for all the above kits. Blood pressure of patients was measured manually by sphygmomanometer.
Statistical analysis of measured trait were done by using one-way analysis of variance (ANOVA) (IBM SPSS, version 2) when $p<0.05$. [9].

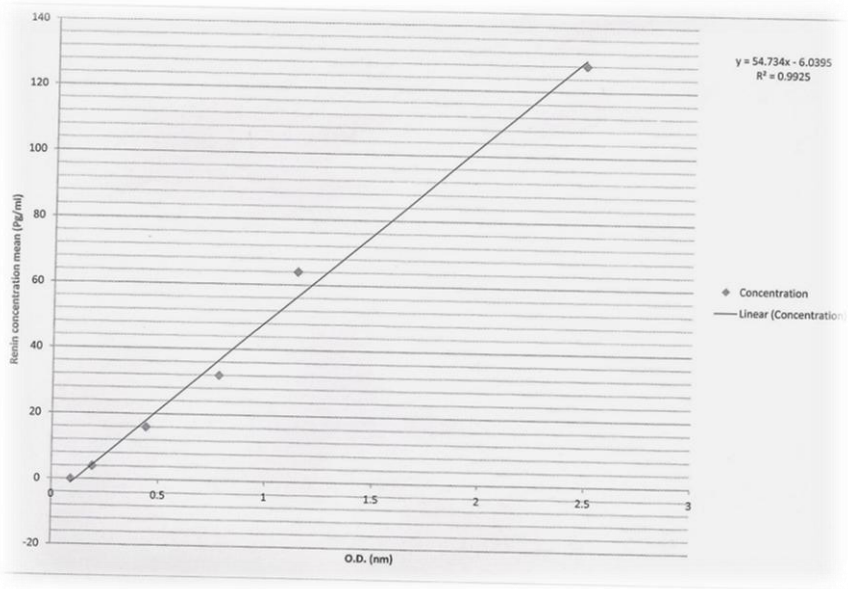

Figure 1-Standard curve of renin enzyme for determination the concentration of enzyme in humans' sera

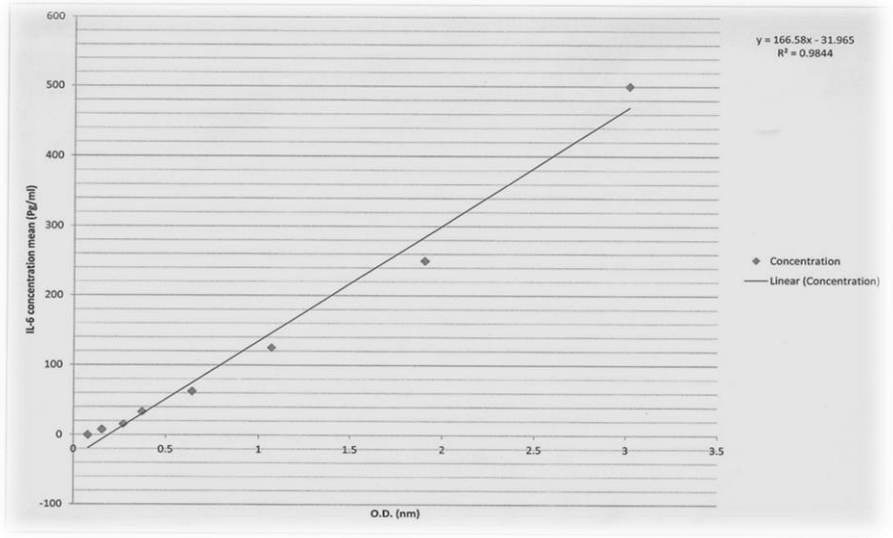

Figure 2-Standard curve of interleukin-6 for determination the concentration of IL-6 in humans' sera

Results and discussion:

Recently, hypertension has truly became a problem which we still face. In Iraqi patients, most of cases were detected during research duration with chronic hypertension belong to genetic factors, kidney diseases and heart diseases (secondary hypertension) and the rest of cases were detected with hypertension belong to unknown another factors (primary hypertension), this research focused on this

type of hypertension and the probability linked to the infection with CMV virus, because this virus may have a direct effect on the renin enzyme one of the possible causative agent of hypertension. Lie and his colleges in 2011 demonstrated a novel link between HCMV infection and essential hypertension by using microarray-based miRNA expression profiling, they compared the miRNA expression in plasma samples from hypertensive patients and healthy control subjects who found that increased HCMV seropositivity and high titers were found in hypertensive group compared with the control group (52.7% versus 30.9%)[10].

Table 1 describes the percentage of CMV-IgM and IgG among patients groups male, female and control, a high percentage of CMV-IgG in female compared to male 75%, 67% respectively, this may be because that female have more probability to be infected by CMV during pregnancy[2].

Table 1-Percentage of CMV – IgM , IgG among patients groups.

	Patients group		**Control group**		**p-value**
	Male	**Female**	**Male**	**Female**	
No. of sample	**88**	**113**	**20**	**20**	---------
Total No.	**201**		**40**		---------
+ ve IgG	67%	75%	50%		Non-Significant
+ ve IgM	30%	47%	Zero		Significant

Furthermore, in this table there is a significant difference between CMV-IgM infection among hypertensive males and females compared to the controls, which indicates a recent infection with this virus, which is normal to be higher in female, and possibility of re-infection cannot be excluded.

It is know that CMV is a common infectious virus, therefore most population show a positive CMV-IgG in there sera which indicates a previous infection. Haarala and his college showed by using a commercial enzyme immunoassay the seropositive in normal women (72.3%) more than the seropositive in normal men (65.9%) this may be due to many reasons like the women are more susceptible than men to be infect with CMV especially during the pregnancy[11].

In the other hand table 2 shows the optical density readings for CMV-IgM and IgG in patients groups compared to the controls, in which CMV-IgG gave a significant difference among patients groups treated (T) group and non-treated(NT) group(0.9±0.03 , 0.5±0.01) respectively compared to control (0.1±0.02), while results of CMV-IgM was not significant among patients groups T and NT (0.3±0.02 , 0.4±0,07) respectively compared to control (0.3±0.01).

Table 2-Optical density readings for CMV – IgM and IgG in patients groups compared to the controls .(the optical density was used instead the titer this belong to the manufacture kit because it was qualitative analysis not quantitative).

Group	**No. of samples**	**IgM**		**IgG**	
		Mean	**S.D**	**Mean**	**S.D**
Treated(T)	172	0.3	0.02	0.9	0.03
Non-treated(NT)	29	0.4	0.07	0.5	0.01
Control	40	0.3	0.01	0.1	0.02
P – value		Non-Significant		Significant	

These results agree with other studies carried out worldwide, Li,C. and his colleges used ELISA and immunofluorescence assay to test for HCMV-IgG among the 6303 participants aged 16-49 years ,

3975 had HCMV infection Of the participants, 54.7% had serologic evidence of HCMV infection and 17.5% had hypertension[12].

Renin is a circulating enzyme with one known substrate angiotensinogen, is a key regulator of the renin-angiotensin aldosterone system(RAAS) and is released from juxtaglomerular cells in kidneys, in response to fall in blood pressure level due to dietary sodium restriction. It establishes a short-term defense mechanism against hypovolemic hypotension[13].

Furthermore, to study the level of renin enzyme and interleukin-6 (IL-6 is a cytokine known to be part of the acute phase response, CMV is known to increase IL-6 gene expression and production in peripheral blood mononuclear cells[14]) in the samples sandwich ELISA test was performed and it is clear in table 3 there is a significant difference between renin enzyme and hypertensive in patients groups T and NT (135±98, 201±102) respectively.

Table 3-Level of Renin enzyme and IL-6 in patients group compared to the control.

Group	No. of samples	Renin		IL-6	
		Mean(pg/ml)	S.D	Mean(pg/ml)	S.D
Treated	172	135	98	190	17
Non-treated	29	201	102	246	43
Control	40	130	90	48	3
P – value		Significant		Non-Significant	

Haarala and his college showed it is possible that high CMV antibody titres are indicators of frequent reactivation of CMV or re-infection with new strains of CMV, leading to stronger immunity in these individuals. CMV activity may lead to an increased renin–angiotensin system (RAS) activation, leading to arterial constriction via the influence of angiotensin-II, a mechanism that may explain the observed increased blood pressure values, which used commercial enzyme immunoassay to detect CMV-IgG antibody titer [11]. Prescott in 2013 showed that CMV led to an increase in expression of the renin enzyme, which has been known to activate the renin-angiotensin system and lead to high blood pressure by studying four groups of laboratory mice to find the link between CMV and hypertension, Prescott found that CMV infection alone led to increase in high blood pressure in mice[6].

Furthermore, table 3 shows a high level of renin enzyme among non-treated group (201±102) compared to treated group (135±98) this may due to the hypertension drugs effect, because many of hypertension drugs have a direct effect on RAS and renin enzyme (like captopril groups) which decrease the level of renin enzyme[15].

The correlation between systolic and diastolic blood pressure with the optical density readings of CMV-IgG was studied in table 4, it is clear that there is a significant positive correlation between CMV-IgG optical density readings and blood pressure among non-treated group, r-value (0.4) for systolic and (0.5) for diastolic blood pressure.

Table 4-The correlation between the optical density readings for CMV – IgG and systolic / diastolic blood pressure among NT, T patients.

	CMV-IgG	
	r-value	p-value
Systolic blood Pressure Non-treated group	+0.4	+ S. (Significant)
Treated group	0.07	N.S (Non-Significant)
Diastolic blood pressure Non-treated group	+0.5	+ S. (Significant)
Treated group	0.08	N. S (Non-Significant)

These findings agree with others who used ELISA and found that the results of CMV seropositivity were in positive correlation with blood pressure [12,16]. Another positive correlation was found between level of renin enzyme and systolic/diastolic blood pressure as showed that in table(5), r-value(0.6) for systolic and diastolic blood pressure as well for the NT group, the T group was non-significant due to the effect of the drugs on renin enzyme as well as blood pressure.

Table 5-The correlation between Renin enzyme level and systolic / diastolic blood pressure among NT , T patients .

	Renin Enzyme	
	r-value	**p-value**
Systolic blood Pressure Non-treated group	0.6	+ S. (Significant)
Treated group	0.1	N.S (Non-Significant)
Diastolic blood pressure Non-treated group	0.6	S. (Significant)
Treated group	0.02	N.S (non-Significant)

Haarala and his college showed that CMV infection induced renin expression in a dose-dependent manner in mouse and human cells [11]. Prescott showed that CMV led to an increase in expression of the renin enzyme which has been known to activate the renin-angiotensin system and lead to high blood pressure [6]. Cheng and his college find that CMV infection alone caused a significant increase in arterial blood pressure measured by microtip catheter technique, by using quantitative real time reverse transcriptase PCR (Q-RT-PCR) and Western blot, they find that CMV stimulated expression of renin in mouse and human cells in an infectious dose-dependent manner. Co-staining and immunofluorescent microscopy analyses showed that mouse CMV infection stimulated renin expression at a single cell level. Further examination of angiotensin-II (Ang II) in mouse serum and arterial tissues with ELISA showed an increased expression of Ang II by mouse CMV infection [5].

Conclusion:

Form this study, it is concluded that previous infection or re-infection with HCMV in Iraqi patients may have a role in increase of arterial blood pressure by enhancing expression of renin enzyme an enzyme directly involved in causing high blood pressure and the protein angiotensin II (powerful vasoconstrictor), which lead to increase of arterial blood pressure, in order to reach a more significant results it is recommend to test more blood samples and using molecular techniques to study the exact effect on the genetic level. That such study may explain more about the pathogenesis of CMV and hypertension, which may affect the drug treatment and vaccination in the future.

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