Screening of Human Cytomegalovirus (CMV) in Diabetic Patients in Najaf Governorate.
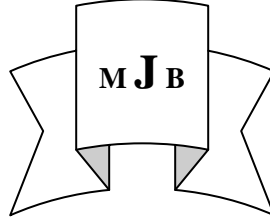
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Abstract

The aim of current study was to study the relationship between the virus and diabetes mellitus through detection of human cytomegalovirus (HCMV) infection in diabetic patients in Najaf governorate by screening of anti-human cytomegalovirus IgM antibodies in the serum of diabetic patients by using of ELISA technique and detection of human cytomegalovirus DNA in blood samples by using of PCR technique.

Blood samples collected from 140 diabetic patients randomly. ELISA technique was used to know the activity of humeral immunity among patients through detection of anti-HCMV IgM antibodies, where the result showed that IgM profile was positive in 49 patients(35 %).

Regarding PCR technique, the study showed that among 140 diabetic patients gave positive results in 27 patients(19.2%); In contrast, the results of control group were negative for anti-HCMV IgM antibodies in both ELISA and PCR technique.

This study concluded that the higher prevalence of seropositivity for human CMV in diabetic patients comparing with normal individuals which means that cytomegalovirus patients with diabetic were at high risk for CMV infections. A higher prevalence of CMV antibodies was observed in diabetic patients of all age-groups as compared with control group.

Key Words

HCMV, Diabetic, ELISA, PCR.

الخلاصة

لقد كان هدف الدراسة معرفة العلاقة بين فايروس (سي أم في) و مرضى السكري(مجموعة الدراسة) من خلال التحري عن وجود الاصابة بالفايروس في مرضى السكري في محافظة النجف بواسطة اجراء مسح لوجود الاجسام المضادة نوع(آي جي أم) في مصول مرضى السكري باستخدام تقنية (الاليزا)، وكذلك من خلال اجراء مسح لوجود الحامض النووي(دي أن أي) لفايروس (سي أم في) في عينات دم نفس المرضى باستخدام تقنية (بي سي آر) .

تم خلال الدراسة جمع ١٤٠عينة دم بصورة عشوائية من مرضى السكري في مدينة الصدر الطبية في النجف. لقد استخدمت تقنية (الاليزا) لمعرفة فعالية المناعة الخلطية بين المرضى من خلال كشف وجود الاجسام المضادة نوع (آي جي أم) والتي كانت نتيجته وجود ٤٩ حالة موجبة (٣٥ %) بينما اظهرت نتائج الدراسة باستخدام تقنية (بي سي آر) وجود ٢٧ حالة موجبة (١٩.٢ %) بينما أظهرت نتائج الدراسة عدم وجود أي حالة موجبة في مجموعة السيطرة باستخدام كلا التقنيتين.

لقد اظهرت الدراسة أن انتشار فايروس (سي أم في) في مجموعة الدراسة (مرضى السكري) كان أعلى بالمقارنة بمجموعة السيطرة مما يعني أن مرضى السكري أكثر خطورة للإصابة بفايروس (سي أم في) كما لوحظ أن وجود الاجسام المضادة لفايروس في دم مرضى السكري(مجموعة الدراسة) كان أكثر انتشارا في جميع الفئات العمرية مقارنة بمجموعة السيطرة.

Introduction

Cytomegalovirus belongs to the family of Alpha herpes viruses and is the largest and most complex member of this family. Owing to several immunoevasive strategies, the virus establishes a symptomless but persistent infection in healthy individuals [1].

Cytomegalovirus like all herpes viruses undergoes latency and reactivation in the host. Although HCMV has been shown to infect a broad spectrum of cells in vivo and has been isolated from saliva, urine, blood and human milk [2, 3].

However, in immune compromised individuals owing to the lack of immunologic control, the virus is able to reactivate and to cause severe CMV disease. Viral activity can be observed in all organs, including the pancreas [4] demonstrating that the virus has a broad cellular tropism. This broad cellular tropism is because widely spread receptors, such as integrins and the epidermal growth factor receptor, serve as entry receptors. [5, 6] These are also found on pancreatic cells making them putative targets for CMV infection. [7]

HCMV is a significant opportunistic pathogen in immunocompromised patients. Primary infection, reactivation of latent virus, and reinfection are possible and are often clinically silent. The onset of infection is marked by spiking pyrexia, which may resolve in a few days, Cytomegalovirus (CMV) can cause severe disease in immunocompromised patients, either via reactivation of latent CMV infection or via acquisition of primary CMV infection. [8, 9]

Materials and Methods

1. Study design:

The present study was conducted in Al-Sadr Medical City in Al-Najaf governorate. The study period was from June 2011 to November 2012.

1. Serum samples were collected from 140 of diabetic patients aged (35 – 65) years whom admitted to the center of diabetic and endocrine glands in AL-Sader Medical City of Al-Najaf governorate. In addition to whole blood samples with EDTA were obtained from the same above mentioned persons for the purpose of detecting DNA of HCMV.

2. Fourteen apparently healthy individuals (male and female) as a control group.

2. ELISA test:

Cytomegalovirus (CMV) IgM ELISA test kit (Bio Check, Inc.).

3. DNA-extraction and amplification kits:

DNA-extraction kits (DNA-Sorb-B) were supplied by Sacace biotechnologies, (Italy).

4. PCR technique:

PCR amplification Kit (CMV 500/800 IC). Target region Major immediate-Early (MIE) gene.

5. Detection of Serum Anti-CMV Antibody

HCMV virus-specific IgM, antibodies were detected by indirect enzyme-linked immunosorbent assay. Sera obtained from our patients and control group were collected and screened for the presence of anti-CMV IgM antibodies by means of a commercial enzyme immunoassay.

6. DNA extraction:

Protocols for Genomic DNA isolation was used according to the leaflet of the (Sacace kit).

7. PCR Amplification and Thermo cycling Conditions

The procedure was applied according to the leaflet of the commercial kit (Sacace, Italy).

8. Agarose Gel Electrophoresis

The amplified PCR products were detected by agarose gel electrophoresis and visualized by staining with ethidium bromide.

9. Statistical Analysis

The Chi-square test was applied to determine the statistical significance of the data.

P-value less than 0.05 were considered as significant [10].

Results

1. Regarding PCR technique: the study showed that among 140 diabetic patients; only 19. % (27 patients) PCR positive result as shown in figure (1).
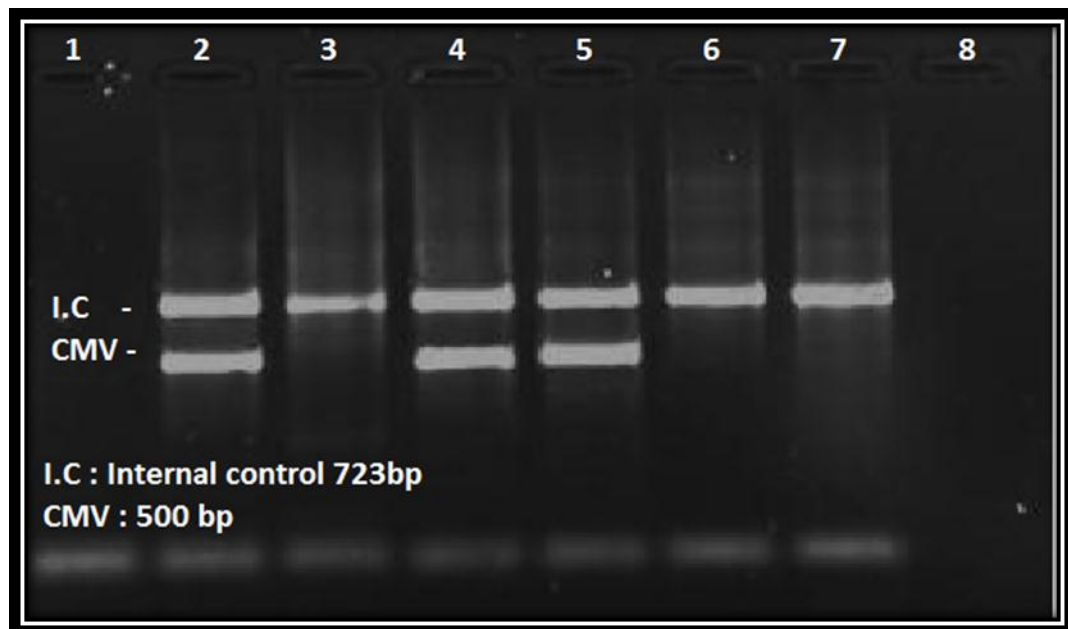

Figure 1 Ethidium bromide-stained agarose gel of PCR amplified products from extracted human cytomegalovirus DNA amplified with primers MIE gene in blood samples of diabetic patients.

Lane (1, 8): Sample show negative control, there is no band detected.

Lane (2): Control positive of human CMV include 2 band 500bp refer to CMV and 723bp refer to internal control.

Lanes (3, 6, and 7): Samples show only internal control bands that are mean negative results.

Lane (4, 5): Sample show two bands (I.C and CMV) refer to positive results.

2. Relationship between PCR and ELISA technique

ELISA test was done for all 140 samples obtained from the diabetic patients in Najaf governorate. The ELISA test was done to detect Anti-HCMV IgM antibodies; there was 35% (49 patients of the 140 samples) were positive whereas 19.2% (27 patients of 140 samples) were positive for HCMV DNA by PCR technique.

3. Correlation between CMV DNA findings and the prevalence of anti-HCMV IgM seropositivity in male and female diabetic patients

In the samples taken from 75 male and 65 female, it had been shown that 27 males (36%) were seropositive anti-HCMV IgM and 16 males (21.3%) PCR positive, while in female samples 22 females (33.8%) were positive anti-HCMV IgM results and 11 females (16.9%) PCR positive. Figure (2)
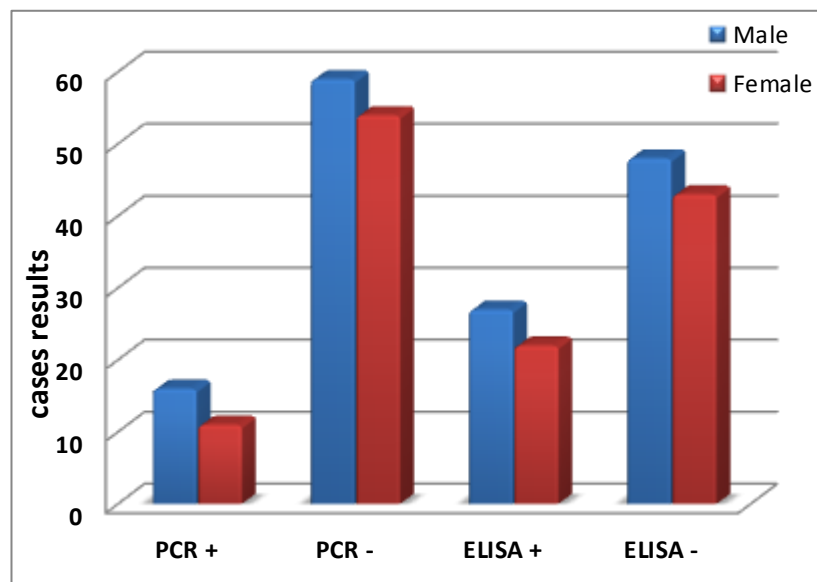

Figure 2 Correlation between CMV DNA findings and the prevalence of anti-HCMV IgM seropositivity in male and female diabetic patients.

4. Correlation between CMV DNA findings and the seropositivity anti-HCMV IgM in healthy male and female (control group)

The correlation between CMV DNA findings with the prevalence of anti-HCMV IgM seropositivity in healthy individuals (control group) revealed negative results for anti-HCMV IgM antibodies in ELISA test , but IgM gave a (4%) positive results in healthy male and PCR results were negative for all. Figure (3)
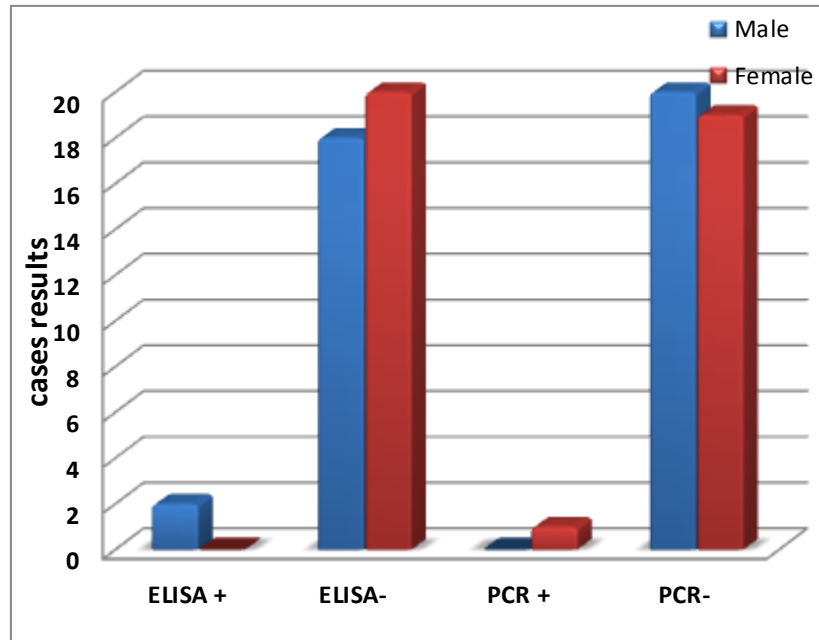
Figure 3 Correlation between CMV DNA findings and the seropositivity anti-HCMV IgM in healthy male and female (control group).

5. Seropositivity of anti-HCMV IgM among diabetic patients in regards to their age groups

From table (1), it was shown that the highest percentage of anti-HCMV IgM antibodies positivity detected in the age group (60-74) was represents 39% (30 patients) of the total samples and the lowest one was the age group (30-44) years which represents 3.9% (3 patients).

Table 1 The results of HCMV detection by ELISA test according to age groups.

Gender	General population		Total	ELISA test		Total
	Male	**Female**		**Male**	**Female**	
30 – 44	11 (8.2%)	5 (3.2%)	16 (22.4%)	5 (18.5%)	1 (5.2%)	6 (12.2%)
45 – 59	26 (19.5%)	24 (15.6%)	50 (35.7%)	10 (37%)	8 (74.3%)	18 (36.7%)
60 - 74	38 (28.5%)	36 (23.4%)	74 (52.8%)	12 (44.4%)	10 (52.6%)	22 (44.8%)
Total	75	65	140	27 (36%)	22 (33.8%)	49 (35%)

6. The results of HCMV detection by PCR test according to age groups

For PCR technique , it was shown that the highest age group in regards to PCR results was the age group (60-74)

and it represent 27.3% (21 patients) from cases while the age group (30-44) years showed the lowest percentage which was 0% (Table 2).

Table 2 The results of HCMV detection by PCR test according to age groups.

Gender	General population		Total	PCR technique		Total
	Male	Female		Male	Female	
30 – 44	11	5	16	1 (6.2%)	0	0
45 – 59	26	24	50	4 (25%)	2 (18.1%)	6 (22.2%)
60 - 74	38	36	74	11 (68.7%)	9 (81.8%)	21 (77.7%)
Total	75	65	140	16 (21.2%)	11 (7.8%)	27 (19.2%)

Discussion

Analysis of PCR results were based on the presence or absence of specific bands of amplified DNA in Agarose gel (2%). The length of specific amplified DNA fragments was: *CMV– 500 bp* and *Internal Control – 723 bp*. Figure (1).

The sample was considered positive for CMV DNA *(*Major Immediate-Early (MIE) gene), if the band of 500 bp was observed on agarose gel. The kit CMV allowed detecting CMV DNA in 100% of the tests with a sensitivity of not less than 500 copies / ml.

The current studies revealed that out of the 140 patients (28.2%) were positive for anti-HCMV IgM antibodies and the seropositivity among the age group (60-74) was higher than other age groups.

The current study has found that the infection rate at the general population was 19.3% (27 patients) by using PCR technique. To our knowledge, this study

was the first at least in the study areas that use PCR tests for diagnosis of asymptomatic HCMV infections and there were no previous known research concerning such number of asymptomatic patients and risk groups in Al-Najaf governorate.

The current study showed that no significant difference for the prevalence of CMV-IgM seropositivity between male and female patients. Figure (2)

Table (1) correlated the rate of anti-HCMV IgM antibodies seropositivity, showed that patients with seropositivity in relation age groups resulted from viral DNA detecting in their blood.

It was seen that there was almost similar results in both males and females regarding all these variables without significant variation in all these factors which might indicate the rate of diabetic patients by one mode or another and this is an important character of HCMV.

Also figure (2) revealed negative results for anti-HCMV IgM antibodies in ELISA test, but IgM gave a (10%) positive results in healthy male and PCR results were negative for all. This results is agreed with study which found that no significant difference for the prevalence of CMV-IgM seropositivity between male and female patients. [11] Showed that diabetic patients are higher than normal people.

Individuals who infect with CMV remain infected for life with latent CMV, which can reactivated at later times to cause CMV disease. [12] This results explain the reactivation of HCMV in case of immunosuppression and chronic diseases. [13]

The high prevalence of CMV infections might be responsible, at least in part, for the immunological disturbances and the susceptibility to other infections observed in diabetic patients [14, 15] whereas CMV was more likely to be associated with autoimmune diabetes, plus other factors, such as viral activation of the clotting cascade and alterations in the expressions of ELAM-1, ICAM-1, and VCAM-1 endothelial Factors. [16]

A strong correlation was reported by *Foy et al.* [15] & *Pak et al.* [17] between the genome of CMV and autoantibodies to islet cells found in the sera of insulin-dependent (type 1) diabetes.

Mel nick et al. found CMV antigen and nucleic acid sequences in arterial smooth muscle cells, suggesting that CMV infection of the arterial wall may be common in patients with severe atherosclerosis. [18] The high level of CMV antibodies was associated with clinically manifested vascular disease, possibly indicating a continuously active or periodically reactivated infection. By extension, one could expect that the accelerated vascular disease seen in diabetic patients on chronic dialysis might be related in part to latent CMV infection, manifested by severe atherosclerosis.

Other study by *Bertram et al.* [11] found an association between seropositivity for CMV and mellitus, this susceptibility to assorted infections in a population with compromised immunity would partially explain the possibility of increased CMV seropositivity in persons with type- 2 diabetes, such as seen in our study. This infection might be latent and not clinically manifested.

Similar study by *Lohr & Oldstone* [19] revealed that in situ nucleic acid hybridization on tissues from 5 randomly selected human-CMV-positive patients showed that the human CMV signal was localized primarily in the islets of Langerhans and not in exocrine cells. Despite the clear viral nucleic acid signal in tissues of human CMV of positive patients, there were no morphological injuries to the islets, no inflammatory cells in the islets, and no perivascular inflammatory cell cuffing. These findings suggest a possible association of human CMV with type- 2 diabetes in human beings.

Conclusions

1- Human cytomegalovirus was present in the blood of diabetic patients at different age groups whereas in control group no virus detected.

2- The PCR is a reliable and applicable tool for detection of HCMV in blood of diabetic patients.

3- The results suggest that diabetic patient more susceptible for human cytomegalovirus infection than healthy persons.

4- Older age group revealed the higher rate of infection in comparison with younger age group.

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