



Relationship between HCMV and Diabetic Mellitus Type 2 of Elderly Patients in Al-Najaf Governorate

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Abstract

The sudden rise mellitus (DM2) and related complications go along with increasing evidence of clinically major age and gender differences. The aim of current study was to study the relationship between the virus and diabetes mellitus type2 through detection of human cytomegalovirus (HCMV) infection in DM2 patients in Najaf governorate by detection of human cytomegalovirus DNA in blood samples by using of PCR technique. Blood samples were collected from 93 diabetic patients randomly. Regarding PCR technique, the study showed that among 93 diabetic patients gave positive results in 39 patients(42%); In contrast, in control group PCR positive result was only 4.4% (4 out of 90) with significant differences . This study concluded that the higher prevalence of HCMV DNA in DM2 patients comparing with normal individuals, which means that patients with DM2 were at high risk for HCMV infections.

Keywords: *HCMV, DM2, PCR.*

Introduction

HCMV is widely disseminated worldwide and can infect entirely age groups [1]. It has the largest and most complex member of Alpha herpes virus family with the size of 150 - 200 nanometers [2]. Human cytomegalovirus (HCMV) is a global reason that can cause infection at any time throughout the course of life and commonly infects individuals from diverse geographical and socio-economic backgrounds [1]. The virus establishes a symptomless but persistent infection in healthy individual's .Because it's owing to several immune-evasive strategies [2].Resembling to all herpes viruses.

Human cytomegalovirus undergoes latency and reactivation in the host, it is able to reactivate and to cause severe CMV disease in immune compromised individuals due to the lack of immunologic control. These viruses have broad cellular tropism because broadly spread receptors, such as integrins and the epidermal growth factor receptor, which serve as entry receptors [3, 4]. Viral activity can be observed in all organs, such as eye, gastrointestinal system, liver and blood

cells [5]. These are also found on pancreatic cells making them supposed targets for CMV infection [6].The overall problem of diabetes mellitus is massive and obvious. The effect on health and economy is extensive, yet this disease is assuming an epidemic proportion worldwide [7]. In the state Cytomegalovirus can cause severe disease in elderly DM2 patients either via reactivation of latent infection or via acquisition of primary infection [8, 9]. This study aimed to see the achievable correlation between CMV infection and type2 diabetes among elderly patients in Al-Najaf stat, Iraq.

Materials and Methods

Study Design

The present study was conducted in Al-Sadr Medical City in Al-Najaf governorate. The study period was from March 2017 to December 2017.

- Whole blood samples with EDTA for the purpose of detecting DNA of HCMV were obtained from 93 of DM2 patients aged (50-

90) years whom admitted to the center of diabetic and endocrine glands in AL-Sadder Medical City of Al-Najaf governorate.

- Nineteen healthy individuals (male and female) as a control group.

Molecular Technique

DNA-extraction and Amplification Kits

DNA-extraction kits (DNA-Sorb-B) were supplied by Sacace biotechnologies, (Italy).PCR amplification Kit (CMV 500/800 IC).Target region Major immediate-Early (MIE) gene.

PCR Technique

A protocol for Genomic DNA isolation was used according to the leaflet of the (Sacace kit).The procedure was applied according to the leaflet of the commercial kit (Sacace, Italy). The amplified PCR products were

detected by agarose gel electrophoresis and visualized by staining with ethidium bromide.

Statistical Analysis

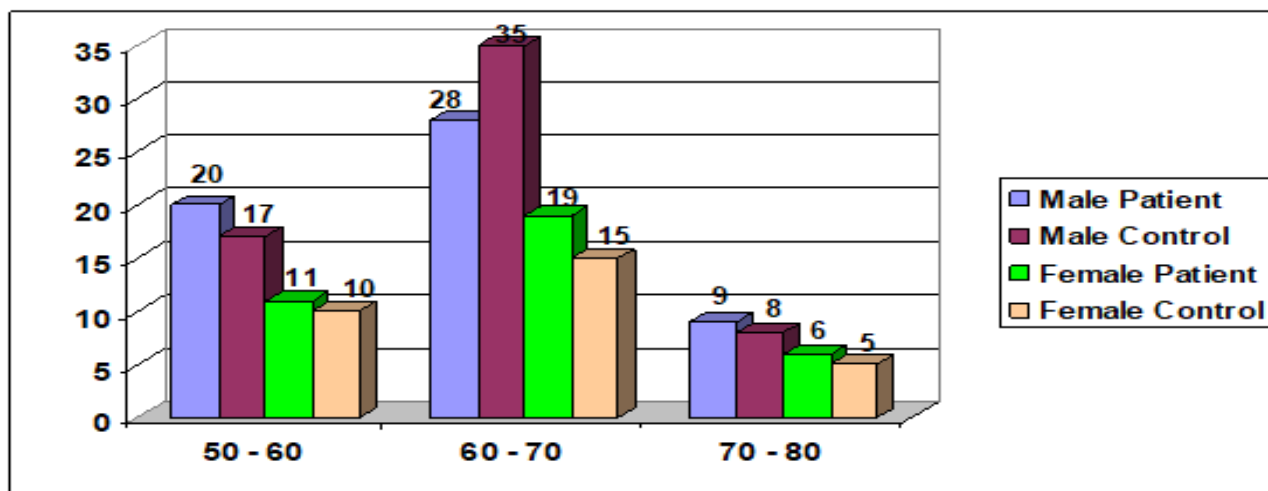
The Chi-square test was applied to determine the statistical significance of the data. P value of <0.05 was considered significant.

Results

The results of collected data on 93 randomly Diabetic Mellitus type 2 cases and 90 control group whom ages were ranged from Fifty to Eighty years, presented that the highest percentage among males and female of DM2 patients was 30% (28 out of 93 cases), 20% (19 out of 93 cases) respectively showed within age group (60-70) years, .with non-significant differences $p > 0.05$ between male and female in both studied group according to age. Table (1) Diagram (1).

Table 1: Diabetic cases according to age groups and gender

Age groups	No. of cases =93		Total	No. of control N=90		Total	p value
	Male	Female		Male	Female		
50 – 60	20 (21.5%)	11 (11.5%)	31 (33%)	17 (19%)	10 (11%)	27 (30%)	P>0.05non- Significant
60 – 70	28 (30%)	19 (20%)	47 (50%)	35 (39%)	15 (17%)	50 (55.5%)	
70 – 80	9 (10%)	6 (6%)	15 (16%)	8 (9%)	5 (6%)	13 (14.5%)	
Total	57 (60%)	36 (38%)	93 (100%)	60 (67%)	30 (33%)	90 (100%)	



HCMV DNA Findings in Both Study & Control Group According to the Age

On the subject of PCR technique: the study revealed that among 93 DM2 elderly

patients; was 42 % (39 from 93 patients) have PCR positive result with the heist percentage 87% (13 out of 15 cases) presented within elderly age group (70-80) years while, in control group PCR positive result was only

4.4%(4 out of 90) with significant differences as shown in Table (2) diagram (2) Figure (1).

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

Age groups	No. of DM2 cases = 93		Total	control N=90		Total	p value
	PCR+	PCR -		PCR+	PCR -		
50 – 60	6 (19%)	25 (81%)	31	0 (0%)	27 (100%)	27	P<0.05 *Sig
60 – 70	20 (43%)	27 (57%)	47	2 (4%)	48 (96%)	50	
70 – 80	13 (87%)	2 (13%)	15	2 (15.4%)	11 (84.6%)	13	
Total	39 (42%)	54 (58%)	93	4 (4.4%)	86 (95.6%)	90	

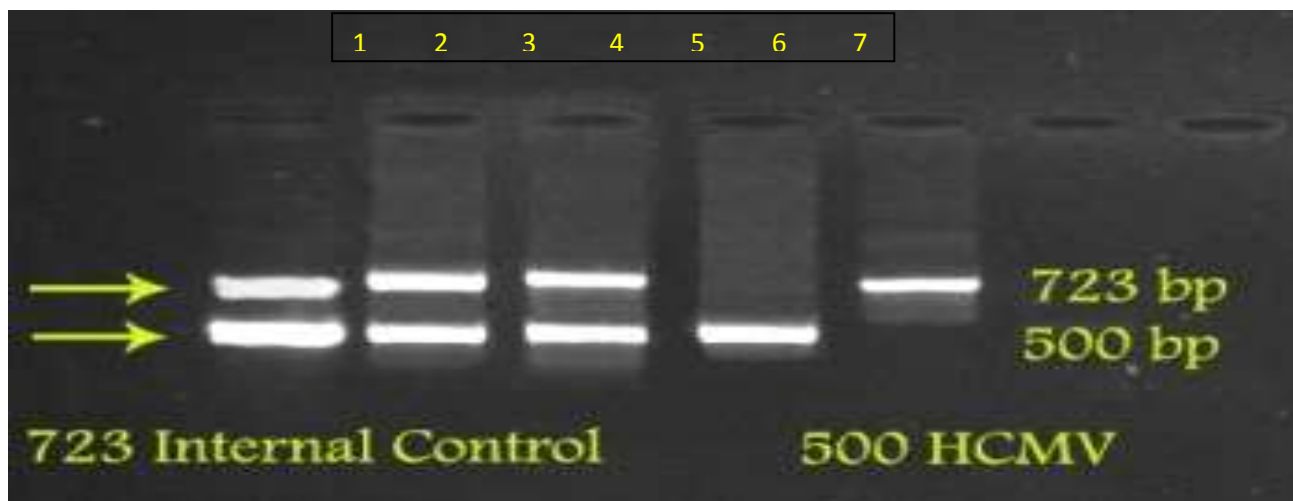
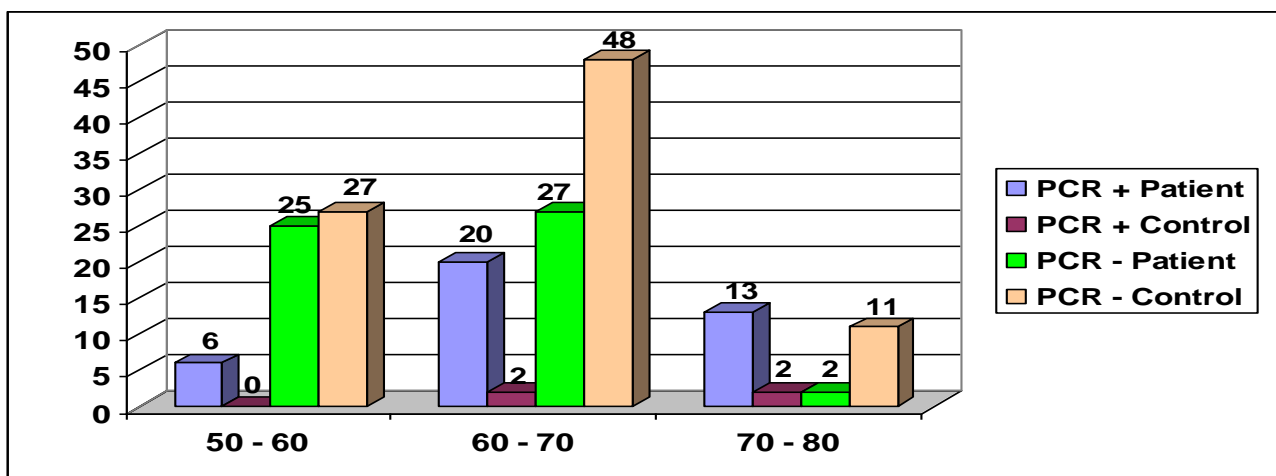


Figure 1: Ethidium bromide-stained agarose gel of PCR amplified products from extracted human cytomegalovirus DNA amplified with primers MIE gene in blood samples of DM2 patients. Lane (6, 7): Sample show negative control, there is no band detected. Lane (1): positive Control of human CMV includes 2 bands 500bp refer to CMV and 723bp refer to internal control. Lanes (5): Sample show negative results. Lane (2, 3, 4): Sample show positive result

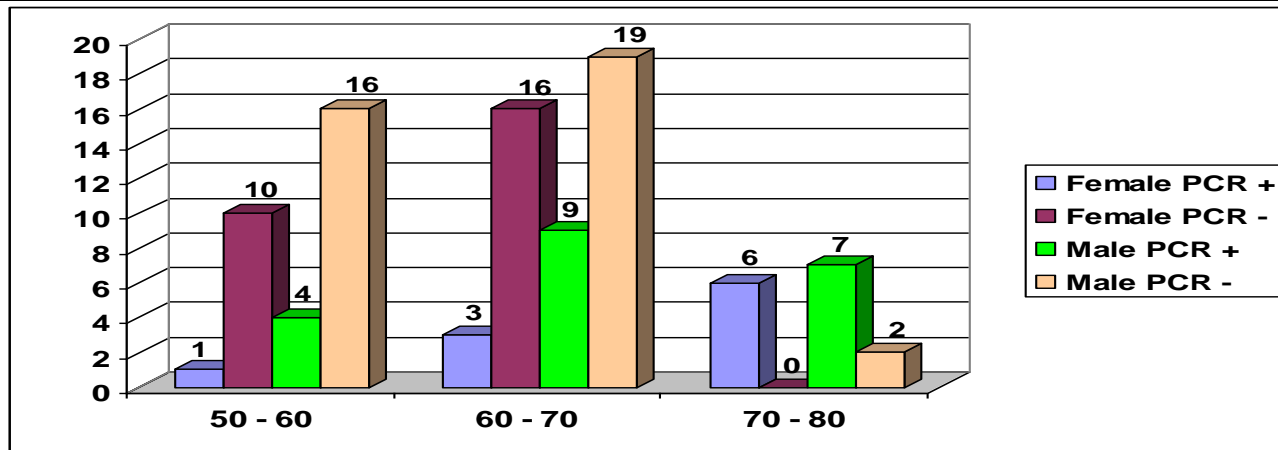
Prevalence of HCMV by PCR Test according to Age Groups and Gender

The result shown that the highest percentage of CMV DNA detection among female with DM2 was 100% (6 out of 6) found within age group (70-80), while it represent 78% (7 out of 9 patients) from male cases within the

same age group (70-80years).On the other hand the age group (50-60) years showed the lowest percentage which was 20% , 9% in male and female respectively, the result revealed significant differences among age group in term to CMV DNA+ detection Table (3) diagram(3).

Table 3: The results of HCMV DNA detection by PCR test according to age groups and gender

Gender	Female		Total	Male		Total	P value
	PCR +	PCR -		PCR +	PCR -		
50 - 60	1 (9 %)	10 (91%)	11 (100 %)	4 (20 %)	16 (80 %)	20 (100 %)	P<0.05* significant
60 - 70	3 (16 %)	16 (84 %)	19 (100 %)	9 (32 %)	19 (68 %)	28 (100 %)	
70 - 80	6 (100 %)	0 (0 %)	6 (100 %)	7 (78 %)	2 (22 %)	9 (100 %)	
Total	10 (28 %)	0 (72 %)	36 (100%)	20 (35 %)	37 (65 %)	57	



Discussion

Cytomegalovirus is one of the most common viruses worldwide; the virus causes severe disease with various complications by stressor of immune system. DM2 additionally referred to as non-insulin dependent DM that accounts for 90-95% of the population with DM. It's communal in most people older than 40yrs and consequences from variable combinations of hypoglycemic agent resistance and defects in hypoglycemic agent secretion [10, 11]. Age are commonly identified risk factors for diabetes mellitus type 2 [12]. A total of 93 authorized incident (DM2) cases and 90 healthy persons as control group were obtained from Al-Sadr Medical City in Al-Najaf governorate, ages of patients' and control group ranges from 50 to 80 years.

Overall, in this study DM2 prevalence is higher in men and women greater than 60 years. Table (1). Also, current study indicates that men are more likely to have (DM2) in all age groups, while the rate of women with diabetes mellitus 2 has increased gradually toward the elderly age group. In agreement to this result the worldwide diabetes prevalence is similar in men and women, but it is slightly higher in greater than 60 years of age [9]. One study found the peak incidence of diabetes in Nigeria and Tanzania to be recorded after age 45-50, and

also to increase with age, similar to the findings in this study.

Also in Nigeria, the risk of diabetes increases 3-4 times after the age of 44 years, 2010 [13]. The study showed significant differences between DM2 patients and the individual in control group. Also, the results showed a significant increase in the rate of infection with the increase in age of people with DM2 compared to younger age groups, this may be due to several factors such as the decline of immunity significantly in the elderly patients due to the presence of many chronic diseases that play an important role in weakening the body's ability to resist the virus, Individuals remain infected for life with latent CMV, which can be reactivated at later times to cause severe CMV disease [14].

This result explains the reactivation of HCMV in case of immunosuppression and chronic diseases [15]. 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 [17, 16] 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 [18].

A strong correlation was reported by Foy et al [16]. This finding, agreed with result of Hjelmessaeth et al, [19] how found significant association between asymptomatic cytomegalovirus infection and increase risk of new onset of DM2. Also, CMV may transmit to the susceptible individuals through blood and tissue harvests. The current study establish that the infection rate among the DM2 patients was 42% (39 out of 93 patients) while only 4.4% (4 out of 90) gave positive result in the control group by using PCR technique with significant differences $P < 0.05$.

Related study by Lohr & Oldstone [20] exposed 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. Although the clear viral nucleic acid signals 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. In smaller proportions, a possible explanation for these findings is that older persons have higher susceptibility to adverse clinical outcomes related to their permanence especially the lower body mass index related to reduced testosterone levels and reduced secretion of the growth hormone [21].

It was understood that there was practically similar results in both males and females as regards all these variables without significant variation in all these factors which might indicate the rate of DM2 patients by one approach or another and this

is an important character of HCMV. Current study revealed that the highest percentage of CMV DNA detection among female and male with DM2 was 100 % (6 out of 6), 68 % (7 out of 9 patients) respectively within age group (70-80), while the age group (50-60) years showed the lowest percentage which was 20 %, 18 % in male and female respectively with non- significant differences (Table 3). Whereas, the result of CMV DNA+ Showed that diabetic type 2 patients are higher than in normal individuals.

Persons who infect with CMV remain infected for life with latent CMV, which can reactivated at later times to cause CMV disease [22]. This result explains the reactivation of HCMV in case of immunosuppression and chronic diseases. . It is crucial to demonstrate the association using a prospective study design, starting with a younger study population, as CMV infection may be a “hidden” cause of morbidity [23].

Conclusions

The PCR is a reliable and applicable tool for detection of HCMV in blood of diabetic patients' type2, CMV was present in the blood of DM2 at different age groups whereas in control group the virus detected only in elderly patient in very little proportion which indicate that diabetic patient more susceptible for human cytomegalovirus infection than healthy persons. Also, older age group revealed the higher rate of infection in comparison with younger age group. Our findings shed light on the pathogenesis of type 2 diabetes in the elderly patients. Further research needs to be accomplished to determine causality in the relationship of CMV infection with type 2 diabetes.

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