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Detection of Human Cytomegalovirus (HCMV) among Male and Female type 2 Diabetic Patients in Thi-Qar Province

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Abstract

Background: Human Cytomegalovirus (HCMV), as a member of the herpesvirus family, is a highly prevalent virus that infects a significant majority of individuals worldwide at some point in their lifetime. HCMV induces a cascade of inflammatory reactions that can result in cellular damage and play a role in the initiation and advancement of diverse inflammatory conditions. Inflammation is a significant determinant for diabetes (diabetes mellitus) and plays a crucial role in the onset and development of various complications associated with diabetes.

Methods: This study included the collection of serum samples for the detection of Human cytomegalovirus antibodies in 200 patients with diabetes type 2. Also included was a control group consisting of 100 individuals of different ages who did not have diabetes. The period of collecting sample was from August 2023 to October 2023. All these serum samples are detected for Human cytomegalovirus antibodies by the enzyme-linked immunosorbent assay method.

Conclusions: According to the results of this study, there was a significant difference in term of anti-human cytomegalovirus IgG antibody between diabetic type 2 patients and control and also there was a high significant difference in term of anti-human cytomegalovirus IgM antibody between those patients and control group. It was also found that there are no significant differences in the detection of anti-human cytomegalovirus IgM antibody among patients in terms of sex, age, residency and family history.

Key words: HCMV: human cytomegalovirus, T2DM: Type 2 diabetes mellitus; Hemoglobin A1C : (HbA1C); Enzyme Liked Immunosorbent Assay : ELISA

Introduction: CMV, a member of the Herpesviridae family, is named from the Greek words "cyto," referring to cells, and "megalo," indicating enormous (1). The HCMV virus possesses a linear, double-stranded DNA genome of approximately 235 kb, enclosed within an icosahedral nucleocapsid. The beta structure capsid can accommodate a genome 60% larger than that of HSV-1. Notably, the three-dimensional structure of the HCMV capsid has a diameter of 130 nm, featuring 16 icosahedral lattices (162 capsomers, 12 pentamers, and 150 hexamers) connected by triplex structures (2). The enveloped viral particle measures 150-200 nm in diameter (3). HCMV is not a highly infectious virus; however, it requires close, even intimate contact with bodily fluids to be transmitted among people (4). The seroprevalence of HCMV varies geographically, ranging from 30 to 100% (5). The longevity of Human cytomegalovirus (HCMV) within the host is maintained through the establishment of latency in specific cell types (6). Reactivation of the virus can occur either through reinfection with an exogenous strain or through endogenous virus reactivation, facilitated by immunological depression or allogeneic immune stimulation (2). Except for uncommon instances of infectious mononucleosis, HCMV infection typically causes no symptoms. This is because a strong immune response to HCMV typically prevents the high viral loads required to cause end organ disease (EOD) in immunocompromised patients .(7)

Type 2 Diabetes Mellitus (T2DM) is a common metabolic illness caused by inadequate insulin secretion from pancreatic β-cells and insulin-sensitive tissue failure .(8)

The distinctive feature of CMV latency triggered unusual expansion of the CMV-specific resting effector population of memory CD8 + T cells, resulting in established chronic inflammation and affecting host immunological mechanisms (9). HCMV infection causes inflammation, which eventually leads to the death of islets β-cells, illustrating the virus's ability to infect and destroy these cells. HCMV infection may cause β-cell deficiency and apoptosis, potentially leading to T2DM (10). Recent research also indicates that the immune system actively induces changes in systemic metabolism as a protective response to viral infection. The lack of control in this system, particularly in relation to diabetes, is thought to be a significant contributor to increased susceptibility to viruses; as a result, people with diabetes are more likely to encounter CMV (11).

Aim: Investigate the correlation between the detection of Human cytomegalovirus and socio-demographic characteristics, such as sex, age, region of residency in patients with type 2 diabetes mellitus.

Study population and methods: This study included the collection of serum samples for the detection of Human cytomegalovirus antibodies in 200 patients with diabetestype 2. Also included was a control group consisting of 100 individuals within different age groups who did not have diabetes. The period of collecting sample was from August 2023 to October 2023. All these serum samples were detected for Human cytomegalovirus antibodies by the enzyme-linked immunosorbent assay method. The study was targeted patients with type 2 diabetes who were receiving care at the Special Center for Endocrine Glands and Diabetes in Al-Nasiriyah city (Thi-Qar province, southern Iraq).

Inclusion criteria: All of the patients in the sample had their information gathered, which includes their Age, sex, residency and family history as stated in the questionnaire are all examples of demographic data.

Exclusion criteria: Patients over 70 years old and under the age of 20 were excluded from the study. Patients with autoimmune diseases or chronic illnesses, as well as those people who have had organ transplants, immunodeficiency disorders, or other known causes of secondary diabetes, are excluded.

Statistical Analysis: Statistical analyses were conducted using SPSS software version 25.0 (SPSS, Chicago). Categorical variables were presented as numbers and percentages and were assessed using the Chi-square test. A p-value below 0.05 was regarded as indicative of a statistically significant difference.

Ethical considerations : To participate in this study, all participants must provide informed verbal consent. The study included patients diagnosed with type 2 diabetes positively detected by A hemoglobin A1C (HbA1C). The Health Office in Thi-Qar Governorate approved the research protocol (ethical number 568, dated July 8, 2023). Prior to participating in the study, all patients and control participants provided informed consent. The samples collected from patients were managed by specialist physicians under direct supervision.

Methods:

Data of collective samples: This study focused on individuals diagnosed with type 2 diabetes who attended the Special Center for Endocrine Glands and Diabetes in Al-Nasiriyah city. The study encompassed a total of 300 participants, consisting of 200 individuals (100 males and 100 females) with type 2 diabetes, aged between 23 and 69 years, and a control group of 100 individuals (47 males and 53 females) without diabetes, aged between 20 and 56 years. Following the acquisition of informed consent from each participant, comprehensive data, including name, age, family history of diabetes, residency, occupation, marital status, pregnancy, BMI, treatment type, duration of diabetes, blood group type, and history of previous COVID-19 infection and vaccination, were gathered through a questionnaire. The data collection period spanned from August 2023 to October 2023.

A total of 200 individuals diagnosed with type 2 diabetes and 100 healthy individuals across various age groups, without diabetes, were selected as the control group. Blood samples (3 ml each) were collected from all participants and placed in gel tubes, which were then centrifuged at 4000 RPM for five minutes. The separated components were divided into two parts: one part, consisting of 0.5 ml of serum, was utilized for the detection of HCMV virus through Enzyme-Linked Immuno-Sorbent Assay (ELISA) for IgG and IgM antibodies. The ELISA tests were conducted following the manufacturer's instructions (DRG, Germany).

Results:

1- Detection of Anti-HCMV IgG antibodies in patients and control group.

Almost all T2DM patients (99 %) were positive for anti-CMV IgG antibodies with (1 %) negative compared to (94 %) of controls were positive with (6 %) negative, with a significant difference, as seen in Table 1.

Table (1): Seroprevalence for anti-HCMV IgG antibodies in patients and controls

Elisa	Diabetic Patients Group *N* = 200	Control Group *N* = 100	P-Value
Positive	198 (99 %)	94 (94 %)	0.054 S
Negative	2 (1 %)	6 (6 %)	

***N*:** number of cases; ***S*:** significant

On the other hand, 14 patients (7 %) were positive for anti-CMV IgM antibodies with (93) negative versus none among controls, with a highly significant difference as seen in Table 2.

2- Detection of Anti-HCMV IgG antibodies in patients and control group.

Table (2): The presence of anti-HCMV IgM antibodies in patients and controls

Elisa	Diabetic Patients Group *N* = 200	Control Group *N* = 100	P-Value
Positive	14 (99 %)	0 (0 %)	0.007 S
Negative	186 (93 %)	100 (100 %)	

***N*:** number of cases; ***S*:** significant

3- Association of Demographic Characteristics with Anti-HCMV IgM Antibody Positivity in T2DM Patients.

The results showed that females are infected more than males in according to anti-HCMV IgM antibody but there was a non-significant statistically difference between them as shown in Table3.

Table (3): Detection of anti-HCMV IgM antibody in patients by ELISA among males and females

Sex	HCMV Positive *N* = 14	HCMV Negative *N* = 186	Total *N*	P-Value
Male	6 (42.86 %)	94 (50.54 %)	100	0.579 NS
Female	8 (57.14 %)	92 (49.46 %)	100	

***N*:** number of cases; ***NS*:** significant

Table 4 shows that there is a non-significant statistical variation between patients who are anti-HCMV IgM positive and negative according to age groups.

4- Detection of anti-HCMV IgM antibody in Diabetic type 2 patients according to age group

Table (4): Detection of anti-HCMV IgM antibody by ELISA among patients according to age

Age Groups	HCMV Positive *N* = 14	HCMV Negative *N* = 186	P-Value
20-30	3 (21.42 %)	8 (4.3 %)	0.103 NS
31-40	2 (14.28 %)	28 (15.05 %)	
41-50	4 (28.57 %)	59 (31.72 %)	
51-60	4 (28.57 %)	62 (33.33 %)	
61-70	1 (7.14 %)	29 (15.59 %)	

***N*:** number of cases; ***NS*:** significant

The results showed no significant difference among patient groups regarding residency among patients. Still, rural residents were more frequent among positive than negative patients in anti-HCMV IgM antibody as seen in Table 5.

5- Detection of anti-HCMV IgM antibody among Diabetic type 2 patients in respect to the residency

Table (5): Detection of anti-HCMV IgM antibody by ELISA among patients in respect to Residency

Residency	HCMV Positive *N* = 14	HCMV Negative *N* = 186	Total N	P-Value
Urban	8(57.14%)	139(74.73%)	147	0.15 NS
Rural	6(42.86%)	47(25.27%)	53	

***N*:** number of cases; ***NS*:** significant

Table 6 shows that there is a non-significant statistical difference among patients who are positive and negative in ELISA according to family history of diabetes.

6- Detection of anti-HCMV IgM antibody by ELISA among patients in accordance to family history

Table (6): Detection of anti-HCMV IgM antibody by ELISA among patients in accordance to family history

Family History	HCMV Positive *N* = 14	HCMV Negative *N* = 186	Total N	P-Value
Positive	5(35.71%)	96(51.61%)	101	0.251 NS
Negative	9(64.29%)	90(48.39%)	99	

***N*:** number of cases; ***NS*:** significant

Discussion

In the current study out of 200 patients with diabetes, (99 %) were positive for anti-human cytomegalovirus IgG antibody and that result is similar to another study (12) in al-Khartoum state where the rate of cytomegalovirus IgG was (96.6 %). The results of the current investigation point to a potential role for anti-CMV IgG antibody in the aetiopathogenesis of type 2 diabetes, and they are in line with other research in this area. One of these studies is a case-control cohort study (13) conducted in Korea the incidence of CMV diseases was significantly higher in the group with type 2 diabetes mellitus (T2DM) compared to the group without T2DM (33.7% vs. 16.2%, P<0.001). In the current study there are (94 %) healthy individuals who are positive in IgG, however, it is unknown if the HCMV directly affect pancreatic beta cells and cause diabetes or the diabetic patients are more prone to HCMV infection.

In this study the anti-human cytomegalovirus IgM was tested for all patients and it was positive in 14 of them which was similar to a study (14) conducted in Kirkuk governorate which antibodies of HCMV IgM only, detected in 26.82 % detected in T2DM patients while in none of controls was positive as in the current study, this maybe attributed to the immunocompromised state in diabetic patients.

In the current study there was a non-significant difference between males and females according to HCMV IgM among patients but females were slightly higher than males and this result is similar to other study (15) conducted in Najaf governorate in which there was a non-significant difference too.

In this study there was a non-significant difference in HCMV IgM according to the age groups among patients but the age groups between 41-50 and 51-60 were slightly higher than other age groups. In other study (16) conducted in Najaf governorate were age group 60-69 were significantly higher than other age groups, that maybe attributed to the lower immunity state which increase with age.

There was a non-significant difference in HCMV IgM regarding residency and lack of reference in residency suggested that the that the immune status in patients residing in rural and urban areas is essentially the same, so there is no difference in the susceptibility to a recent infection or reactivation of infection between them.

In the current study there was a non-significant variation in HCMV IgM regarding the family history among patients and lack of reference suggested that the susceptibility to a recent infection or reactivation of infection is the same among patients with or without a family history.

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