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Detection of human cytomegalovirus among type 2 diabetic patients with evaluation of physiological parameters

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ABSTRACT

Background: Prolonged HCMV infection leads to the development of a pro-inflammatory environment, which subsequently promotes the initiation and progression of chronic inflammatory disorders. Inflammation is a key factor in the development of diabetes and is also responsible for the emergence of various complications related to the disease.

Aim: The objective of this study is a detection of the HCMV UL57 gene by real-time PCR. Also, to assess the levels of anti-CMV virus IgG and IgM in the serum of individuals from Iraq diagnosed with Type 2 diabetes mellitus, alongside evaluating various markers such as sex, BMI, complete blood count, serum creatinine, blood urea, and lipid profile (cholesterol and triglyceride levels).

Methods: The study involved collection of serum samples from 200 type 2 diabetes patients, also included a control group of 100 people of various ages without diabetes to detect Human Cytomegalovirus antibodies by each of real-time PCR and ELISA. Apart from the parameters assessed in this study, which encompassed BMI and complete blood count analysis, lipid profile (cholesterol, triglyceride), serum creatinine, and blood urea.

Results: The study's findings indicate that there was a highly significant difference in detection of the HCMV UL57 gene by real-time PCR among diabetic patients in compared to non-diabetic individuals. There was also a significant difference in the anti-human cytomegalovirus IgG antibody level between the patient and control groups as well as a highly significant difference in the anti-human cytomegalovirus IgM level. It was found that there are no significant differences in the detection of anti-human cytomegalovirus IgM antibody among patients in terms of BMI, complete blood count, cholesterol, triglyceride, serum creatinine and blood urea.

Keywords: HCMV: human cytomegalovirus, Real-time PCR, T2DM: Type 2 diabetes mellitus, ELIS

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INTRODUCTION

Cytomegalovirus (CMV), a member of the herpesvirus family, is a highly prevalent pathogen that infects a significant majority of individuals worldwide at some point in their lifetime [1]. The occurrence of HCMV-specific antibodies in adults is reported to be as high as 100% in certain countries in Africa and Asia, while in industrialized nations it typically ranges from 35% to 80% on average [2]. Cytomegalovirus comprises the largest amount of genetic material among the human herpesviruses, with a DNA genome size of 240 kilobase pairs (kpb), which is considerably larger than that of the herpes simplex virus [3]. Infection is transmitted through direct contact with infectious individuals. Transmission can occur either vertically, through the placenta from mother to fetus, or horizontally, through sexual intercourse or contact with fluids such as saliva, breast milk, maternal genital secretions, or blood [4]. Additionally, it can be transmitted through the transplantation of stem cells or solid organs [5]. Despite other herpes viruses, which tend to remain localized, human cytomegalovirus may establish latency in a range of cell types [6]. Human cytomegalovirus infection may occur in persons who have never been exposed to the virus before (primary infection) or in people who have been exposed previously (repeat infection); recurrent infection can occur when endogenous latent virus is reactivated or when an exogenous virus is re-infected [7]. Various data suggest that persistent CMV infection could potentially contribute to the onset of type 2 diabetes. CMV may expedite immune-senescence by enhancing the generation of late-differentiated CD4+ and CD8+ T-cells, which secrete pro-inflammatory cytokines, thereby fostering a more pro-inflammatory milieu. [8]. Viral activity can be noticed in all organs, such as eye, gastrointestinal system, liver and blood cells. These are also located on pancreatic cells making them supposed targets for CMV infection [9]. Inflammation generated by HCMV infection subsequently leads to death of islets β-cells, revealing that HCMV may infect and destroy β-cells. Therefore, HCMV infection, via increasing the deficit and increased death of β-cells, may be linked with T2DM [10]. Type 2 diabetes mellitus is one of the most frequent metabolic diseases globally that occurs because of a pair of essential indicators: impaired insulin responsivity of tissues and hypo-insulinemia generation by pancreatic islets [11]. Hyperglycemia, a hallmark of type 2 diabetes, is generally brought on by the combined existence of insulin resistance and reduced beta cell activity [12]. Some studies also indicate that T2DM patients are more sensitive to occurrence of viral infection as diabetes inhibits recovery. Moreover, hyperglycemia frequently inhibits coagulation, fibrin activity, body fat and endothelial function [13].

METHODS

Samples investigated: This study involved obtaining serum samples from 200 patients with type 2 diabetes who were receiving care at the Special Center for Endocrine Glands and Diabetes in Al-Nasiriyah city to detect the presence of Human cytomegalovirus antibodies. In addition, a control group comprising 100 individuals of varying ages who were not suffering from diabetes was also incorporated. Samples were collected between August and October of 2023.

Exclusion criteria: The study excluded individuals who were 70 years of age or older. Exclusion criteria include patients suffering from autoimmune diseases, chronic illnesses, organ

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transplant recipients, individuals with immunodeficiency disorders, or those with other known causes of secondary diabetes.

5 ml of venous blood was collected from patients and controls and separated in two parts: one part was collected in gel tube for serum after centrifugation to be used for ELISA and biochemical analysis and the other part was collected in EDTA tube to be used for CBC and detection by real-time PCR.

All the participants' information was gathered, and their BMI was calculated.

Detection of HCMV IgG Antibodies by ELISA: 3 ml of blood were centrifuged at 4000 RPM for five minutes and 500 μl of serum were used for detection of HCMV IgG by ELISA using DRG Germany ELISA kit and according to the manufacturer's instructions.

Detection of HCMV IgM Antibodies by ELISA: 3 ml of blood were centrifuged at 4000 RPM for five minutes and 500 μl of serum were used for detection of HCMV IgM by ELISA using DRG, Germany ELISA kit and according to the manufacturer's instructions.

Biochemical analysis: 500 μl of serum were used for cholesterol, triglyceride, serum creatinine and blood urea analysis by using the automated method (Abbott Architect c4000, Japan).

Complete blood count test: The Mindray BC-5000 CBC machine was used to assess the hematological parameters using 1 ml of whole blood.

DNA extraction: The viral genomic DNA was obtained from the whole blood of 70 patients and 30 controls following the instructions provided by the manufacturer, the viral nucleic acid extraction package supplied by SIMBIOLAB/Iran was utilized for this purpose. The eluted DNA was subsequently kept at a temperature of -20°C until the PCR assay was carried out.

Real-Time PCR: The present study employed the real-Time PCR technique to amplify the UL57 gene of HCMV. The primers utilized in the current investigation were acquired from Macrogen, Korea. The forward primer used was 5'-CATCACGCTATTTTCGCGGGC-3', while the reverse primer employed was 5'-CGGTGATCGGTTGCGTTGGTC-3'(14). The Go Taq® G2 master mix kit, used for RT PCR, was acquired from Promega, USA. Each reaction comprised 10 μl of green master mix, 5 μl of extracting DNA, 2 μl of forward and reverse primers, 0.3 μl of CXR reference dye (carboxy-X-rhodamine), and 0.7 μl of Nuclease free water. The final volume in the reaction tube was 20 μL. The thermocycling conditions were mentioned in Table 1.

Table 1: Real time PCR conditions

Steps	Temperature (°C)	Time min\sec	Cycles
Initial Denaturation	95	10:00	1
Denaturation	95	00:30	40
Annealing	57	00:30	
Extension	72	00:30	

Statistical Analysis: The statistical analyses were carried out utilizing SPSS software version 25.0 (SPSS, Chicago). Normality testing for continuous data was conducted using the Shapiro-Wilk test. Data demonstrating a normal distribution were depicted as mean and standard deviation and analyzed using the Student t-test. Categorical variables were represented as

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numbers and percentages and assessed using the Chi-square test. A significance level of $p < 0.05$ was considered indicative of statistically significant differences.

Ethical considerations: To participate in this study, all individuals must provide informed verbal consent. The study included participants diagnosed with type 2 diabetes. The research protocol (ethical number 168, dated July 8, 2023) had been approved by the Thi-Qar Governorate Health Office. Prior to participation in the study, all patients and control subjects submitted informed consent. The samples collected from patients were managed by professional physicians under direct supervision.

RESULTS

Serological results: Almost all patients (99 %) were positive for HCMV IgG and 94 % of controls were positive with a significant difference (Figure 1).

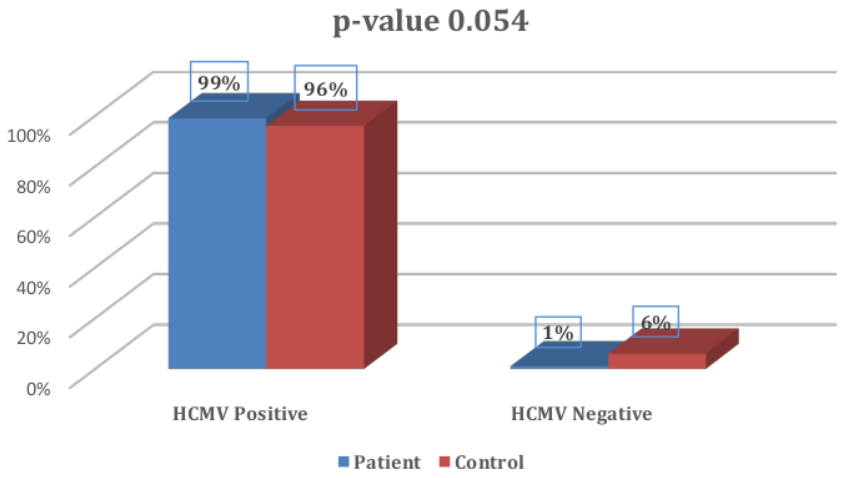

Figure 1: Seroprevalence of HCMV IgG among patients and control

While only 7 % of patients were positive for HCMV IgM and none of controls with a highly significant difference (Figure 2).

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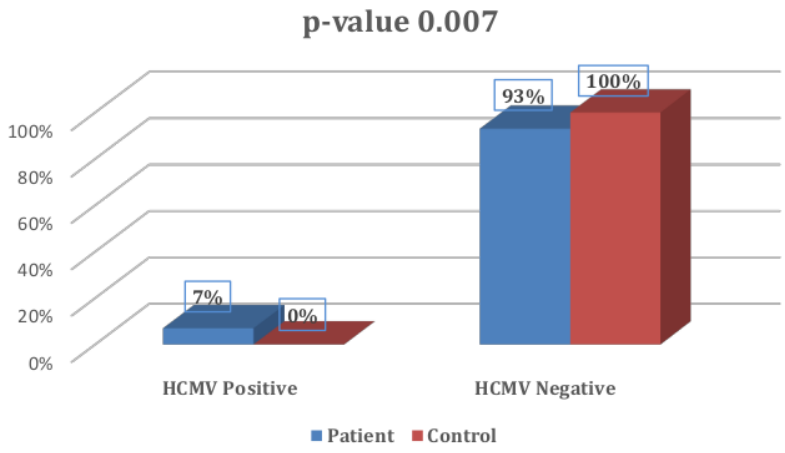

Figure 2: Detection of HCMV IgM among patients and controls

Biochemical analysis results: The results found that there was a non-significant difference among patients who are positive and negative for HCMV IgM according to lipid profile (cholesterol and triglyceride) tests (Table 1).

Table 2: Association of lipid profile with anti-CMV IgM antibody positivity in T2DM patients

Variables	CMV negative (n=186)	CMV positive (n=14)	p- value
TC, mg/dl Mean±SD Range	174.8±40.52 74-315	192.5±44.1 135-199	0.113 NS
TG, mg/dl Mean±SD Range	117.12±112.8 27-979	223.5±146.8 66-573	0.140 NS

n: number of cases; NS: significant

The results found that there was a non-significant variation among patients who are positive and negative to HCMV IgM according to renal function (serum creatinine and blood urea) tests (Table 2)

Table 3: Association of renal function with anti-CMV IgM antibody positivity in T2DM patients

Variables	**CMV negative (n=186)**	**CMV positive (n=14)**	**p- value**
Urea, mg/dl Mean±SD Range	25.1±8.0 7.0-56	21.71±4.17 12-27	0.119 NS

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Creatinine, mg/dl Mean±SD Range	0.82±0.16 0.46-1.37	0.81±0.14 0.59-1.07	0.697 NS

n: number of cases; NS: significant

Body mass index results: The results found that there was a non-significant difference among patients who are positive and negative for HCMV IgM according to BMI (Table 3).

Table 4: Association of BMI with anti-CMV IgM antibody positivity in T2DM patients

Variable	CMV negative (n=186)	CMV positive (n=14)	p- value
BMI, kg/m2 Mean±SD Range	29.03±6.3 16-58	30.57±4.91 22-40	0.367 NS

n: number of cases; NS: significant

Complete blood count results: Total WBC and neutrophil percentage were slightly higher in patients positive for anti-CMV IgM (9.46±3.23×109/L and 55.17%±18.94, respectively) than those negative for Anti-CMV IgM (8.44±2.22×109/L and 59.04±8.64, respectively). However, the differences were not significant. Other hematologic indices were very close between the two groups with no significant differences (Table 4).

Table 5: Association of hematologic indices with anti-CMV IgM antibody positivity in T2DM patients.

Variables	**CMV negative (n=186)**	**CMV positive (n=14)**	**p- value**
WBC×10^9/L Mean±SD Range	8.44±2.22 3.15-16.13	9.46±3.23 2.93-17.48	0.112 NS
Neutrophil, % Mean±SD Range	59.04±8.64 36.5-82.3	55.17±18.94 5.22-82.3	0.150 NS
Lymphocyte, % Mean±SD Range	32.52±8.59 13.5-65.1	32.55±11.97 20.9-65.1	0.882 NS
Monocyte, % Mean±SD Range	5.77±1.64 3.0-14.0	5.68±1.29 3.7-7.7	0.826 NS
Eosinophil, % Mean±SD Range	2.56±1.71 2.0-10.6	2.88±3.76 0.7-15.5	0.544 NS
Basophil, % Mean±SD Range	0.03±0.06 0.0-4.0	0.03±0.06 0.0-2.0	0.952 NS

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Hb, g/Dl Mean±SD Range	 13.41±1.61 8.9-17.8	 13.57±1.77 10.1-17.7	0.344 NS
RBC×10^9/L Mean±SD Range	 4.92±0.57 3.64-7.13	 5.06±0.56 4.2-6.03	0.723 NS

n: number of cases; NS: significant

Molecular results: The results showed that 94.29 % of patients where positive in real time PCR and 50 % of controls were positive with a highly significant difference. Table 6.

Table 6: percentage of detection of HCMV UL57 gene among patients and controls using real time PCR

Real Time PCR	Control		Patient	
	Count	%	Count	%
Positive	66	94.29	15	50
Negative	4	5.71	15	50
$CalX^2$= 48.0 $TabX^2$= 3.84 DF= 1 p. value < 0.001^{Sig}				

Cal: calculated value; Tab: tabulated value; DF: degree of freedom; Sig: significant

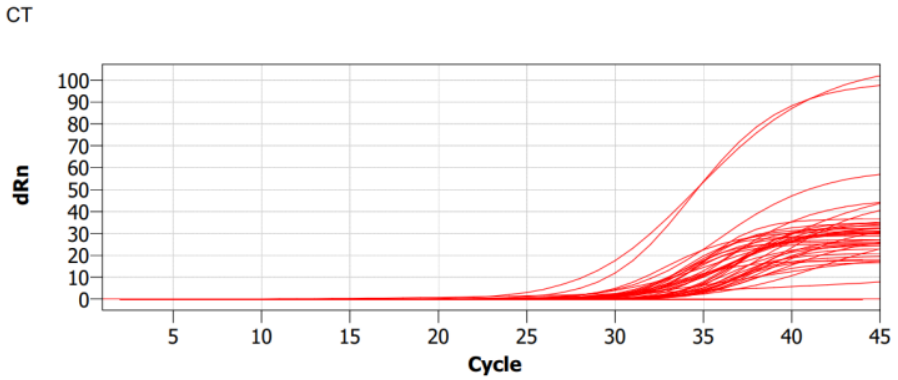

Figure 3a: Amplification curve Run (1) of patients HCMV UL57 gene

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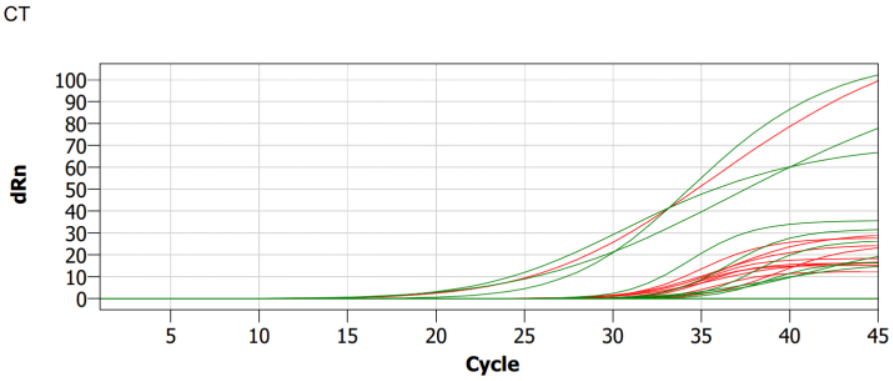

Figure 3b: Amplification curve Run (2) of patients (red) and controls (green) HCMV UL57 gene.

DISCUSSION

Human cytomegalovirus (HCMV) has been proposed as a potential contributor to the development of diabetes mellitus, particularly type 2 diabetes. Several studies have suggested a correlation between HCMV infection and an increased risk of diabetes, although the exact mechanisms underlying this association remain under investigation [14]. One potential mechanism is the ability of HCMV to induce chronic inflammation. Prolonged HCMV infection can lead to the establishment of a pro-inflammatory environment, which has been implicated in the pathogenesis of diabetes. Chronic inflammation is known to impair insulin signaling and promote insulin resistance, key features of type 2 diabetes [15].

Furthermore, HCMV infection has been linked to alterations in glucose metabolism. Studies have shown that HCMV infection can affect glucose uptake and utilization in various cell types, including adipocytes and skeletal muscle cells. These metabolic changes may contribute to the development of insulin resistance and ultimately diabetes [15]. Additionally, HCMV infection has been associated with other risk factors for diabetes, such as obesity and dyslipidemia. HCMV has been detected in adipose tissue, where it may exacerbate inflammation and insulin resistance, further predisposing individuals to diabetes [14].

However, while numerous studies have reported associations between HCMV infection and diabetes, not all studies have found consistent results, and the causal relationship between HCMV infection and diabetes remains to be fully elucidated [15]. Further research is needed to clarify the mechanisms by which HCMV may contribute to the development of diabetes and to determine the clinical significance of HCMV screening and treatment in individuals at risk for diabetes [16]. In this case-control study we found that 99 % of patients were positive to anti-HCMV IgG and 94 % of control group with a significant difference. This result agreed with a previous study [17] in Texas which found that 97.6 % of patients with diabetes had anti-HCMV IgG antibodies. The high occurrence of HCMV among diabetic patients can be attributed to the role of HCMV IgG in the development of type 2 diabetes. A case-control study [18] conducted in Korea found that the rate of HCMV diseases was notably higher in the group with type 2

diabetes mellitus (T2DM) compared to the group without T2DM. Moreover, the organism had been found in the arterial walls of diabetic patients more than non-diabetic individuals [19]. Nevertheless, it remains uncertain if the pancreatic HCMV virus directly impacts beta cells and inhibits the secretion of insulin, leading to diabetes, or if individuals with type 2 diabetes mellitus are more vulnerable to HCMV infection.

In the current study the anti-HCMV IgM was detected in 7 % of patients and none of controls with a highly significant difference and this result is in line with other studies, one conducted in Kirkuk governorate [20] which antibodies of anti-HCMV IgM only detected in 26.82 % of T2DM patients and none of controls and other study conducted in Diyala [21] also with a highly significant difference. These results may be due to the immunocompromised condition in diabetic patients. As shown in table 3 there was a non-significant difference between the groups according to cholesterol and in term of triglyceride and this result is in line with other study conducted in Baghdad [22] with a non-significant association too. In another study by Mendy *et al*. [23] there was a significant difference in cholesterol and triglyceride between the two groups regarding anti-HCMV IgG.

In the current study there was a non-significant association in blood urea and serum creatinine between the two groups regarding anti-HCMV IgM which is like the result [22] that there was a non-significant difference between the two groups regarding anti-HCMV IgM. In [23] and [24] studies regarding anti-HCMV IgG positivity there was a non-significant correlation according to renal function.

As seen in table 3 there was a non-significant correlation in anti-HCMV IgM according to BMI which is also similar with [22] that there was a non-significant association between the groups regarding BMI.

In table 4 the results showed that neutrophils and WBCs were slightly higher in patients who are positive to anti-HCMV IgM other than negative to it, but the difference was not significant as with the rest of parameters. Our result that correlated to hematological parameters could be novel because of lack of reference regarding anti-HCMV IgM and T2DM. However, in [24] which was according to anti-HCMV IgG positivity there was a non-significant difference in WBCs, RBCs, platelets and Hb.

As in table 6 the result of real time PCR assay showed that 94.28 % of patients with T2DM were positive to the presence of HCMV UL57 gene compared to the result of controls which was 50 % and this result is highly significant and it's in line with other study conducted in Najaf governorate that the rate of detection by PCR in patients was 42 % compared to controls (4.4 %) with a significant difference [9]. In a study conducted by Taylor and Oldstone, nucleic acid sequences that are unique to HCMV were identified in 44% of pancreatic tissue samples obtained from patients diagnosed with type 2 diabetes mellitus. A total of 49 non-diabetic controls did not possess any nucleic acid sequence [25]. These findings indicated a potential correlation between human HCMV and type 2 diabetes.

CONCLUSION

To conclude the HCMV may have a role in the etiopathogenesis of T2DM. The results was non-significant with other clinical parameters. May be needs more studies and different techniques to prove the correlation.

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