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Prevalence of Cytomegalovirus among Women with Type 2 Diabetes and Estimation of Oxidative Stress Status and Levels of Some Interleukin in Kirkuk

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Annotation: Introduction & aim: The human cytomegalovirus (HCMV) can cause cellular damage by triggering a series of inflammatory responses. Determining the levels of certain interleukins and oxidative stress indicators in women in Kirkuk City who had both type 2 diabetes and CMV was the goal of the current investigation.

Materials & Methods: In this investigation, 190 patients with type 2 diabetes had serum samples taken in order to identify human cytomegalovirus antibodies. A control group of one hundred people without diabetes in various age ranges was also included. Samples were gathered between January and May of 2025. Using the enzyme-linked immunosorbent test technique, human cytomegalovirus antibodies were found in all of these serum samples. Patients with type 2 diabetes undergoing treatment at Kirkuk City, Iraq's Special Center for Endocrine Glands and Diabetes were the study's target population.

Results: the results referred that the Cytomegalovirus infection was found in 167 (87.9%) of the 190 women with T2DM. It is found that the highest age group exposed to

infection is 41-50 years, where the percentage reached 37.7%, while the age group 51-60 years is the lowest, if it reached 12.6%. There were no significant differences (P=0.185) between the age groups. Malondialdehyde (MDA) levels in serum of T2DM patients and T2DM & CMV demonstrated significant (P <0.05) elevated compared with control women. While, the levels of glutathione and catalase exhibited a significant (P <0.05) reduced in T2DM patients and T2DM & CMV compared with healthy women. For interleukins, IL-1beta and IL-17 in serum of T2DM patients and T2DM & CMV demonstrated significant (P <0.05) elevated compared with control women.

Conclusions: The study concludes that there is a widespread prevalence of cytomegalovirus among women with type 2 diabetes, and that infection with the virus increases the risk of oxidative stress and raises the concentrations of interleukin in patients.

Keywords: CMV, T2DM, interleukins, oxidative stress, glutathione.

Introduction

In adulthood, cytomegalovirus (CMV), also known as human herpesvirus-5, or "HHV5," is rather prevalent and a member of the Beta-herpesvirinae family of the Herpesviridae (1). According to current knowledge, it can result in a broad spectrum of clinical syndromes, ranging from asymptomatic infection in healthy hosts to serious and sometimes lethal illness in immunocompromised people, including transplant patients (2,3). Most parts of the world are endemic for human cytomegalovirus. In various geographic locations, the sero-prevalence of HCMV ranges from 30% to 100% (4). The prevalence of CMV among women in Iraq has been determined by a number of studies; in Babylon, it ranges from 77.3% to 95.7%, whereas in Kirkuk, it reached 100% (5,6,7). The significantly higher prevalence rate in this study is in line with Iraq's high prevalence rate of over 95%. While screening pregnant women for CMV antibodies is still debatable, varying rates of exposure to the virus and, consequently, varying prevalence rates in different regions, even within the same nation, may emphasize the need for new policies for the control and management of CMV infection (8). The widespread metabolic

disease known as type 2 diabetes mellitus (T2DM) is brought on by insulin-sensitive tissue failure and insufficient insulin release from pancreatic β-cells (9). HCMV infection results in inflammation, which ultimately causes islets β-cells to die, demonstrating the virus's capacity to infect and kill these cells. Apoptosis and β-cell insufficiency brought on by HCMV infection may result in type 2 diabetes (10). According to recent studies, the immune system actively alters systemic metabolism in order to defend against viral infection. People with diabetes are more prone to contract CMV because of the lack of control in this system, which is believed to be a major factor in their greater vulnerability to viruses (11). One of the earliest and fastest host defense mechanisms against invading microorganisms is the innate immune response. Innate signaling processes that result in the expression of antiviral effectors and the secretion of immunologically active factors are first triggered by pattern recognition receptors (PRRs), which are initially in charge of identifying pathogen- and danger-associated molecular patterns (PAMPs and DAMPs, respectively) (12, 13). Important antimicrobial responses that have been largely conserved include the innate immune response's interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) signaling pathways (14). Numerous studies have been conducted in the field of IL-1 and TNF-α signaling, leading to a significant understanding of the signaling processes involved in these pathways (15, 16). Numerous bacteria (17) and viruses (18) have developed mechanisms to control (both stimulate and repress) the IL-1 and TNF-α signaling pathways, demonstrating the significance of these pathways for infectious organisms. A low-grade inflammatory response is brought on by elevated levels of pro-inflammatory cytokines like TNF-α, IL-6, and IL-17 (19). T cells, Th17 cells, and neutrophils are the main producers of the inflammatory cytokine interleukin-17, commonly referred to as IL-17A. The synthesis of additional pro-inflammatory cytokines and chemokines that mediate immune responses is stimulated by interleukin-17. It is unknown what part IL-17A plays in CMV infection (20). Determining the levels of certain interleukins and oxidative stress indicators in women in Kirkuk City who had both type 2 diabetes and CMV was the goal of the current investigation.

Materials & Methods

In this investigation, 190 patients with type 2 diabetes had serum samples taken in order to identify human cytomegalovirus antibodies. A control group of one hundred people without diabetes in various age ranges was also included. Samples were gathered between January and May of 2025. Using the enzyme-linked immunosorbent test technique, human cytomegalovirus antibodies were found in all of these serum samples. Patients with type 2 diabetes undergoing treatment at Kirkuk City, Iraq's Special Center for Endocrine Glands and Diabetes were the study's target population.

Inclusion criteria

Demographic information collected from the study included the age, place of residence, and family history of all women with type 2 diabetes between the ages of 20 and 60.

Exclusion criteria

The study did not include patients younger than 20 or older than 70. Individuals who have had organ transplants, immunodeficiency disorders, autoimmune diseases, chronic illnesses, or other established causes of secondary diabetes are not included.

Blood collection

After a fifteen-minute centrifugation at 300 rpm, five milliliters (ml) of venous blood were drawn using disposable plastic syringes. Before analysis, the separated sera were frozen at -20 OC.

Measurements

- Cytomegalovirus-IgG (CMV-IgG): ELISA Kit (SUNLONG, China) uses Sandwich-ELISA to measure CMV-IgG in human serum and plasma.
- Malondialdehyde (MDA): ELISA Kit (SUNLONG, China) uses Sandwich-ELISA to measure MDA in human serum and plasma.
- Glutathione (GSH): ELISA Kit (SUNLONG, China) uses Sandwich-ELISA to measure GSH in human serum and plasma.
- Catalase (CAT): ELISA Kit (SUNLONG, China) uses Sandwich-ELISA to measure CAT in human serum and plasma.
- Intetlukin-1 beta (IL-1β): ELISA Kit (SUNLONG, China) uses Sandwich-ELISA to measure IL-1β in human serum and plasma.
- Intetlukin-17 (IL-17): ELISA Kit (SUNLONG, China) uses Sandwich-ELISA to measure IL-17 in human serum and plasma.

Statistical analysis

The independent t-test, spearman test, and ANOVA table were used to express significant changes at a probability threshold of 0.05 using the SPSS version of the computer program. A M ± SE was used to represent the results (21, 22).

Results & Discussion

Table 1 shows the prevalence of cytomegalovirus among patients with type 2 diabetes. Cytomegalovirus infection was found in 167 (87.9%) of the 190 women with T2DM, while 23 (22.1%) were negative for CMV-IgG using ELISA.

Table (1): prevalence of CMV-IgG among T2DM

Disease	CMV-IgG +ve	CMV-IgG –ve	Total
T2DM	167(87.9%)	23(22.1%)	190(100.0%)

The current study's findings are consistent with a study by Hasan and Dunya (23), which found that 82.67% of T2DM patients had a serum level of CMV-IgG antibody that was positive for anti-CMV IgG, compared to 31.43% of controls who had seropositivity for this virus. This difference was highly significant ($p < 0.01$). The results of this study are in line with prior research in this area and imply that anti-CMV-IgG antibodies may play a role in the aetiopathogenesis of type 2 diabetes. The association between CMV and T2DM is explained by age and other diabetes risk factors. According to one of these studies, which was published by Schmidt et al. (24) and found that among those who were cytomegalovirus-seropositive, T2DM crude odds were 47% higher than cytomegalovirus-seronegative. However, the correlation was significantly diminished and no longer significant after controlling for age and other factors.

Table 2 shows the distribution of the number of samples depending on age. It is found that the highest age group exposed to infection is 41-50 years, where the percentage reached 37.7%, while the age group 51-60 years is the lowest, if it reached 12.6%. There were no significant differences (P=0.185) between the age groups.

Table (2): prevalence of CMV-IgG according to age

Disease	No.	%	P value
20-30	37	22.2%	0.185
31-40	46	27.5%	
41-50	63	37.7%	
51-60	21	12.6%	
Total	167	100.0%	

The extremely high seroprevalence (87.9%) in this study was the reason for the lack of a meaningful association between the CMV-seroprevalence and the various age groups of women. CMV sero-prevalence in women aged 18–45 years was 51.7%, according to a study conducted in Germany by Lachmann et al. (25) that found that age was the primary factor that substantially linked with CMV seroprevalence. In contrast to women under 40, women over 40 had a greater seroprevalence of CMV, according to other North American studies. In contrast, a Mexican study of pregnant women revealed that the frequency was higher among those aged 20 to 30 than among those under 20. In Europe, however, there was no correlation seen between infection and age (26, 27).

Oxidative and antioxidant enzymes

Table (3) show the concentrations of Oxidative and antioxidant enzymes in T2DM women with CMV and healthy women, where MDA levels in serum of T2DM patients (36.24 ±4.85) and T2DM & CMV (41.6 ±5.01) demonstrated significant (P <0.05) elevated compared with control women (8.05 ±1.43), as shown in figure (1). the levels of GSH exhibited a significant (P <0.05) reduced in T2DM patients (3.1±0.92) and T2DM & CMV (3.9±0.37) compared with healthy women (16.5 ± 2.15), as shown in figure (2). the levels of catalase exhibited significant (P <0.05) reduced in T2DM patients (2.41±0.47) and T2DM & CMV (2.15±0.26) compared with healthy women (5.19±1.62), as shown in figure (3).

Table (3): the levels of Oxidative and antioxidant enzymes in studied groups

Parameter \ Groups	Control (100)	T2DM (23)	T2DM & CMV (167)	P-Value
MDA (ng/ml)	8.05 ±1.43	36.24 ±4.85	41.6 ±5.01	0.001
GSH (ng/ml)	16.5 ± 2.15	3.1±0.92	3.9±0.37	0.001
Catalase (ng/ml)	5.19±1.62	2.41±0.47	2.15±0.26	0.001

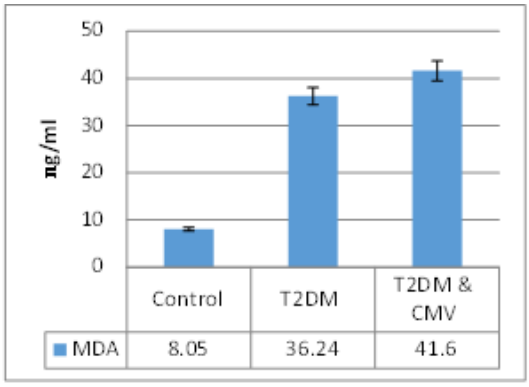

Figure (1): MDA levels in patients and control.

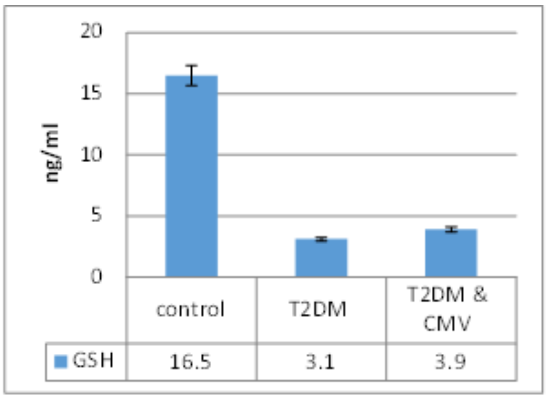

Figure (2): GSH levels in patients and control.

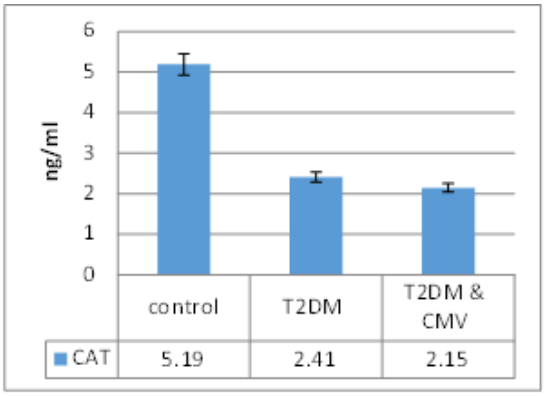

Figure (3): catalase levels in patients and control.

In my current study, we measured antioxidants (catalase and glutathione) and lipid oxidative damage marker malondialdehyde (MDA) in T2DM women and compared the results with those of healthy women in order to examine the oxidative status. A drop in GSH and catalase and an increase in MDA indicated that the diabetic group had been subjected to more oxidative stress than the non-diabetic group. These results aligned with earlier research by Najafi et al. (28), Rani & Mythili (29) that showed diabetic patients had greater levels of ROS indicators like MDA and lower levels of antioxidants. According to Vincent et al. (30), persistent hyperglycemia in diabetes mellitus (DM) promotes the overproduction of reactive oxygen species (ROS), which in turn targets lipids within cells and leads to an increase in the production and release of lipid peroxidation products such as MDA (29, 31). Additionally, the body's antioxidant defense systems are compromised by chronic hyperglycemia in DM, which lowers TAC (30). Catalase activity and fasting blood glucose were found to be significantly positively correlated in the study by Pieme et al. (32) but glutathione and fasting blood glucose were shown to be significantly negatively correlated. However, there was no link between FPG and TAC in our investigation. In contrast, MDA levels were high and glutathione and catalase levels were low in T2DM women who tested positive for CMV. According to earlier studies, a viral infection causes server oxidative stress, which is consistent with the findings of our investigation (33, 34).

interleukins

Table (4) show the concentrations of some interleukins in T2DM women with CMV and healthy women, where IL-1β levels in serum of T2DM patients (56.11 ±7.87) and T2DM & CMV (62.4 ±13.18) demonstrated significant (P <0.05) elevated compared with control women (6.15 ±0.38), as shown in figure (4). the levels of IL-17 exhibited a significant (P <0.05) elevated in T2DM patients (489.5±26.2) and T2DM & CMV (518.2±35.31) compared with healthy women (95.83 ± 10.67), as shown in figure (5).

Table (4): the levels of some interleukins in studied groups

Groups / Parameter	Control (100)	T2DM (23)	T2DM & CMV (167)	P-Value
IL-1beta (pg/ml)	6.15 ±0.38	56.11 ±7.87	62.4 ±13.18	0.001
IL-17 (pg/ml)	95.83 ± 10.67	489.5±26.2	518.2±35.31	0.001

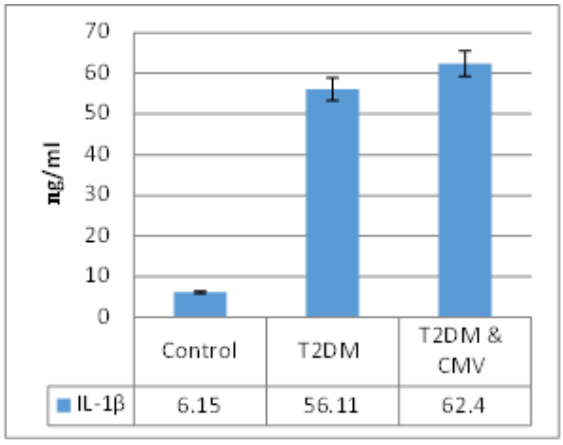

Figure (1): IL-1β levels in patients and control.

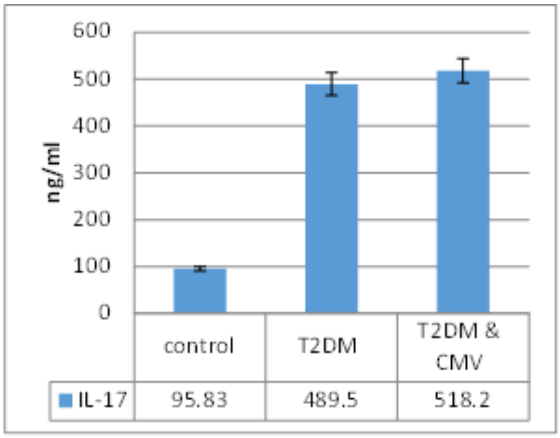

Figure (2): IL-17 levels in patients and control.

These few studies (35,36) found that IL-1β levels were directly correlated with FPG and HbA1c levels and were primarily higher in the T2DM group. The vast age scale included in the meta-analysis, variations in the patients' disease duration, or variations in the study assay or design could all be contributing factors to the substantial heterogeneity between the studies, according to the meta-analysis. Other inflammatory cytokines, such as IL-6, IL-8 (37), IL-33, and IL-18, are activated by elevated IL-1β levels in type 2 diabetes, and the pro-inflammatory milieu is amplified

(38). According to recent research, it temporarily raises insulin production, which may be detrimental to metabolism (39). However, it was discovered that CMV infection increased IL-1beta in women with type 2 diabetes. This finding is in line with that of Iwata et al. (40), who observed that CMV infection raised IL-1beta levels in patients' serum. According to Chenet et al. (41) who suggested that IL-17 may contribute to the pathophysiology of type 2 diabetes in conjunction with other inflammatory cytokines, it was discovered that CMV infection in women with the disease led to increased levels of IL-17. Thus, IL-17 may contribute to local inflammation and work in concert with these inflammatory cytokines to cause the death of β cells in the pancreas. (42).

Conclusions

The study comes to the conclusion that women with type 2 diabetes have a high incidence of cytomegalovirus, and that infection with the virus boosts the risk of oxidative stress and increases interleukin levels in patients. Additionally, no discernible variations were observed in the age factor, indicating that CMV infection can occur at any age.

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