ORIGINAL ARTICLE

Cytomegalovirus Infection and Glutamic Acid Decarboxylase Antibodies in Type 2 Diabetic patients

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ABSTRACT

Background: At present, whether human cytomegalovirus (HCMV) infection is associated with type 2 diabetes mellitus (T2DM) is debatable.
Aim: To obtain information about the prevalence of CMV IgG antibodies among type 2 diabetic patients and to identify age, gender, and laboratory differences.
Methods: This cross-sectional study was carried out among DM type2. Seven hundred eighty-three sera type 2 diabetic patients were tested for the presence of HCMV-IgM&IgG antibodies, GAD, and cytomegalovirus DNA by PCR.Chi-square test (χ2-test) was applied for testing the significant association between different variables.
Results: The study included 783 diagnosed cases of type 2 diabetic mellitus**,** 261 of them (33%) were HCMV-IgG positive. Further investigations of HCMV-IgG seropositive samples revealed that 121(46%) tested positive to HCMV-IgM with a significant statistical differences of HCMV IgM within gender (P=0.02) and age groups (P<0.001).PCR test for diagnosis of HCMV showed that 81(31%) of samples were positive. Searching for presence of GAD antibodies in all HCMV IgG positive samples revealed that 100(38%) of patients were tested positive, however, statistically there was no significant association between positive anti-GAD antibodies with age groups and gender (P>0.05)**.**
Conclusion: Our study determined that the higher prevalence of HCMV IgG in DM2 patients, depending on the results of IgM & PCR testes, more than one-third of HCMV IgG seropositive were having recent HCMV infection, about one-third of type two diabetic patient were tested positive to anti-GAD antibodies.
Keywords: HCMV, type 2 DM, anti-GAD antibodies,

INTRODUCTION

he worldwide prevalence of type 2 diabetes mellitus (T2DM) is estimated to have multiplied over the past decades and now includes rapidly increasing numbers of younger age groups. The condition is considerably more complex with much earlier onset, since there is a higher overall risk of life-time complications [1]. T2DM prevalence has risen faster in low and middle-income countries than in high-income countries[2]. Cytomegalovirus (CMV)is a virus found around the world.Itis a ubiquitous beta-herpes virus and herpesviridae that infects the majority of humans[3]. It is a common virus that infects people of all ages.CMV infection iscommonly asymptomatic in healthy individual,but can cause severe disease in immunocompromised children or adultsand in newborn. A person can also be re-infected with a different strain (variety) of the virus[4].Active CMV infection or reactivation from a latent state is considered potentially a cofactor for inflammatory disease [5].CMV may accelerate immune responses by prompting the accumulation of late-differentiated CD8+ and CD4+ T-cells which produce pro-inflammatory cytokines and thereby create a more pro-inflammatory background that might be causing T2DMonset[6].A Glutamic Acid Decarboxylase(GAD)is an enzyme that present in the pancreas and the nervous system,its aids the body produce a specific neurotransmitter called gamma-aminobutyric acid (GABA), which is an amino acid that decreases the extent of communication to and from the nerves.GAD can also trigger the immune system to produce autoantibodies against healthy cells[7]. GAD antibodies test is helping to discover whether someone has either Latent Autoimmune Diabetes of Adulthood (LADA) or type 1 diabetes. The test is done to define which type of diabetes someone has.It is particularly useful for adults more than 30 years who get diabetes where diagnosis of T2DM is uncertain[8].This research aims toobtain information about the prevalence of CMV IgG antibodies among type 2 diabetic patientsand to identify age, gender, and laboratory differences.

MATERIALS AND METHOD

This was a cross-sectional study that conducted in diabetes and endocrine gland center in AL-Sader teaching hospital in Al-Najaf Province during the period from January to July2019.The caseswereascertained as type 2 DM andchecked throughtheir hospitalrecords. All type 2 diabetic patients attendedthediabetic outpatientclinic during the period of the study were included.

We tested seven hundred eighty-three sera from type 2 diabetic patients aged from 20 to 70 years, two hundred sixty-one of them were founding Gpositive for HCMV .IgGsero positive samples were collected to determine glutamic acid decarboxylase (GAD) antibodies and HCMVIgM antibodies. Anti-GAD antibody and specific anti-cytomegalovirus antibody (IgG and IgM) were detected by the ELISA test kit. (BioCheck,Inc.). Wholly blood samples with EDTA were detecting cytomegalovirus DNA. DNA extraction kit and PCR amplification kit are (DNA –Sorb-B)where supplied by Sacaca Biotechnology, Italy. DNA isolation and PCR amplification and thermocycling

conditions and procedures were done according to the instruction of the kit. The PCR product was amplified and detected by ethidium bromide staining visualization in agarose gel electrophoresis. The researcher took approval from Al-Najaf Health Directorate before the collection of data. The purpose of the research was clarified toall participants and formal consent was took fromthem.

Statistical analysis: Data were stored and analyzed through the Statistical Package for the Social Science, SPSS (version 20) program. The results were represented through tables and graphs including frequency and percentage.Chi-square test (χ2-test) was applied for testing the significant association between different categorical variables. Statistical significance was considered if the p-value equal to or less than 0.05.

RESULTS

The studied sample compromised 783 type 2 diabetic patients, 33% of them (261/783) were HCMV IgG positive. Their ages ranged from 20 to 69 years, about one-third of them (32.2%)werewithin the age group (50-59) years.The results showed that121(46%)of the patients seropositive to HCMV-IgM antibody, whereas 81(31%), and 100(38%)of patients were tested positive to GAD antibody, and PCR respectively(Table1).Table(2) demonstrated that 76 (62.8%) of males and 45(37.2%) of females were seropositive to GAD antibodies, although statistically there is no significant association(P>0.05). Furthermore, testing of HCMV IgM positivity revealed that males were higher in frequency than females, 69.%vs 31.0% with significant statistical difference (P=0.02).

More than one third (40%)of HCMV IgM positive patients were within the age group (60-69) years,(figure1).Statistical analysis showed a highly significant differences of HCMV IgM positivity between different age groups (P<0.001).Seropositivity to GAD antibodies was highly observed within age groups (50-59), and (60-69) years (figure2). However, there were no statistical significant differences between different age groups and the result of GAD antibody testing (p>0.05). PCR testing revealed that 100 patients areHCMV positive, whodistributed within different age groups, more than one-quarter of them (28%) was within age group (50-59) years, (figure3). Statistically, there were no significant differencesbetween age groups according to the result of the PCR test (P>0.05).

Table1: Demographic and laboratory features ofHCMV IgGPositive Diabeticpatient (N=261).

Features	n	%age
Gender		
Male	159	61
Female	102	39
Age groups		
20 – 29	12	4.6
30 – 39	45	17.2
40 – 49	54	20.7
50 – 59	84	32.2
60 – 69	66	25.3
GAD antibody		
GAD +	81	31.0
GAD –	180	69.0
HCMV-IgM		
IgM +	121	46.0
IgM–	140	54.0
PCR		
PCR+	100	38.0
PCR–	161	62.0

Table 2: Comparison HCMV seropositive males and females according to the result of GAD, and HCMV IgM antibodies.

	Male%	Female%	X2	P value
GAD +	76 (62.8)	45 (37.2)	0.34	0.56
GAD -	83 (59.3)	57 (40.7)		
IgM +	83 (69.0)	38(31.0)	5.58	0.02*
IgM -	76(54.3)	64 (45.7)		
Total	159 (60.9)	102 (39.1)		

*significant

Figure 1 :The results of HCMV detection by IgM antibodies according to age groups.

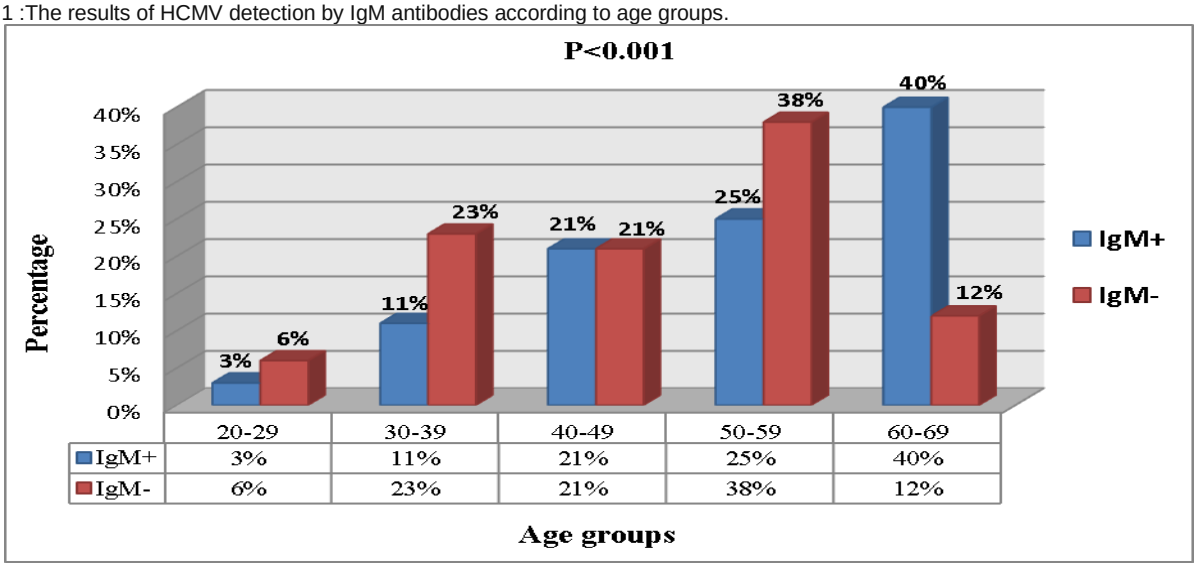


Figure 2: The results of GAD diabetic patients antibodies according to age groups.

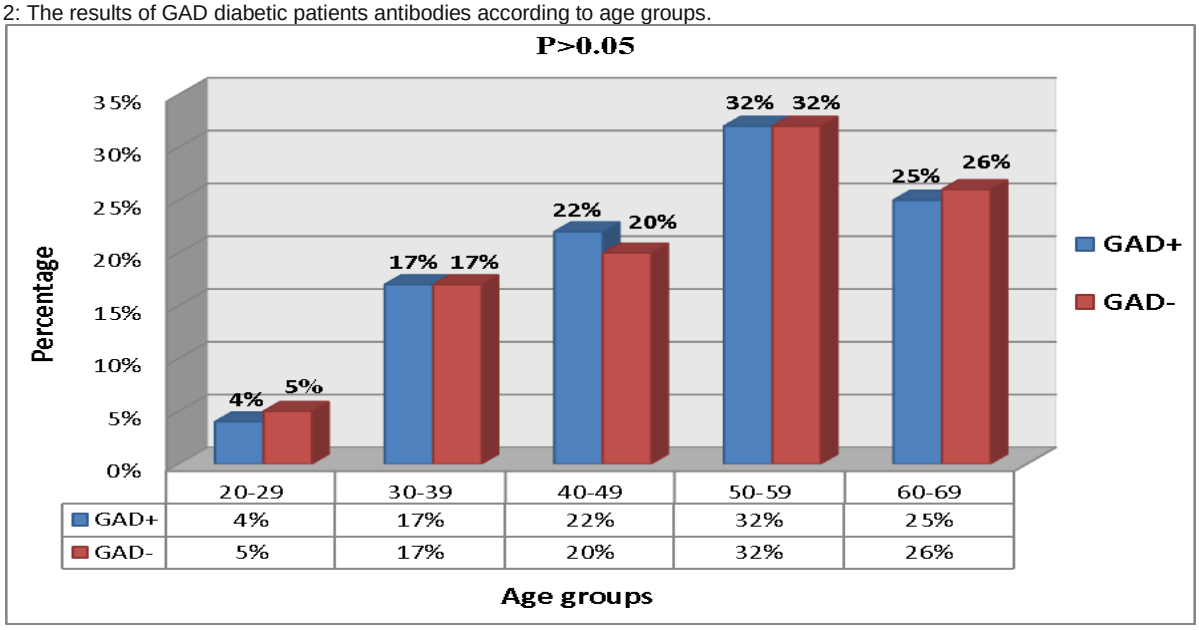
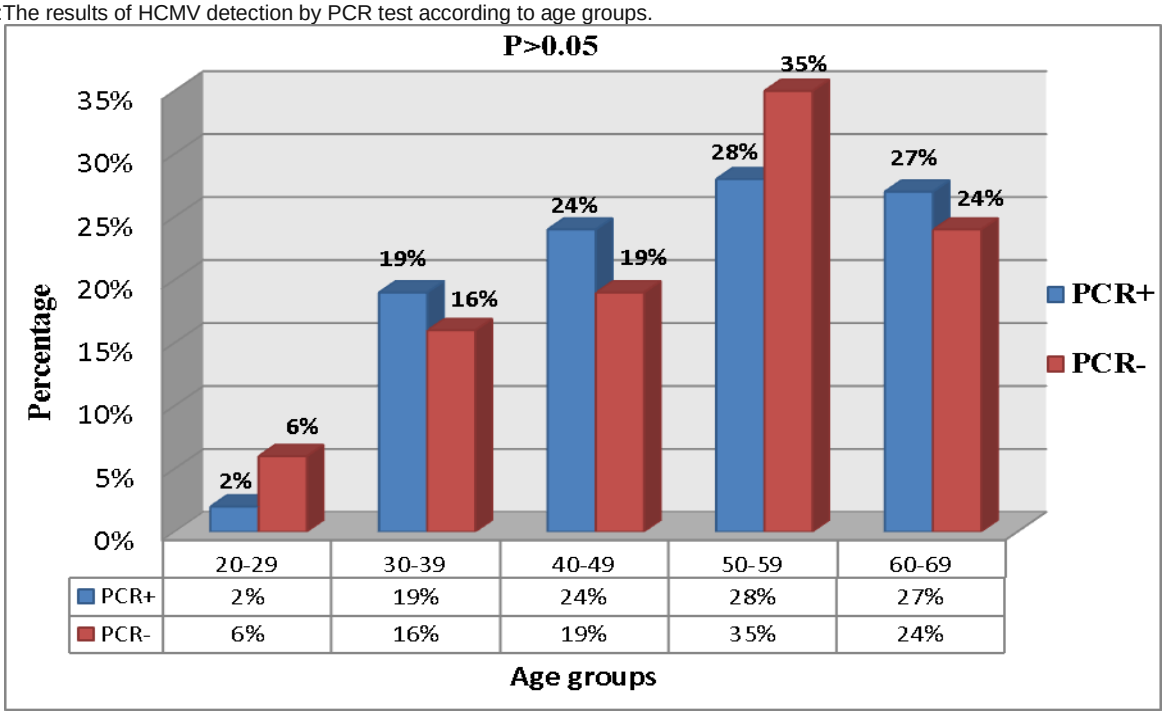


Figure 3:The results of HCMV detection by PCR test according to age groups.



DISCUSSION

CMV is a highly prevalent herpes virus that infects persons from 30% to 100% in worldwide populations[9]. The evidence of high prevalence of human cytomegalovirus (HCMV) infection in T2DM patients is the existing of HCMV DNA in b cells of pancreas suggests that viral infection plays a significant role in the establishment of T2DM[10].

Our findings demonstrated that the high prevalence of CMV infections (33%) coincided with T2DM, which was similar to findings of other studies conducted in Iraq[11,12] and globally[13,14,15]. A previous research estimating anti-CMV IgG found that a higher frequency of CMV seropositive status and titer among T2DM patients than healthy controls (OR, 2 to 12). Likewise, CMV seropositivity was related with higher glucose levels[16]. However, these researches may have substantial limitations and are underpowered due to rare CMV events,the small sample size, and because the specific subjects do not reflect the universal population.

The pathophysiological mechanisms of post-CMV T2DM stay questionable, in spite of the fact thatresearchers[17] showed that CMV genes localized in the pancreatic islets of Langerhans cells without

cytopathicinflammation[18]. Arecent study reported that direct pancreatic β-cell injury was associated with the immunologic consequences of CMV infection, supported by T lymphocyte infiltration[19]. Therole of CMV infection in the development of T2DM could be due todirect and/or indirect pancreatic damages throughthe effect of CMV infection on the failure of islet allografts to support the active CMV replication[20]. In the contrast, the materials that used in the envelope components like viperin, was assumed to change the pathways of lipid and glucose metabolism[21].

Researchers reported different evidences that support a possiblerelationship between CMVand diabetes.CMV nucleic acids have been demonstrated more often in the arterial walls of diabetic patients versus controls[22]. Also, CMV DNA has been identified in the pancreas of individuals with T2DM; however, it is uncertain whether CMV damages b cells in the pancreas and reduces insulin release, leading to diabetes, or whether diabetic persons are more prone to CMV infection[23]. Another mechanism may be explained by immunological response to CMV infection, including T cells cross-reacting with a CMV-binding protein, activating cytokine production and inflammatory response[24].

The current research showed that higher HCMV IgG seropositivity among male beyond the age group (50-59) years this was similar to findings of previous studies[11,12,14].

In this study, HCMV-IgM was tested in all IgG seropositive type 2 diabetic patients, 46% of them were positive for IgM. which was similar to findings as those reported by Bertram& Irina[25].However; current results found that HCMV-IgM positive test was higher than PCR (46%) vs (38%), this could be attributed to False-positive IgM reactions that might be due to antigenic cross-reactivity among the herpes viruses, including CMV and EBV[26]. Regarding gender distribution, Previous researches[12,13] reported no significant differences in the rate of recent CMV infection among diabetic patients, current findings showed that HCMV IgMseropositivity was significantly higher in males than females(69%) vs (31%), (P=0.02).

Testing of HCMV IgM according to different age groups revealed that seropositivity was higher in percentage (40%) among the older age groups (60-69) years, statistically, there weresignificant differences of HCMV IgM between different age groups(p<0.001). This finding may be attributed to lower immunity in the older patients, or due to associated chronic conditions that play a role in decreasing the body's ability to resist the virus, Individuals remain infected for life with latent CMV, which have reactivated later[12].

According to the result of the PCR test.the highest percentage of CMV- DNA detection among age group (50-59) years than other age groups with no significant differences(P>0.05). The PCR is a reliable and applicable tool for the detection of HCMV in the blood of the diabetic patients. Quantitative PCR shows to be superior, faster and highly sensitive than measurement of antibodies titer for the diagnosis of CMV infection[27].

In the present study, GAD-Ab was tested in all HCMV IgG positive type 2 diabetic patients, the results showed that 31% of patients were positive for GAD which represents higher than findings of Iraqi research that recorded anti-GAD IgG frequency of 14.8%[28]. Other researchesshowed prevalence of anti-GAD IgG among type two DM ranged from 12.6% to 37%[29].Type 2 diabetic patients who were tested positive for GAD auto-antibody were distributed within different age groups with the highest percentage within age group (50-59) years, although there were no significant statistical association between CMV infection and age groups(P>0.05).One factor could be explained the current result that having inherited TCF7L2, which is the risk allele of the gene with the strongest relationship with T2DM, it has been found to reduce insulin secretion and it was as common in LADA as in T2DM[30].

The current research has important limitations. First, since it is a cross-sectional study so the temporal relationship cannot be inferred, we did not distinguish whetherCMV infection occurs before or after type two DM. This research was more complicated by the using IgG seropositivity for diagnosis of CMV infection because IgG indicates an old infection, however, further confirmatory tests were done including PCR and IgM. Large sample size (783) type 2 diabetic patients is one of the strengths of this study.

CONCLUSION

High prevalence of HCMVIgG antibodies among type two diabetic patients, more than one-third of HCMV IgG seropositive were having recent HCMV infection depending on the results of IgM& PCR testes, there are gender and age differences in distribution of recent CMV infection, about one-third of type two diabetic patient were tested positive to anti-GAD antibodies, although there was no significant association with gender and age groups.

Conflicts of interest: None of the authors have any conflicts of interest relevant to this research subject.

Ethical clearance: The study was conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki. The study protocoland subject information were reviewed and approved by a local Ethics Committee.

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