

Received: 2015.09.23  
Accepted: 2015.12.11  
Published: 2015.12.30

# High Human Cytomegalovirus IgG Level is Associated with Increased Incidence of Diabetic Atherosclerosis in Type 2 Diabetes Mellitus Patients

Authors' Contribution:  
Study Design A  
Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
Literature Search F  
Funds Collection G

ABCDEF 1,2 **Jun Zhang**  
AD 3 **Yuan-yuan Liu**  
AB 4 **Hui-ling Sun**  
AB 3 **Shan Li**  
AD 3 **Hai-rong Xiong**  
AD 3 **Zhan-qiu Yang**  
AB 4 **Guang-da Xiang**  
AFG 1,2 **Xiao-jing Jiang**

1 Graduate School, Southern Medical University, Guangzhou, Guangdong, P.R. China  
2 Department of Infectious Diseases, Wuhan General Hospital of Guangzhou Military Command, Wuhan, Hubei, P.R. China  
3 State Key Laboratory of Virology, Institute of Medical Virology, School of Medicine, Wuhan University, Wuhan, Hubei, P.R. China  
4 Department of Endocrinology, Wuhan General Hospital of Guangzhou Military Command, Wuhan, Hubei, China

**Corresponding Author:** Xiao-jing Jiang; e-mail: xjjiang2003@126.com

**Source of support:** This study was supported by the National Natural Science Foundation of China (No: 81171586) and the Natural Science Foundation of Hubei Province (2010CDB09205)

**Background:** At present, whether human cytomegalovirus (HCMV) infection is associated with type 2 diabetes mellitus (T2DM) is debatable. The effect of active HCMV infection on glucose regulation has been poorly studied. Although HCMV infection is correlated with atherosclerosis in cardiovascular disease, the role of HCMV infection in the development of diabetic atherosclerosis in T2DM is unclear and is usually neglected by endocrinologists. The aim of this study was to assess the effects of HCMV infection on glucose regulation and the development of diabetic atherosclerosis in T2DM patients.





**Material/Methods:** A total of 222 hospitalized T2DM patients were enrolled. Nested polymerase chain reactions were used to detect HCMV DNA extracted from peripheral blood leukocytes. Quantitative real-time PCR was used to determine viral load. HCMV IgG antibody concentrations were analyzed by chemiluminescence immunoassay.

**Results:** HCMV active infection, viral load, and HCMV IgG titers were not correlated with glucose regulation. Binary logistic regression demonstrated that the highest quartile of HCMV IgG concentration (>500 U/ml) was correlated with the incidence of diabetic atherosclerosis (OR: 8.0, 95%CI: 2.3–27.2), and that titer >127U/ml of HCMV IgG is an independent predictor for the development of diabetic atherosclerosis in T2DM patients (OR: 4.6, 95%CI: 1.9–11.3) after adjustment for all potential confounding factors.

**Conclusions:** Active HCMV infection is unlikely to influence glucose regulation in T2DM. However, HCMV IgG titers are associated with the incidence of diabetic atherosclerosis, and titer >127U/ml of HCMV IgG might be an independent risk factor for the development of diabetic atherosclerosis in T2DM patients.

**MeSH Keywords:** **Atherosclerosis • Cytomegalovirus • Diabetes Mellitus, Type 2**

**Full-text PDF:** <http://www.medscimonit.com/abstract/index/idArt/896071>

 3665  4  1  61



## Background

T2DM is one of the most prevalent chronic diseases worldwide and accounts for 90–95% of all diabetes cases. It encompasses individuals who have insulin resistance and usually have relative rather than absolute insulin deficiency. Genetic and environmental factors are both involved in the development of T2DM. However, the underlying mechanism of T2DM is poorly understood, and the present prevention and treatment of the disease are not effective. The evidence of high prevalence of human cytomegalovirus (HCMV) infection in T2DM patients and HCMV DNA existing in  $\beta$  cells suggest that viral infection plays an important role in the development of the T2DM [1,2].

HCMV is a member of the beta-herpesvirus family, which contains a large double-stranded DNA genome [3]. HCMV is ubiquitous, with seropositivity rates ranging from 40% to 100% in adults globally [4]. Like other herpesviruses, HCMV establishes latent infection after primary infection and remains in hosts throughout life. HCMV diseases are related to host immune status. Among immunocompetent hosts, HCMV infection is mostly an asymptomatic infection. However, as an important opportunistic pathogen, it is a major cause of morbidity and mortality among immunocompromised patients, such as patients with acquired immunodeficiency syndrome (AIDS) and recipients of solid organ and stem cell transplantation [5–7]. Congenital HCMV infection is a leading non-genetic cause of sensorineural hearing loss in children.

Previous research has found that nucleic acid sequences specific for HCMV can be located in the islets of the pancreas [2]. HCMV infection can result in inflammation by inducing immune reactions, eventually those leading to apoptosis of islets  $\beta$ -cells [8,9], suggesting that HCMV can infect and damage  $\beta$ -cells. Therefore, HCMV infection, by causing the deficit and increased apoptosis of  $\beta$ -cells, may be associated with T2DM [10]. However, the effect of human cytomegalovirus infection on T2DM remains unclear and controversial [1,2,11–13]. Clinical studies have shown an association between HCMV infection and T2DM [1,11]. HCMV seropositivity with detectable IgG antibodies to HCMV correspond with hemoglobin A1C (HbA1c) and non-fasting glucose levels in patients with T2DM [11]. However, other studies have not found this same correlation [12–14]. Moreover, little is known about the role of acute HCMV infection in development of T2DM.

Previous studies [15,16] confirmed that accumulated HCMV burden, which normally presents as high HCMV IgG titers, is linked to a variety of chronic diseases or symptoms, including increased rate of cognitive decline [17], functional impairment (e.g., in activities of daily living) [18], frailty in older women [19], and all-cause and cardiovascular disease mortality [19,20]. High HCMV IgG titer also has a significant association with hypertension and

coronary artery disease in cardiovascular disease (CVD) [21–23]. CVD is a common and severe complication in T2DM patients. Metabolisms, hypertension, ageing, overweight, and obesity have been confirmed to be risk factors for CVD in diabetes patients [24,25]. However, the effect of high HCMV IgG titers on development of atherosclerosis in T2DM patients has received little research attention and remains undefined.

Given the high prevalence of HCMV infection in people with T2DM worldwide [1,11,13,14], and in view of the controversial and unclear relationship between HCMV infection and T2DM, we conducted a cross-sectional study in patients with T2DM. Our aims were to assess the effects of active HCMV infection on glucose regulation in people with T2DM and to study the role of HCMV infection in development of diabetic atherosclerosis among people with T2DM.

## Material and Methods

### Patients and data collection

A total of 222 hospitalized patients with diabetes mellitus type 2 treated at the Department of Endocrinology, Wuhan General Hospital of Guangzhou Military Command were recruited into the study between March 2014 and July 2014. The diagnosis of T2DM was based on the Diagnosis and Classification of Diabetes Mellitus formulated by the American Diabetes Association in 2013 [26]. Patients with diagnosed malignant disease, autoimmune disease other than diabetes mellitus, or immunosuppression were excluded. The diagnosis of diabetic atherosclerosis was determined by ultrasound examination of arteries. Hypertension was diagnosed as a sitting systolic blood pressure  $\geq 140$  mm Hg and/or a diastolic blood pressure  $\geq 90$  mm Hg, or having medication history of using antihypertensive drugs. All participants voluntarily joined this study and provided informed consent. This study was approved by the Ethics Committee of Wuhan General Hospital of Guangzhou Military Command.

Clinical data were collected, including age, sex, duration of diabetes mellitus, body mass index (BMI), smoking, and history of hypertension. Blood samples were taken to test fasting blood glucose (FBG), postprandial blood glucose (PBG), C-peptide, hemoglobin A1C (HbA1c), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), and low-density lipoprotein cholesterol (LDL-c). Blood samples were also used for determining HCMV infection, including HCMV DNA and IgG antibody.

### DNA extraction and polymerase chain reaction (PCR)

DNA from peripheral blood leukocytes (PBL) (separated by Lymphoprep™, Axis-Shield) was extracted according to the

**Table 1.** Characteristics of 222 T2DM patients stratified by with and without HCMV active infection.

Characteristics	HCMV DNA		P
	Positive (n=45)	Negative (n=177)	
Age (years)	54.1±14.9	59.1±13.2	0.028*
Duration of diabetes (months)	72.0 (24.0–126.0)	72.0 (24.0–144.0)	0.795**
FBG (mmol/L)	8.1 (6.6–10.9)	7.5 (5.6–10.2)	0.142**
PBG (mmol/L)	12.8 (9.9–16.7)	13.6 (10.2–17.3)	0.630**
HbA1c (%)	8.9 (6.8–10.6)	8.2 (6.7–10.4)	0.690**
C peptide (mmol/L)	0.5 (0.4–0.9)	0.5 (0.3–0.8)	0.231**
2 h C peptide (nmol/L)	1.2 (0.8–1.7)	1.4 (0.8–1.7)	0.698**

FBG – fast blood glucose; PBG – postprandial blood glucose; HbA1c – hemoglobin A. \* Continuous variables with normal distribution were compared with Independent-Samples T Test; \*\* Continuous variables with skewed distribution were compared with a nonparametric test (Wilcoxon on rank sum test). Values are given as mean ±SD or median [range].

manufacturer's protocols (Axygen, Hangzhou, China). The nested PCR procedure and primers for amplification of HCMV IE gene were identical to our previous study (in Chinese) [27].

Samples determined to be HCMV DNA-positive were further examined for viral load by quantitative PCR performed on a CFX96 Real-time System device (BIO-RAD, USA). Primers for the HCMV (strain AD169) IE gene amplification were designed using Primer Premier 5.0 software and synthesized by the Sangon Biotech Company (Shanghai) as primer 1 (5'-tttagcagggccttagcct-3') and primer 2 (5'-gctgcatgatgtgagcaaggg-3'). Each reaction sample had a final volume of 15 µl, consisting of 7.5 µl of Master Mix, 0.3 µl primers, 4.9 µl of distilled water, and 2 µl of the respective DNA. PCR amplification was performed using these steps: initial denaturation at 94°C for 3 min, followed by 40 cycles of (denaturation at 94°C for 15 s, annealing at 62°C for 15 s, and elongation at 72°C for 15 s), and a final elongation at 72°C for 15 min. A standard graph was constructed and the HCMV DNA copy number was calculated according to a previously published protocol [23].

### Measurement of HCMV IgG titers

HCMV IgG titers were measured by use of a commercial chemiluminescence test kit (Anti-Cytomegalovirus Antibody, immediate early, clone 6F8.2, Roche Diagnostics GmbH) according to the manufacturer's directions. Among the 222 patients, IgG titers were measured successfully in 206 participants (16 patients did not have HCMV IgG concentration tested due to hemolysis); 204 were seropositive and the concentration of HCMV IgG was >1 U/ml (the remaining 2 participants were <1 U/ml, reflecting no prior HCMV infection).

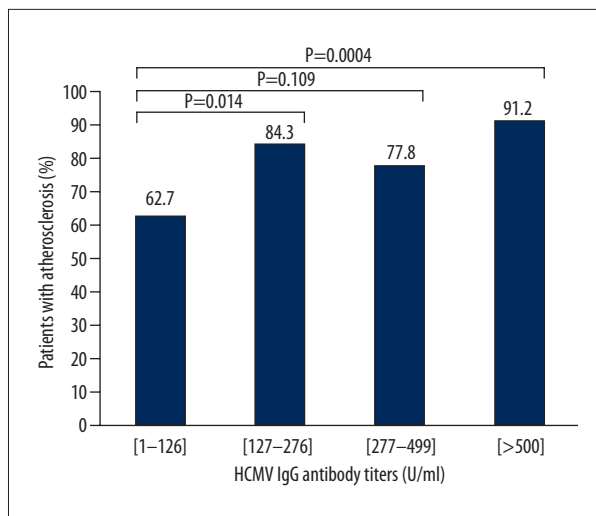
### Statistical analysis

For categorical variables, Fisher's exact test and the chi squared test were used. For continuous variables, the *t* test, Kruskal-Wallis ANOVA, and Mann-Whitney U test were used, as appropriate. Binary logistic regression was used to evaluate the risk of atherosclerosis. Two-tailed *p* values of below 0.05 were considered significant. The analysis was done using SPSS version 20.0 statistical software (IBM Corporation, Armonk, NY). Statistical methods partially refer to this study [20,28].

## Results

### The prevalence of HCMV infection and the effects of active HCMV infection on glucose regulation in the T2DM population

In the present study, the seroprevalence of HCMV IgG was 99.0% (204/206), indicating a very high prevalence of HCMV infection in the T2DM population. To determine if there is an association between HCMV infection and T2DM, we first investigated the effect of active HCMV infection on glucose regulation in T2DM. Detection of HCMV DNA in serum by nested PCR is considered as a marker of active HCMV infection. The incidence of active HCMV infection was 20.3% (45/222). The patients with active HCMV infection were significantly younger than the patients without HCMV active infection (54.1±14.9 and 59.1±13.2, respectively) (*P*=0.028). We also compared the duration of the T2DM, FBG, PBG, HbA1c, and C peptide or 2-h C peptide, and no statistically significant differences were observed between the 2 groups (with and without active HCMV infection) (Table 1).



**Figure 1.** Incidence of diabetic atherosclerosis with different CMV IgG titers in T2DM.

### The effect of HCMV IgG antibody titers on diabetic atherosclerosis in T2DM patients

Active HCMV infection showed no correlation with the glucose regulation according to the aforementioned results. Therefore, we further investigated whether persistent HCMV infection had an effect on the diabetic atherosclerosis of T2DM, using HCMV-specific IgG as an indicator for long-term HCMV infection. HCMV IgG titers were detected in 206 serum samples from 206 T2DM patients; 99.0% (204/206) of patients showed evidence of HCMV infection (HCMV IgG titer >1 U/ml). Patients seropositive for HCMV IgG were further categorized into 4 groups according to quartiles of HCMV antibody concentrations (U/ml): group 1 with 51 patients (HCMV antibody concentrations 1–126 U/ml), group 2 with 51 patients (127–276 U/ml), group 3 with 45 patients (277–499 U/ml), and group 4 with 57 patients (>500 U/ml). The prevalence of diabetic atherosclerosis in different HCMV IgG concentration groups was studied and we found that the prevalence of the atherosclerosis was significantly different among these 4 groups ( $P=0.002$ ). We further examined the incidence of diabetic atherosclerosis within the groups, and significant differences were observed between group 1 and group 2 ( $P=0.014$ ), as well as between group 1 and group 4 ( $P=0.0004$ ). However, no significant difference was found between group 1 and group 3 ( $P=0.109$ ). Patients with higher HCMV IgG concentrations were more likely to develop atherosclerosis (Figure 1).

### Analysis of factors affecting diabetic atherosclerosis in T2DM patients

According to the results shown above, a significant difference was found in atherosclerosis morbidity among the 4 groups with different HCMV IgG titers, suggesting that HCMV IgG

concentration is associated with the development of diabetic atherosclerosis. We further analyzed the potential confounding risk factors of atherosclerosis in T2DM patients with and without atherosclerosis. Therefore, clinical data (sex, age, smoking, hypertension, BMI, TC, TG, HDL-c, LDL-c, and lipoprotein) of 222 patients with T2DM were studied. The data showed that the median HCMV IgG titers were higher in the atherosclerosis group than in the group without atherosclerosis [313.6 (159.2 – >500) U/ml vs. 159.2 (60.3–366.1) U/ml,  $P=0.00045$ ]. The patients with atherosclerosis ( $61.4\pm 11.3$  years) were older than the patients without atherosclerosis ( $45.7\pm 14.6$  years), ( $P<0.001$ ). The group of patients with atherosclerosis had a longer duration of T2DM ( $P=0.001$ ) and were more likely to have hypertension than the patients without atherosclerosis ( $P=0.006$ ). In addition, BMI, smoking, TC, TG, LDL-c, lipoprotein, and sex did not differ significantly between patients with and without diabetic atherosclerosis, except for HDL-c (Table 2).

### High HCMV IgG titer is an independent risk factor for the development of diabetic atherosclerosis

To determine whether HCMV IgG titer is an independent risk factor for the development of diabetic atherosclerosis, we used binary logistic regression to adjust for potential confounding factors. According to the above results, risk factors for atherosclerosis, such as age, duration of DM, hypertension, HDL-c, and HCMV IgG, were included in a multiple forward stepwise logistic regression analysis. In this analysis, age (in years) was categorized as: group 1 (19–49), group 2 (50–58), group 3 (59–67), and group 4 (68–88), according to quartile. Duration of DM (in months) was dichotomized as group 1 (1–24), group 2 (25–72), group 3 (73–132), and group 4 (133 and above) according to quartile. The variables of HDL-c and hypertension were defined as abnormal or normal. HCMV IgG titer was initially categorized into 4 groups according to quartile: IgG titers (U/ml) <126, 127–276, 277–499, and >500.

Higher IgG titer was an independent risk factor of atherosclerosis after adjustment for confounding risk factors (except for age) compared with the lowest HCMV IgG quartile (<126 U/ml). After full adjustment (further adjusted for age), patients with HCMV IgG titers >500 U/ml and 127–276 U/ml IgG titer were significantly associated with atherosclerosis. However, the p-value (0.073) was on the boundary for HCMV IgG titers of quartile 3 (277–499 U/ml) compared with the lowest quartile after adjustment for age (OR=2.6, 95%CI: 0.9–7.5). We further categorized the IgG titers into 2 groups (<126 U/ml and >127 U/ml); HCMV antibody titers were significantly associated with diabetic atherosclerosis both in the unadjusted analysis and after adjustment for all confounding factors analysis ( $P=0.001$  and  $P=0.001$ , respectively), and patients with HCMV IgG titers >127 U/ml have a median-fold of 4.6 (95%CI: 1.9–11.3) increased incidence of atherosclerosis. Therefore, titer >127

**Table 2.** Clinical characteristic and biological data in T2DM patients with and without diabetic atherosclerosis.

Characteristics	Patients with atherosclerosis (n=175)		Patients without atherosclerosis (n=47)		P
	Mean	Range	Mean	Range	
Age (years)	61.4	±11.3	45.7	±14.6	<0.001*
Duration of DM (months)	84.0	(24.0–156.0)	48.0	(3.0–84.0)	0.001**
HCMV IgG titer (U/ml)	315.4	(162.1–500.0)	152.9	(60.3–366.1)	0.0003**
BMI (kg/m <sup>2</sup> )	25.2	±4.1	25.1	±3.9	0.863*
Hypertension (%)	106	(60.6)	17	(36.2)	0.003***
Smoke (%)	40	(22.9)	10	(21.3)	0.818***
HCMV (%)	33	(18.9)	12	(25.5)	0.312***
Man (%)	95	(54.3)	29	(61.7)	0.363***
TC (mmol/L)	4.8	(4.0–5.8)	4.9	(3.9–5.5)	0.593**
TG (mmol/L)	1.3	(0.9–2.0)	1.4	(0.9–2.2)	0.589**
HDL-c (mmol/L)	1.1	(0.9–1.3)	1.0	(0.9–1.1)	0.008**
LDL-c (mmol/L)	2.36	±0.79	2.27	±0.99	0.561*
Lipoprotein (mg/L)	122.0	(45.5–280.5)	103.5	(52.3–200.0)	0.490**

TC – total cholesterol; TG – triglycerides; HDL-c – high density lipoprotein (HDL) cholesterol; LDL-c – low density lipoprotein (LDL) cholesterol. \* Continuous variables with normal distribution were compared by Independent-Samples T Test; \*\* Continuous variables with skewed distribution were compared by a nonparametric test (Wilcoxon rank sum test). Values are given as mean ±SD or median [range]; \*\*\* Proportions were compared by a  $\chi^2$  test.

**Table 3.** Hazard ratio for incidence of atherosclerosis by HCMV IgG titers.

	Model 1		Model 2		Model 3	
	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
HCMV IgG titers (U/ml)						
1–126	1 (reference)*		1 (reference)**		1 (reference)***	
127–276	3.2 (1.2–8.2)	0.016	4.5 (1.513.4)	0.006	4.1 (1.3–13.1)	0.017
277–499	2.1 (0.85.1)	0.113	3.2 (1.2–8.8)	0.026	2.6 (0.9–7.5)	0.073
>500	6.2 (2.1–18.2)	0.001	8.1 (2.526.1)	< 0.001	8.0 (2.3–27.2)	0.001

OR – odds ratio; CI – confidence interval. Binary logistic regression was used for analyzing the role of HCMV IgG levels in the incidence of atherosclerosis. Model 1 – unadjusted for all the confounder factors; Model 2 – adjust for duration of diabetes, hypertension, HDL-C; Model 3 – further adjusted for age. \* P=0.005; \*\* P=0.002; \*\*\* P=0.006.

**Table 4.** The titer >127 U/ml of HCMV IgG is an independent risk factor for incidence of diabetic atherosclerosis.

	Model 1		Model 2	
	OR (95% CI)	P	OR (95% CI)	P
HCMV IgG titers (>127 U/ml)*	3.4 (1.6–6.9)	0.001	4.6 (1.9–11.3)	0.001

OR – odds ratio; CI – confidence interval. Binary logistic regression was used for examined the HCMV IgG Levels as an independent risk factors of atherosclerosis. \* Reference is HCMV antibody titers <127 U/ml; Model 1 – unadjusted for all the confounder factors; Model 2 – adjust for all the confounder factors.

U/ml of HCMV IgG is an independent risk factor for the development of diabetic atherosclerosis (Tables 3, 4).

## Discussion

HCMV is ubiquitously distributed worldwide and 40–100% of adults carry the virus with detectable HCMV IgG in their serum. After primary infection, HCMV establishes latency with sporadic reactivation or exogenous reinfection in the host throughout life. In the present study, the prevalence of HCMV infection in a Chinese T2DM population was quite high and the serological positivity of HCMV IgG reached 99.0%. Epidemiology data showed that seroprevalence of HCMV IgG was 94.9% in blood donors in the same region in China in 1991 [29]. This suggests that the prevalence of HCMV infection is probably higher in T2DM patients than in the general population. In addition, it has been reported that the prevalence of HCMV infection in T2DM patients is also high in other countries: 77% in the USA [13], 89.3% in the Netherlands [11], and 77% in the Czech Republic [14]. These findings raise the question of whether HCMV is one of the pathogenic agents causing T2DM. Previous studies showed controversial results regarding the pathogenic role of HCMV infection in T2DM. In various populations, a clear positive association between HCMV infection and T2DM has not yet been defined [1,11,13]. Researchers found that individuals with HCMV seropositivity were more likely to have T2DM than were HCMV-seronegative participants (17.2% vs. 7.9%,  $P=0.016$ ) among people age 85 and older [11]. An investigation in patients undergoing hemodialysis showed that T2DM patients had a higher seroprevalence of HCMV IgG (97.6%) than in patients without T2DM [1], suggesting that HCMV infection may be associated with T2DM. However, another cross-sectional study in a multi-ethnic population (Hispanic, African-American, white, and Chinese) found no association between HCMV IgG seropositivity and occurrence of T2DM, suggesting HCMV has no etiological role in the development of T2DM [13]. In addition, most studies reported that HCMV seropositivity had no correlation with glucose regulation in T2DM patients [1,13,14,30], except for the Leiden 85-plus study, which showed that T2DM patients with HCMV seropositivity had a significantly higher level of HbA1c and higher non-fasting glucose than the sero-negative patients [11]. Therefore, information regarding the long-term clinical effect of persistent HCMV infection in T2DM patients is limited. Moreover, to the best of our knowledge, little is known about the effect of active HCMV infection on glucose regulation in T2DM patients. To date, only Liang Hao et al. have evaluated the role of active HCMV infection in development of T2DM; they reported that T2DM patients with positive HCMV DNA in their serum had a significantly lower fasting C peptide level than those with HCMV-negative DNA, but no significant differences in blood glucose and insulin level were found between the 2 groups [31]. This suggests that active HCMV infection may

play a role in T2DM. In consideration of the few studies and the debatable results on the effect of active HCMV infection on T2DM, it is important to determine the role of active HCMV infection in the development of T2DM and the long-term clinical effect of persistent HCMV infection on T2DM.

In the current study, to determine if active infection indicated by sporadic reactivation and exogenous reinfection of HCMV might affect glucose regulation, we used nested PCR to amplify HCMV DNA in PBL of T2DM patients. HCMV DNA was detectable in the serum of 20.3% of patients. We found that HCMV DNA was more frequently detectable in the young group than in the older age groups. It is possible that exogenous reinfection is more easily acquired in younger populations due to lifestyle behaviors that importantly affect the epidemiology of HCMV infection. Exogenous reinfection can more easily occur when there is close contact with children less than 3 years old and when there is sexual exposure to multiple partners. Previous studies showed that HCMV viral load is highly correlated with the risk of HCMV disease in immunocompromised individuals [32–34]. In the present study, although active HCMV infection was more frequently detectable in young patients, no significant relationship was found with HCMV active infection or HCMV viral load with glucose regulation, including level of HbA1c, FBG, PBG, and C-peptide in T2DM patients. These findings suggest that active HCMV infection does not acutely impair glucose regulation in T2DM patients.

HCMV antibody may be associated with cumulative viral burden [15,16]. Previous research showed that antibody levels increase over time in individuals after viral infection [15]. Higher HCMV IgG titers are induced by frequent HCMV reactivations or long-term HCMV infection, which are generally observed in older individuals [16,35]. Our research showed that higher HCMV IgG titers in the T2DM patients aged 19–88 years had no impact on FBG, PBG, HbA1c, C peptide, or 2-h C peptide. It seems that HCMV infection is not correlated with glucose regulation in T2DM patients, and although a positive association between HCMV IgG and glucose regulation was observed in an 85-year-old individual [11], this discrepancy might be due to the differences in study populations. Cumulative viral burden is induced by long-term HCMV infection and frequent reactivation of HCMV. In addition, the impairment of pancreatic endocrine function caused by the chronic inflammation and immune response of HCMV infection also needs time to develop [36]. HCMV infection probably requires the coexistence of multiple risk factors, which is most common in the elderly; these factors importantly contribute to T2DM. Therefore, HCMV infection may more strongly influence glucose regulation in an 85-year-old than in middle-aged people with T2DM.

HCMV has a broad cell tropism and can infect endothelial cells, epithelial cells, fibroblasts cells, smooth muscle cells,

and macrophages [37,38], which are considered important in pathogenesis of vascular disease [39,40]. HCMV antigen and DNA in atherosclerotic vessels were confirmed in previous studies [41–43], suggesting that HCMV infection may damage vascular cells. A previous study demonstrated that the mechanistic contributions of HCMV in atherosclerosis involve not only “pro-atherosclerotic” effects caused by the HCMV pathogen residing in the arterial wall [44,45], but also by the increasing occurrence of systemic inflammation [46–50]. HCMV may also damage the vasculature by an immune response caused by molecular mimicry that occurs because the antigens expressed by HCMV infection are homologous to peptides expressed on uninfected host cells [51–53]. Thus, HCMV infection may play a role in progression of atherosclerosis via the aforementioned mechanisms.

Cell-specific anti-cytomegalovirus response has been found to be associated with CVD related to atherosclerosis. Recent studies have reported that male patients undergoing vascular surgery for atherosclerosis had a higher HCMV IgG titer than a matched control group of patients with high cholesterol levels [54]. Moreover, high anti-HCMV IgG titer detected by enzyme-linked immunosorbent assay (ELISA) was correlated with the carotid intima-media thickness (IMT) and elastic pressure modulus in patients with carotid atherosclerosis [55]. Therefore, higher HCMV IgG titers may predict post-coronary balloon angioplasty restenosis [56] and may become a predictor for the highest quartile of carotid intima-media thickness in HIV-infected patients with low HIV viral load after anti-virus treatment [21]. However, in the study of Puz, anti-HCMV IgG titers were not associated with atherosclerotic lesions, symptomatic stenosis, or unstable plaque in the carotid arteries [57].

Although most of the aforementioned studies demonstrated a positive relationship between high HCMV IgG level and atherosclerosis in patients with CVD, little is known about whether HCMV IgG level is an independent risk factor for diabetic atherosclerosis in T2DM. Patients with T2DM are likely to have poor glycemic control, dyslipidemia, smoking history, and hypertension; these promoters have been confirmed to be associated with atherosclerotic cardiovascular disease [58–60]. Indeed, studies found that patients with dyslipidemia, such as LDL-C  $\geq 2.6$  mmol/L, TG  $\geq 2.3$  mmol/L and HDL-C  $\leq 0.88$  mmol/L, have a significantly increased incidence of CVD [24,25]. A 1997 study by Frank et al. showed that patients with diabetes mellitus type 1 or 2 with clinical manifestations of atherosclerosis had a higher prevalence of HCMV IgG and higher IgG antibody titers than patients without atherosclerosis [61].

## References:

1. Roberts BW, Cech I: Association of type 2 diabetes mellitus and seroprevalence for cytomegalovirus. *South Med J*, 2005; 98: 686–92
2. Lohr JM, Oldstone MB: Detection of cytomegalovirus nucleic acid sequences in pancreas in type 2 diabetes. *Lancet*, 1990; 336: 644–48
3. Dolan A, Cunningham C, Hector RD et al: Genetic content of wild-type human cytomegalovirus. *J Gen Virol*, 2004; 85: 1301–12

In the present study we found that patients with higher HCMV IgG titers corresponded to the incidence of atherosclerosis in T2DM. Our positive results agree with the study of Frank et al. [61], although the ethnic groups and methods for determining HCMV IgG titer were different. Our regression analysis demonstrated that HCMV IgG titer is an independent risk factor for atherosclerosis in T2DM patients after adjustment for important covariates, such as age, duration of DM, hypertension, and HDL-c. Patients with T2DM often have hyperglycemia and dyslipidemia, which are major contributors to atherosclerosis. Our logistic regression analysis results suggest that T2DM patients with HCMV infection are more likely to develop atherosclerosis. Risk factors such as hypertension, smoking, dyslipidemia, and aging, which have been generally been the focus of endocrinologists in preventing atherosclerosis, are increasing the occurrence of diabetic atherosclerosis in T2DM. However, HCMV infection is poorly understood and should receive greater attention. Importantly, we found that titer  $>127$  U/ml of HCMV IgG is an independent risk factor for diabetic atherosclerosis in T2DM patients (OR=4.6, 95%CI: 1.9–11.3). This could help guide preventive treatment in order to avoid the serious consequences of atherosclerosis in T2DM patients. This might have the potential to lead to a new treatment for vascular complications, directed at antiviral therapy of HCMV or prevention by vaccination in T2DM patients.

## Conclusions

The present study demonstrates that active infection and reactivation of HCMV have no influence on clinical manifestations or glucose regulation in T2DM patients. However, high anti-HCMV IgG titers are associated with incidence of atherosclerosis in patients with T2DM. Titer  $>127$  U/ml of HCMV IgG might be an independent risk factor for increasing diabetic atherosclerosis in T2DM. The interpretation of our results is limited because this was a cross-sectional study. Therefore, a large-scale prospective investigation is necessary to further confirm and extend our findings.

## Acknowledgments

This study was supported by the National Natural Science Foundation of China (No. 81171586), and the Natural Science Foundation of Hubei Province (2010CDB09205). The authors wish to acknowledge the statistical expertise of Ying Ju at Huazhong University of Science and Technology Press, Ltd.

4. Cannon MJ, Schmid DS, Hyde TB: Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. *Rev Med Virol*, 2010; 20: 202–13
5. Jacobson MA, Mills J: Serious cytomegalovirus disease in the acquired immunodeficiency syndrome (AIDS). Clinical findings, diagnosis, and treatment. *Ann Intern Med*, 1988; 108: 585–94
6. Rubin RH, Tolkoff-Rubin NE, Oliver D et al: Multicenter seroepidemiologic study of the impact of cytomegalovirus infection on renal transplantation. *Transplantation*, 1985; 40: 243–49
7. Winston DJ, Ho WG, Champlin RE: Cytomegalovirus infections after allogeneic bone marrow transplantation. *Rev Infect Dis*, 1990; 12(Suppl.7): S776–92
8. Rabinovitch A, Suarez-Pinzon WL: Cytokines and their roles in pancreatic islet beta-cell destruction and insulin-dependent diabetes mellitus. *Biochem Pharmacol*, 1998; 55: 1139–49
9. Hiemstra HS, Schloot NC, van Veelen PA et al: Cytomegalovirus in autoimmunity: T cell crossreactivity to viral antigen and autoantigen glutamic acid decarboxylase. *Proc Natl Acad Sci USA*, 2001; 98: 3988–91
10. Butler AE, Janson J, Bonner-Weir S et al: Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes*, 2003; 52: 102–10
11. Chen S, de Craen AJ, Raz Y et al: Cytomegalovirus seropositivity is associated with glucose regulation in the oldest old. Results from the Leiden 85-plus Study. *Immun Ageing*, 2012; 9: 18
12. Hattersley AT, Lo YM, Read SJ et al: Failure to detect cytomegalovirus DNA in pancreas in type 2 diabetes. *Lancet*, 1992; 339: 459–60
13. Lutsey PL, Pankow JS, Bertoni AG et al: Serological evidence of infections and Type 2 diabetes: The MultiEthnic Study of Atherosclerosis. *Diabet Med*, 2009; 26: 149–52
14. Roubalova K, Broz J, Hrubá D et al: Prevalence of active infection with *Chlamydia pneumoniae* and human cytomegalovirus in patients with type II diabetes mellitus. *Folia Microbiol (Praha)*, 2007; 52: 287–90
15. Mehta SK, Stowe RP, Feiveson AH et al: Reactivation and shedding of cytomegalovirus in astronauts during spaceflight. *J Infect Dis*, 2000; 182: 1761–64
16. McVoy MA, Adler SP: Immunologic evidence for frequent age-related cytomegalovirus reactivation in seropositive immunocompetent individuals. *J Infect Dis*, 1989; 160: 1–10
17. Aiello AE, Haan M, Blythe L et al: The influence of latent viral infection on rate of cognitive decline over 4 years. *J Am Geriatr Soc*, 2006; 54: 1046–54
18. Aiello AE, Haan MN, Pierce CM et al: Persistent infection, inflammation, and functional impairment in older Latinos. *J Gerontol A Biol Sci Med Sci*, 2008; 63: 610–18
19. Wang GC, Kao WH, Murakami P et al: Cytomegalovirus infection and the risk of mortality and frailty in older women: a prospective observational cohort study. *Am J Epidemiol*, 2010; 171: 1144–52
20. Roberts ET, Haan MN, Dowd JB, Aiello AE: Cytomegalovirus antibody levels, inflammation, and mortality among elderly Latinos over 9 years of follow-up. *Am J Epidemiol*, 2010; 172: 363–71
21. Masia M, Robledano C, Ortiz De La Tabla V et al: Increased carotid intima-media thickness associated with antibody responses to varicella-zoster virus and cytomegalovirus in HIV-infected patients. *PLoS One*, 2013; 8: e64327
22. Jeong SJ, Han SH, Kim CO et al: Association between human cytomegalovirus antibody levels, and essential hypertension and functional status in elderly Koreans. *Geriatr Gerontol Int*. 2014 [Epub ahead of print]
23. Tang N, Li JW, Liu YM et al: Human cytomegalovirus infection is associated with essential hypertension in Kazakh and Han Chinese populations. *Med Sci Monit*, 2014; 20: 2508–19
24. Scott R, O'Brien R, Fulcher G et al: Effects of fenofibrate treatment on cardiovascular disease risk in 9,795 individuals with type 2 diabetes and various components of the metabolic syndrome: the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study. *Diabetes Care*, 2009; 32(3): 493–98
25. Ginsberg HN, Elam MB, Lovato LC et al: Effects of combination lipid therapy in type 2 diabetes mellitus. *N Engl J Med*, 2010; 362(17): 1563–74
26. American Diabetes Association: Diagnosis and classification of diabetes mellitus. *Diabetes Care*, 2013; 36(Suppl.1): 67–74
27. Yuan-yuan L, Shan L, Ning L et al: Prevalence of human cytomegalovirus infection in hospitalized children. *Military Medical Journal of South China*, 2014; 1–4 [in Chinese]
28. Strandberg TE, Pitkala KH, Tilvis RS: Cytomegalovirus antibody level and mortality among community-dwelling older adults with stable cardiovascular disease. *JAMA*, 2009; 301: 380–82
29. Yan XB, Wang HQ, Chen LS: Analysis of sero-positive of antibody specific for human cytomegalovirus among blood donors in Wuhan area of China. *Chinese Journal of Blood Transfusion*, 1991; 4: 124–25 [in Chinese]
30. Casarin RC, Duarte PM, Santos VR et al: Influence of glycemic control on Epstein-Bar and Cytomegalovirus infection in periodontal pocket of type 2 diabetic subjects. *Arch Oral Biol*, 2010; 55: 902–6
31. Liang H, Liang YZ, Chen H et al: Role of cytomegalovirus infection in the pathogenesis of type 2 diabetes mellitus. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi*, 2003; 17: 351–53
32. Cope AV, Sabin C, Burroughs A et al: Interrelationships among quantity of human cytomegalovirus (HCMV) DNA in blood, donor-recipient serostatus, and administration of methylprednisolone as risk factors for HCMV disease following liver transplantation. *J Infect Dis*, 1997; 176: 1484–90
33. Gor D, Sabin C, Prentice HG et al: Longitudinal fluctuations in cytomegalovirus load in bone marrow transplant patients: relationship between peak virus load, donor/recipient serostatus, acute GVHD and CMV disease. *Bone Marrow Transplant*, 1998; 21: 597–605
34. Aitken C, Barrett-Muir W, Millar C et al: Use of molecular assays in diagnosis and monitoring of cytomegalovirus disease following renal transplantation. *J Clin Microbiol*, 1999; 37: 2804–7
35. Mocarski ES Jr: Immunomodulation by cytomegaloviruses: manipulative strategies beyond evasion. *Trends Microbiol*, 2002; 10: 332–39
36. Dandona P, Aljada A, Bandyopadhyay A: Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol*, 2004; 25: 4–7
37. Sinzger C, Grefte A, Plachter B et al: Fibroblasts, epithelial cells, endothelial cells and smooth muscle cells are major targets of human cytomegalovirus infection in lung and gastrointestinal tissues. *J Gen Virol*, 1995; 76(Pt.4): 741–50
38. Sinzger C, Jahn G: Human cytomegalovirus cell tropism and pathogenesis. *Intervirol*, 1996; 39(5–6): 302–19
39. Streblov DN, Soderberg-Naucler C, Vieira J et al: The human cytomegalovirus chemokine receptor US28 mediates vascular smooth muscle cell migration. *Cell*, 1999; 99: 511–20
40. Loenen WA, Bruggeman CA, Wiertz EJ: Immune evasion by human cytomegalovirus: lessons in immunology and cell biology. *Semin Immunol*, 2001; 13: 41–49
41. Melnick JL, Petrie BL, Dreesman GR et al: Cytomegalovirus antigen within human arterial smooth muscle cells. *Lancet*, 1983; 2: 644–47
42. Melnick JL, Hu C, Burek J et al: Cytomegalovirus DNA in arterial walls of patients with atherosclerosis. *J Med Virol*, 1994; 42: 170–74
43. Hendrix MG, Salimans MM, van Boven CP, Bruggeman CA: High prevalence of latently present cytomegalovirus in arterial walls of patients suffering from grade III atherosclerosis. *Am J Pathol*, 1990; 136: 23–28
44. Ibanez CE, Schrier R, Ghazal P, Wiley C, Nelson JA: Human cytomegalovirus productively infects primary differentiated macrophages. *J Virol*, 1991; 65: 6581–88
45. Epstein SE, Zhou YF, Zhu J: Infection and atherosclerosis: emerging mechanistic paradigms. *Circulation*, 1999; 100: e20–28
46. Smith PD, Saini SS, Raffeld M et al: Cytomegalovirus induction of tumor necrosis factor-alpha by human monocytes and mucosal macrophages. *J Clin Invest*, 1992; 90: 1642–48
47. Almeida GD, Porada CD, St Jeor S, Ascensao JL: Human cytomegalovirus alters interleukin-6 production by endothelial cells. *Blood*, 1994; 83: 370–76
48. Zhu J, Quyyumi AA, Norman JE et al: Cytomegalovirus in the pathogenesis of atherosclerosis: the role of inflammation as reflected by elevated C-reactive protein levels. *J Am Coll Cardiol*, 1999; 34: 1738–43
49. Hermansson A, Ketelhuth DF, Strodtzoff D et al: Inhibition of T cell response to native low-density lipoprotein reduces atherosclerosis. *J Exp Med*, 2010; 207: 1081–93
50. Schieffer B, Selle T, Hilfiker A et al: Impact of interleukin-6 on plaque development and morphology in experimental atherosclerosis. *Circulation*, 2004; 110: 3493–500
51. Bason C, Corrocher R, Lunardi C et al: Interaction of antibodies against cytomegalovirus with heat-shock protein 60 in pathogenesis of atherosclerosis. *Lancet*, 2003; 362: 1971–77



52. Lunardi C, Dolcino M, Peterlana D et al: Endothelial cells' activation and apoptosis induced by a subset of antibodies against human cytomegalovirus: Relevance to the pathogenesis of atherosclerosis. *PLoS One*, 2007; 2: e473
53. Xu Q, Wick G: The role of heat shock proteins in protection and pathophysiology of the arterial wall. *Mol Med Today*, 1996; 2: 372-79
54. Adam E, Melnick JL, Probstfield JL et al: High levels of cytomegalovirus antibody in patients requiring vascular surgery for atherosclerosis. *Lancet*, 1987; 2: 291-93
55. Espinola-Klein C, Rupprecht HJ, Blankenberg S et al: Are morphological or functional changes in the carotid artery wall associated with *Chlamydia pneumoniae*, *Helicobacter pylori*, cytomegalovirus, or herpes simplex virus infection? *Stroke*, 2000; 31: 2127-33
56. Blum A, Giladi M, Weinberg M et al: High anti-cytomegalovirus (CMV) IgG antibody titer is associated with coronary artery disease and may predict post-coronary balloon angioplasty restenosis. *Am J Cardiol*, 1998; 81: 866-68
57. Puz P, Lasek-Bal A, Ziaja D et al: Inflammatory markers in patients with internal carotid artery stenosis. *Arch Med Sci*, 2013; 9: 254-60
58. Miettinen H, Lehto S, Salomaa V et al: Impact of diabetes on mortality after the first myocardial infarction. The FINMONICA Myocardial Infarction Register Study Group. *Diabetes Care*, 1998; 21(1): 69-75
59. Haffner SM, Lehto S, Ronnema T et al: Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med*, 1998; 339(4): 229-34
60. Almdal T, Scharling H, Jensen JS, Vestergaard H: The independent effect of type 2 diabetes mellitus on ischemic heart disease, stroke, and death: A population-based study of 13,000 men and women with 20 years of follow-up. *Arch Intern Med*, 2004; 164(13): 1422-26
61. Visseren FL, Bouter KP, Pon MJ et al: Patients with diabetes mellitus and atherosclerosis; a role for cytomegalovirus? *Diabetes Res Clin Pract*, 1997; 36: 49-55