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Impact of Cytomegalovirus Disease on New-Onset Type 2 Diabetes Mellitus: Population-Based Matched Case-Control Cohort Study

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Background: A latent cytomegalovirus (CMV) cause chronic inflammation through undesirable inflation of cell-mediated immune response. CMV immunoglobulin G has been associated with cardiovascular disease and type 1 diabetes mellitus. We evaluated impact of CMV diseases on new-onset type 2 diabetes mellitus (T2DM).
Methods: From the Korean Health Insurance Review and Assessment Service claim database of entire population with 50 million, we retrieved 576 adult case group with CMV diseases diagnosed with International Statistical Classification of Diseases and Related-Health Problems 10th Revision (ICD-10) B25 code between 2010 and 2014 after exclusion of patients with T2DM to 2006. The 2,880 control patients without T2DM from 2006 to cohort entry point were selected between 2010 and 2014 by age, sex matching with case group. The subjects without new-onset T2DM were followed until 2015. T2DM, hypertension (HTN), dyslipidemia (DYS), and end-stage renal disease (ESRD) were coded as ICD-10.
Results: The frequency of new-onset T2DM in case group was significantly higher than that in control (5.6% vs. 2.2%, $P<0.001$). The group with T2DM ($n=95$) had higher incidence of CMV diseases than the group without T2DM ($n=3,361$) (33.7% vs. 16.2%, $P<0.001$). In multivariate regression model adjusted by age, sex, lower income, HTN, and DYS, the incidence rate (IR) of T2DM in case group was significantly higher than that in the control group (IR per 1,000, 19.0 vs. 7.3; odds ratio, 2.1; 95% confidence interval, 1.3 to 3.2). The co-existence of HTN, DYS, and ESRD with CMV diseases did not influence the IR of T2DM.
Conclusion: CMV diseases increase the patients' risk of developing T2DM.

Keywords: Cytomegalovirus; Diabetes mellitus, type 2; Disease; Population

INTRODUCTION

The genes of human cytomegalovirus (CMV) chronically exists in the host myeloid lineage including CD34+ hematopoietic progenitor cells as extrachromosomal plasmid after asymptomatic or self-limited primary lytic infection that occurs during childhood and adolescence [1-3]. Unlike other viruses of *Herpesviridae* family, latently established CMV can express a handful of transcripts such as latency-associated unidentified nuclear antigen (LUNA), UL81-82 antisense transcript, and latency-associated homolog of interleukin-10 (LAcmvIL-10) together with periodic active replication events during the lifelong [2,4-7]. The outstanding feature of sleepless latency leads to the extraordinary expansion of CMV-specific resting effector memory CD8+ T-cell subpopulation and then chronic inflammatory condition as well as dysregulation of host immune mechanisms [7-12]. This long-term pathophysiologic mechanisms of CMV acquisition may be the direct cause of chronic

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inflammatory cardiovascular diseases (CVDs) or autoimmune diseases including systemic lupus erythematosus and systemic sclerosis [13,14].

Although the pathogenesis of type 2 diabetes mellitus (T2DM, non-insulin-dependent diabetes mellitus [DM]) is quite complex and involves various cross-linked molecules including adipocytokines, receptors, and genetic pathways as well as immune system, T2DM seemed to be a fundamentally chronic low-grade inflammatory metabolic disease with clinical detrimental effects through various micro- and macrovascular complications [15-21]. Lohr and Oldstone [22] detected the CMV immediate-early and late gene products using reverse transcription polymerase chain reaction (PCR) and *in situ* hybridization in pancreatic tissues of T2DM patients. Recent studies suggested that viperin (endoplasmic reticulum-associated, interferon-inducible virus inhibitory protein), which is directly induced by CMV, may play a role in lipid and glucose metabolism through interaction with the CMV mitochondrial inhibitor of apoptosis (vMIA) protein [23,24].

Considering these molecular biological experiments, we hypothesized that CMV diseases may contribute to the development of T2DM. Although only a few clinical studies evaluating the association between CMV and T2DM were performed nearly 2 decades ago, these studies evaluated CMV seroepidemiology measured by anti-CMV immunoglobulin G (IgG) antibody test or titer, to determine previous CMV exposure and latent status by humoral immunity, with age being a variable influence [25-29]. The anti-CMV IgG is not useful for evaluating CMV-specific cell-mediated immunity (CMV-CMI), which plays a major role in immunosenescence and immune exhaustion caused by CMV [30]. The active replication of whole CMV genes can be delicately categorized into CMV infection and diseases, defined as detection of DNA (DNAemia) or competitive virions (viremia) in the peripheral blood and cytopathic inflammatory end-organ tissue-invasive disease, respectively [30]. The CMV infection or diseases, in relation to more powerful immune boosting of CMV-CMI than anti-CMV IgG serostatus, will better reflect the chronic inflammatory dysregulation phenomenon by CMV-related indirect effect [30].

The impact of CMV infection or diseases on new-onset T2DM had been primarily evaluated as posttransplant DM in adult solid organ transplant recipients [31,32]. However, little is known about the causal connection of CMV diseases to T2DM development in the entire population including both transplant and non-transplant patients. Therefore, we performed the general population-based matched case-control cohort study in both immunocompetent and immunocompromised patients to explore whether the CMV diseases contributes to the development of T2DM.

METHODS

Data resource and management process

Our study used the database warehouse of the Korean Health Insurance Review and Assessment Service claims, including the detailed information about all kinds of healthcare utilization in the entire Korean population [33]. A total of 1,188 unduplicated patients with CMV diseases between 2010 and 2014 were extracted using a unique code (V104) for the relief reduction of patients with rare intractable diseases, operated by the South Korean National Health Insurance Service (NHIS) [33]. The healthcare providers should submit the specific document to the NHIS to register this particular payment relief. The V104 code corresponds to B25 (cytomegalovirus disease), B25.0 (cytomegaloviral pneumonitis), B25.1 (cytomegaloviral hepatitis), B25.2 (cytomegaloviral pancreatitis), B25.8 (other cytomegaloviral diseases), and B25.9 (cytomegaloviral disease, unspecified) according to the World Health Organization's 2016 International Statistical Classification of Diseases and Related-Health Problems 10th Revision (ICD-10). To apply the V104 code, the clinicians should confirm the CMV diseases through histopathological exam, culture, shell viral assay, and pp65 antigen test. The presumptive or suspected diagnosis with imaging studies or clinical decision is not permissible. The V104 code does not include congenital CMV infection (ICD-10 code: P35.1) and cytomegaloviral mononucleosis (ICD-10 code: B27.1). The study was approved with waiver of informed consent by the Institutional Review Board of Gangnam Severance Hospital (IRB no. 3-2017-0341) and the NHIS. This study was performed according to the Declaration of Helsinki, the ethical principles for medical research involving human subjects.

Study design and patient selection

We performed the whole population-based matched case-control cohort study. The patients with V104 code of CMV diseases comprised the case group and those without this code comprised the control group. Five hundred and seventy-six case patients of the 1,188 with CMV diseases were finally selected

between 2010 and 2014 after excluding 285 patients with previous T2DM before first CMV diagnosis date back to 2006 and another 327 who were aged <20 years. The 2,880 control patients without T2DM between 2006 and cohort entry point were randomly stratified matched by age and sex with the case patients in the same cohort entry year, with a 5:1 ratio. This cohort start and end date were January 1, 2011 and December 31, 2015. The event in the cohort was the development of new-onset T2DM in both case and control group. If subject had the event, their follow-up were ceased. All subject without event were followed until December 31, 2015. If patients were died before event occurrence, they were censored (Fig. 1).

Definition

Lower income status was defined as an annual household income of lower than 25% based on the results of the 2010 South Korea Population and Housing Census. Recurred or refractory CMV diseases cases were defined as patients who received repeat treatments of ganciclovir and/or valganciclovir between 30 days and 1 year after the first CMV diagnosis [34]. We simply expressed the refractory or recurred disease as refractory CMV diseases. Chronic medical diseases were matched with the ICD-10 code of T2DM (E11–E14), hypertension (I10–I13 and I15), dyslipidemia (E78 including E78.0–E78.9), chronic obstructive pulmonary disease (COPD) (J44 including J44.0, J44.1, J44.8, and J44.9), ischemic heart diseases (IHDs) (I20-I25), cerebral infarction (I63, I64), heart failure (HF) (I50), and end-stage renal disease (ESRD) (N18.5). For T2DM diagnosis, we additionally used the anti-diabetic drug description with codes of insulins, sulfonylureas, metformin, meglitinides, thiazolidinediones, dipeptidyl peptidase-4 inhibitors, and α-glucosidase inhibitors along with above ICD-10 codes [35].

Statistical analysis

We used the McNemer test and paired *t*-test to compare the nominal and continuous variables between the two groups, respectively. We performed the multivariate logistic regression analyses with model 1 (M1, not adjusted), model 2 (M2, adjusted by age and sex), and model 3 (M3, adjusted by age, sex, lower income status, hypertension, and dyslipidemia) to evaluate the impact of adult CMV diseases on new-onset T2DM. The incidence probability of T2DM was analyzed using Kaplan-Meier curves and log-rank test. The two-tailed *P* values of ≤0.05 were considered significant. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA).

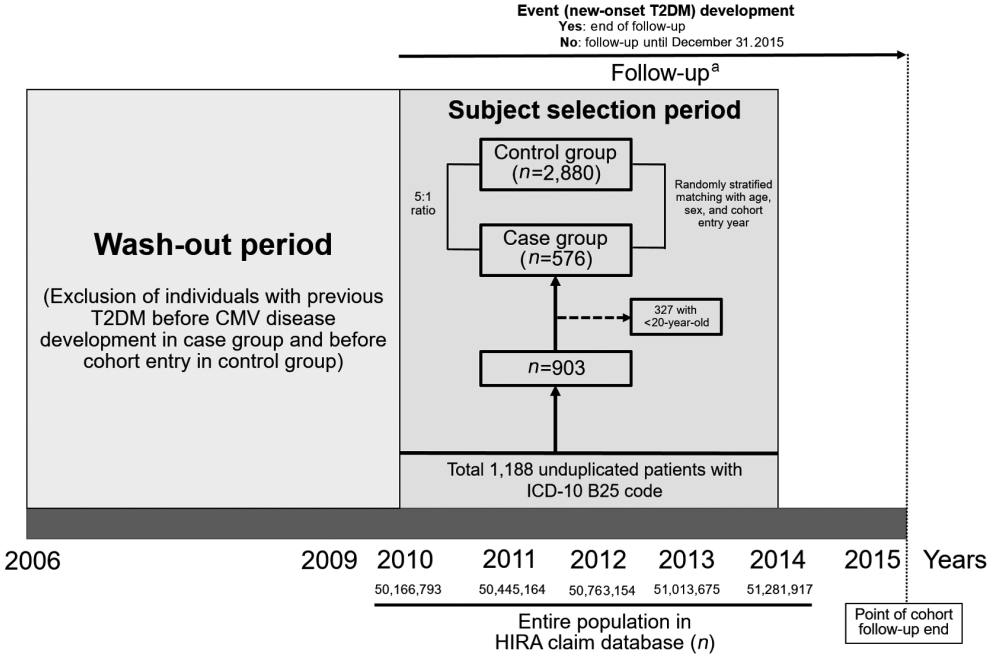

Fig. 1. The process of subject selection by case and control group. T2DM, type 2 diabetes mellitus; CMV, cytomegalovirus; ICD-10, International Statistical Classification of Diseases and Related-Health Problems 10th Revision; HIRA, the Korean Health Insurance Review and Assessment Service. [a]If patients were died before event occurrence, they were censored.

Table 1. Comparison of baseline characteristics between adult individuals with and without cytomegalovirus disease in the matched cohort

Characteristic	CMV diseases			Refractory CMV diseases		
	Yes (n=576)	No (n=2,880)	P value	Yes (n=88)	No (n=488)	P value
Male sex	315 (54.69)	1,575 (54.69)	>0.999[a]	54 (61.36)	261 (53.48)	0.172[a]
Age, yr-old	50.95±15.99	50.95±15.98	>0.999[b]	51.02±14.13	50.93±16.32	0.962[b]
≥60	164 (28.47)	820 (28.47)	>0.999[a]	21 (23.86)	143 (29.30)	0.298[a]
Lower income status	154 (26.74)	670 (23.26)	0.074[a]	26 (29.55)	128 (26.23)	0.518[a]
Hypertension	226 (39.24)	571 (19.83)	<0.001[a]	29 (32.95)	197 (40.37)	0.190[a]
Dyslipidemia	132 (22.92)	323 (11.22)	<0.001[a]	19 (21.59)	113 (23.16)	0.748[a]
COPD	163 (28.30)	363 (12.60)	<0.001[a]	23 (26.14)	140 (28.69)	0.625[a]
Ischemic heart diseases	147 (25.52)	250 (8.68)	<0.001[a]	23 (26.14)	124 (25.41)	0.807[a]
Cerebral infarction	45 (7.81)	121 (4.20)	<0.001[a]	6 (6.82)	39 (7.99)	0.784[a]
Heart failure	64 (11.11)	57 (1.98)	<0.001[a]	7 (7.95)	57 (11.68)	0.361[a]
ESRD	148 (25.69)	3 (0.10)	<0.001[a]	17 (19.32)	131 (26.84)	0.137[a]
Follow-up duration, yr	2.92±1.48	3.01±1.43	0.173	2.50±1.53	2.99±1.47	0.004[b]

Values are presented as number (%) or mean±standard deviation.
CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; ESRD, end-stage renal disease.
[a]McNemar test, [b]Paired t-test.

RESULTS

Frequencies of chronic diseases between case and control group

The overall follow-up duration of all participants was about 3 years. There was no significant difference in the proportion of patients aged ≥60 years and with lower income status between case and control group. The case group had significantly higher frequency of new-onset T2DM compared to the control group (5.6% vs. 2.2%, P<0.001) (Table 1). When we divided the total patients (n=3,456) into group with (n=95) new-onset T2DM and group without T2DM (n=3,361), the frequency of CMV diseases was found to be higher in the group with T2DM (33.7% vs. 16.2%, P<0.001) (Supplementary Table 1). The percentages of patients with hypertension, dyslipidemia, COPD, IHD, cerebral infarction, HF, and ESRD were common in the case group (all of P<0.001) (Table 1).

Impact of CMV diseases on the development of new-onset T2DM: subgroup analyses

The incidence rate (IR) per 1,000 T2DM patients in the case group was nearly two-fold higher than that in the control group at M1 (odds ratio [OR], 2.61; 95% confidence interval [CI], 1.69 to 3.96), M2 (OR, 2.60; 95% CI, 1.68 to 3.95), and M3 (OR, 2.06; 95% CI, 1.31 to 3.17) (Table 2). Kaplan-Meier curve showed that the incidence probability of T2DM in the case group was significantly higher than that in the control (P< 0.001) (Fig. 2A). In the case group, the subgroup aged ≥60 years had a significantly lower risk of developing T2DM than those aged <60 years (M3 [OR, 1.11; 95% CI, 0.50 to 2.20] vs. [OR, 3.63; 95% CI, 1.97 to 6.65]; P=0.032, respectively). However, there was no significant difference in the OR of sex, lower income status, hypertension, dyslipidemia, COPD, IHD, cerebral infarction, HF, and ESRD (Table 2).

Difference between refractory and non-refractory CMV diseases in T2DM

In a further analysis conducted in three groups with refractory CMV diseases, the patients with refractory CMV diseases (M3: OR, 4.01; 95% CI, 1.76 to 7.69) had a significantly higher IR of T2DM than those without CMV diseases (reference) or with non-refractory CMV diseases (M3: OR, 1.77; 95% CI, 1.07 to 2.82). There was no significant difference in the ORs between the three groups according to sex and age (P=0.256 and P= 0.114, respectively) (Supplementary Table 2). In the Kaplan-Meier curve of the three groups, patients with refractory CMV diseases had the highest IR of T2DM (P<0.001) (Fig. 2B).

Table 2. Multivariate logistic regression models to examine the effect of adult cytomegalovirus disease on new-onset of type 2 diabetes mellitus in various subgroups

	CMV group	Total no.	T2DM no.	Total FU duration, yr	IR[a] of T2DM	Model 1	Model 2	Model 3	*P* value for interaction
Total	No	2,880	63	8,664.4	7.27	1 (reference)	1 (reference)	1 (reference)	-
	Yes	576	32	1,681.4	19.03	2.61 (1.69–3.96)	2.60 (1.68–3.95)	2.06 (1.31–3.17)	
Sex									0.408
Male	No	1,575	33	4,737.1	6.97	1 (reference)	1 (reference)	1 (reference)	
	Yes	315	19	915.7	20.75	2.97 (1.66–5.16)	2.96 (1.66–5.16)	2.17 (1.19–3.84)	
Female	No	1,305	30	3,927.3	7.64	1 (reference)	1 (reference)	1 (reference)	
	Yes	261	13	765.7	16.98	2.21 (1.12–4.15)	2.21 (1.11–4.14)	1.95 (0.96–3.74)	
Age, yr-old									0.032
<60	No	2,060	26	6,357.6	4.09	1 (reference)	1 (reference)	1 (reference)	
	Yes	412	23	1,228.9	18.72	4.55 (2.58–7.98)	4.59 (2.60–8.04)	3.63 (1.97–6.65)	
≥60	No	820	37	2,306.8	16.04	1 (reference)	1 (reference)	1 (reference)	
	Yes	164	9	452.5	19.89	1.24 (0.56–2.45)	1.25 (0.57–2.47)	1.11 (0.50–2.20)	
Income									0.422
Other	No	2,210	47	6,659.1	7.06	1 (reference)	1 (reference)	1 (reference)	
	Yes	422	25	1,202.9	20.78	2.91 (1.77–4.69)	2.81 (1.70–4.52)	2.33 (1.40–3.80)	
Lower 25%	No	670	16	2,005.2	7.98	1 (reference)	1 (reference)	1 (reference)	
	Yes	154	7	478.5	14.63	1.85 (0.71–4.33)	2.02 (0.77–4.75)	1.36 (0.50–3.36)	
HTN									0.116
No	No	2,309	30	7,088.7	4.23	1 (reference)	1 (reference)	1 (reference)	
	Yes	350	14	1,043.4	13.42	3.16 (1.62–5.84)	3.17 (1.63–5.87)	2.92 (1.49–5.44)	
Yes	No	571	33	1,575.7	20.94	1 (reference)	1 (reference)	1 (reference)	
	Yes	226	18	638.0	28.21	1.36 (0.75–2.38)	1.48 (0.80–2.66)	1.47 (0.79–2.64)	
DYS									0.384
No	No	2,557	45	7,769.3	5.79	1 (reference)	1 (reference)	1 (reference)	
	Yes	444	22	1,328.2	16.56	2.87 (1.69–4.71)	2.76 (1.63–4.54)	2.22 (1.29–3.70)	
Yes	No	323	18	895.1	20.11	1 (reference)	1 (reference)	1 (reference)	
	Yes	132	10	353.2	28.32	1.39 (0.62–2.96)	1.36 (0.57–3.06)	1.40 (0.58–3.20)	
COPD									0.242
No	No	2,517	54	7,594.3	7.11	1 (reference)	1 (reference)	1 (reference)	
	Yes	413	19	1,212.8	15.67	2.20 (1.27–3.64)	2.26 (1.31–3.75)	1.69 (0.96–2.86)	
Yes	No	363	9	1,070.1	8.41	1 (reference)	1 (reference)	1 (reference)	
	Yes	163	13	468.6	27.74	3.30 (1.42–7.99)	3.52 (1.51–8.57)	3.35 (1.42–8.22)	
IHD									0.625
No	No	2,630	50	7,954.5	6.29	1 (reference)	1 (reference)	1 (reference)	
	Yes	429	21	1,271.3	16.52	2.62 (1.54–4.30)	2.70 (1.59–4.44)	2.02 (1.17–3.37)	
Yes	No	250	13	709.9	18.31	1 (reference)	1 (reference)	1 (reference)	
	Yes	147	11	410.1	26.83	1.50 (0.66–3.34)	1.51 (0.64–3.47)	1.46 (0.62–3.34)	

(*Continued to the next page*)

Table 2. Continued

	CMV group	Total no.	T2DM no.	Total FU duration, yr	IR[a] of T2DM	Model 1	Model 2	Model 3	*P* value for interaction
HF									0.642
No	No	2,823	60	8,519.4	7.04	1 (reference)	1 (reference)	1 (reference)	
	Yes	512	28	1,503.6	18.62	2.64 (1.66–4.09)	2.71 (1.70–4.20)	2.12 (1.32–3.34)	
Yes	No	57	3	145.0	20.69	1 (reference)	1 (reference)	1 (reference)	
	Yes	64	4	177.8	22.50	1.11 (0.25–5.66)	1.02 (0.19–5.73)	1.08 (0.19–6.17)	
Cerebral infarction									0.591
No	No	2,759	59	8,314.2	7.10	1 (reference)	1 (reference)	1 (reference)	
	Yes	531	28	1,563.7	17.91	2.52 (1.59–3.91)	2.58 (1.62–4.00)	2.03 (1.26–3.20)	
Yes	No	121	4	350.2	11.42	1 (reference)	1 (reference)	1 (reference)	
	Yes	45	4	117.6	34.00	2.79 (0.66–11.81)	2.48 (0.57–10.68)	3.40 (0.77–15.21)	
ESRD									-
No	No	2,877	63	8,656.2	7.28	1 (reference)	1 (reference)	1 (reference)	
	Yes	428	20	1,249.0	16.01	2.19 (1.29–3.56)	2.10 (1.24–3.41)	1.92 (1.13–3.12)	
Yes	No	3	0	8.2	0.00	-	-	-	
	Yes	148	12	432.4	27.75	-	-	-	

Values are presented as number or odds ratio (95% confidence interval). Model 1, non-adjusted; Model 2, age- and sex-adjusted; Model 3, age-, sex-, lower income-, hypertension-, and dyslipidemia-adjusted.
CMV, cytomegalovirus; T2DM, type 2 diabetes mellitus; FU, follow-up; IR, incidence rate; HTN, hypertension; DYS, dyslipidemia; COPD, chronic obstructive pulmonary disease; IHD, ischemic heart disease; HF, heart failure; ESRD, end-stage renal disease.
[a]Per 1,000.

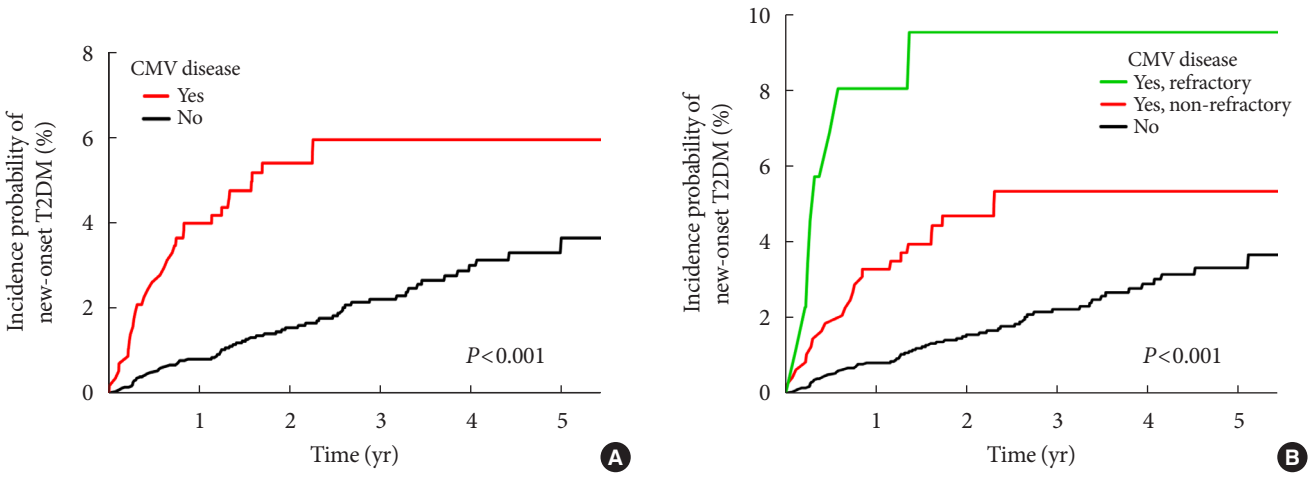

Fig. 2. Kaplan-Meier curve for new-onset type 2 diabetes mellitus (T2DM) incidence according to cytomegalovirus (CMV) disease. (A) Comparison of patients with and without CMV diseases. (B) Comparison of three groups including refractory and non-refractory CMV diseases (*P* value of log-lank test).

DISCUSSION

This study showed that the occurrence of CMV diseases increases the incidence of new-onset T2DM, with a relative risk of ≥200%, which was higher than the CMV-attributable risk of CVD evaluated by meta-analyses (22% and 67%) [14,36]. This result was identical with additional analysis from the matched cohort of 3:1 ratio (1,488 of control group and 496 of case

group) with age, sex, cohort entry year, lower income status, hypertension, and dyslipidemia as 2.01 of OR (Supplementary Table 3). Interestingly, patients aged <60 years had a higher risk of T2DM after CMV diseases. However, the existence of chronic inflammatory diseases such as hypertension, dyslipidemia, COPD, IHD, cerebral infarction, HF, and ESRD did not influence the incidence of post-CMV T2DM. These findings were consistently derived from different regression models adjusted by clinical factors of usual risks for T2DM [37] and confounding variables showing higher frequencies in patients with CMV diseases (Table 1). The Kaplan-Meier curve indicated that most post-CMV T2DM cases developed shortly after CMV diseases, in particular, within 1 year.

Majority of epidemiological studies evaluating the causal relationship between CMV and metabolic diseases have focused on CVD, especially coronary heart disease (CHD), type I DM, and autoimmune diabetes [13,14,36,38]. The meta-analysis conducted by Ji et al. [14] revealed that CMV infection was associated with high risk of CHD. However, only seven (12.7%) among the total 55 studies including the meta-analysis reported the detection of CMV DNAemia by PCR [14]. Interestingly, the OR reported in seven studies was higher than that in studies using CMV seroprevalence (8.12 vs. 1.56, respectively) [14]. A few studies measuring anti-CMV IgG showed that T2DM patients had higher frequency of CMV seropositivity and titer (OR, 2 to 12) than healthy subjects [26,27]. In addition, CMV seropositive status was associated with higher glucose levels [26]. However, these studies may have considerable limitations and are underpowered due to the small sample size and rare CMV events and because the specific subjects do not reflect the universal population.

The pathophysiological mechanisms of post-CMV T2DM remain uncertain. Although Lohr and Oldstone [22] revealed that CMV genes existed in the pancreatic islets of Langerhans cells without cytopathic inflammation and Numazaki et al. [39] investigated that CMV was found to replicate in *in vitro* human fetal pancreatic islet cells in the early 1990s, further studies had not been reported. Yoneda et al. [38] recently reported that the immunologic indirect effect of CMV infection was associated with direct pancreatic β-cell injury, showing T lymphocyte infiltration. The reliability of the experiments conducted in human samples was confirmed although the absence of CMV in normal controls [22,38]. The discrete impact of CMV infection on the failure of islet allografts to support the active CMV replication may be an attributing factor to the development of T2DM through direct and/or indirect pancreatic damages from CMV [40]. On the contrary, we may presume that the substances used in the envelop composition such as viperin, which is essential to extracellular budding and shedding of complete CMV virion, might alter the lipid and glucose metabolism pathways [24].

Our new findings of post-CMV T2DM discrepancy by age group and early development after CMV diseases could provide novel insights for the causative effects of CMV replication in chronic inflammatory diseases including T2DM. Another unique analysis for the refractory nature of CMV diseases, which may suggest prolonged active CMV replication, had earlier development of and more strong relationship with T2DM. The complete lytic reactivation of CMV resulting in end-organ cytopathic disease or DNAemia, especially in the younger population who have more prominent immunologic response than the elderly with profound immunosenescence or subjects who have uncontrolled CMV replication, could lead to deeper deterioration of immune homeostasis. The comprehensive measurement of CMV-specific immunologic profiles using mass cytometry (cytometry by time of flight [CyTOF]) and genetic evaluation for alteration of glucose metabolism by CMV are warranted. If information regarding causative mechanisms in CMV replication and T2DM would be accumulated along with further epidemiologic studies in various ethnic populations, the development of CMV vaccine could be targeted to prevent several chronic inflammatory diseases including T2DM in this era of aging population.

This study has some limitations, same as those of large-scale studies using coding data processing. Therefore, the data collection method could cause the selected disease to be underestimated. However, our analyses can overcome this drawback through a matched case-control design. In addition, the frequencies of CMV diseases incidence according to ICD-10 code showed that our database from the entire population had the majority of B25.8 and B25.9 code (1,025 of 1,188, 86.3%), which may indicate the confirmatory CMV diagnosis driven by pathology in gastrointestinal tract such as esophagus, stomach, duodenum, and colon. The absence of rare cytomegaloviral pancreatitis as well as the minority of cytomegaloviral pneumonitis (75 of 1,188, 6.3%), which could be presumptive or over-diagnosed with chest computed tomography scan or quantitative nucleic acid amplification test in bronchoalveolar lavage without standardization and universal cut-off level for proper diagnosis, will guarantee the validity of our database for

the confirmatory CMV tissue-invasive end-organ disease (Supplementary Table 4) [30]. Secondly, our subjects were restricted by South Korean nationality. A previous study showed that the effect of CMV infection on CHD might be different among ethnic groups, with increased risk among Asian populations [14]. In addition, the refractory CMV diseases were identified simply by repeated administration of anti-CMV drugs. The huge database could not allow a more precise definition of clinical information-based refractory cases in each CMV patient. Finally, our cohort includes some proportion of solid organ transplantation recipients of about 20% among total individuals with CMV diseases (Supplementary Table 5). However, the patients with new-onset T2DM had higher frequencies of several chronic inflammatory diseases, including hypertension, dyslipidemia, IHD, cerebral infarction, and ESRD, in the well-known relation to T2DM (Supplementary Table 1) [37]. These basic characteristics will ensure the good quality in this cohort. In addition, the low IR of CMV end-organ disease in general population may make our population-based study worthy to evaluate the relationship of CMV and T2DM.

In spite of these limitations, our study has several strengths and outstanding features: (1) first assessment of the T2DM incidence after CMV diseases including tissue-invasive end-organ damage, but not the CMV serostatus in T2DM patients, (2) long wash-out period for ≥4 years that can discriminate the casual relationship between CMV diseases and T2DM as well as can analyze the post-CMV T2DM, and (3) largest sample size among studies reporting about the relation between T2DM and CMV.

In conclusion, our data indicate that CMV diseases increase the development of T2DM. These results suggest that active CMV replication may play an important role in T2DM pathogenesis.

SUPPLEMENTARY MATERIALS

Supplementary materials related to this article can be found online at https://doi.org/dmj.2018.0167.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

Conception or design: K.D.H., S.H.H.
Acquisition, analysis, or interpretation of data: S.G.Y., K.D.H., S.H.H.
Drafting the work or revising: S.G.Y., K.H.L., Y.L., D.E.K.
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REFERENCES

1. Kumar A, Herbein G. Epigenetic regulation of human cytomegalovirus latency: an update. Epigenomics 2014;6:533-46.
2. Poole E, Sinclair J. Sleepless latency of human cytomegalovirus. Med Microbiol Immunol 2015;204:421-9.
3. Cannon MJ, Schmid DS, Hyde TB. Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. Rev Med Virol 2010;20:202-13.
4. Mason GM, Jackson S, Okecha G, Poole E, Sissons JG, Sinclair J, Wills MR. Human cytomegalovirus latency-associated proteins elicit immune-suppressive IL-10 producing CD4+ T cells. PLoS Pathog 2013;9:e1003635.
5. Bego M, Maciejewski J, Khaiboullina S, Pari G, St Jeor S. Characterization of an antisense transcript spanning the UL81-82 locus of human cytomegalovirus. J Virol 2005;79:11022-34.
6. Jenkins C, Abendroth A, Slobedman B. A novel viral transcript with homology to human interleukin-10 is expressed during latent human cytomegalovirus infection. J Virol 2004;78:1440-7.
7. Nikolich-Zugich J, van Lier RAW. Cytomegalovirus (CMV) research in immune senescence comes of age: overview of the 6th International Workshop on CMV and Immunosenescence. Geroscience 2017;39:245-9.
8. La Rosa C, Diamond DJ. The immune response to human CMV. Future Virol 2012;7:279-93.
9. Willis EL, Eberle R, Wolf RF, White GL, McFarlane D. The ef-

fects of age and cytomegalovirus on markers of inflammation and lymphocyte populations in captive baboons. PLoS One 2014;9:e107167.
10. Holtappels R, Pahl-Seibert MF, Thomas D, Reddehase MJ. Enrichment of immediate-early 1 (m123/pp89) peptide-specific CD8 T cells in a pulmonary CD62L(lo) memory-effector cell pool during latent murine cytomegalovirus infection of the lungs. J Virol 2000;74:11495-503.
11. Karrer U, Sierro S, Wagner M, Oxenius A, Hengel H, Koszinowski UH, Phillips RE, Klenerman P. Memory inflation: continuous accumulation of antiviral CD8+ T cells over time. J Immunol 2003;170:2022-9.
12. Ouyang Q, Wagner WM, Wikby A, Walter S, Aubert G, Dodi AI, Travers P, Pawelec G. Large numbers of dysfunctional CD8+ T lymphocytes bearing receptors for a single dominant CMV epitope in the very old. J Clin Immunol 2003;23:247-57.
13. Halenius A, Hengel H. Human cytomegalovirus and autoimmune disease. Biomed Res Int 2014;2014:472978.
14. Ji YN, An L, Zhan P, Chen XH. Cytomegalovirus infection and coronary heart disease risk: a meta-analysis. Mol Biol Rep 2012;39:6537-46.
15. Esser N, Legrand-Poels S, Piette J, Scheen AJ, Paquot N. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. Diabetes Res Clin Pract 2014;105:141-50.
16. Grossmann V, Schmitt VH, Zeller T, Panova-Noeva M, Schulz A, Laubert-Reh D, Juenger C, Schnabel RB, Abt TG, Laskowski R, Wiltink J, Schulz E, Blankenberg S, Lackner KJ, Munzel T, Wild PS. Profile of the immune and inflammatory response in individuals with prediabetes and type 2 diabetes. Diabetes Care 2015;38:1356-64.
17. Griffin SJ, Leaver JK, Irving GJ. Impact of metformin on cardiovascular disease: a meta-analysis of randomised trials among people with type 2 diabetes. Diabetologia 2017;60:1620-9.
18. Abdel-Moneim A, Bakery HH, Allam G. The potential pathogenic role of IL-17/Th17 cells in both type 1 and type 2 diabetes mellitus. Biomed Pharmacother 2018;101:287-92.
19. Szabo M, Mate B, Csep K, Benedek T. Genetic approaches to the study of gene variants and their impact on the pathophysiology of type 2 diabetes. Biochem Genet 2018;56:22-55.
20. Kerru N, Singh-Pillay A, Awolade P, Singh P. Current anti-diabetic agents and their molecular targets: a review. Eur J Med Chem 2018;152:436-88.
21. Ashoori MR, Rahmati-Yamchi M, Ostadrahimi A, Fekri Aval S, Zarghami N. MicroRNAs and adipocytokines: promising biomarkers for pharmacological targets in diabetes mellitus and its complications. Biomed Pharmacother 2017;93:1326-36.
22. Lohr JM, Oldstone MB. Detection of cytomegalovirus nucleic acid sequences in pancreas in type 2 diabetes. Lancet 1990;336:644-8.
23. Seo JY, Yaneva R, Hinson ER, Cresswell P. Human cytomegalovirus directly induces the antiviral protein viperin to enhance infectivity. Science 2011;332:1093-7.
24. Seo JY, Cresswell P. Viperin regulates cellular lipid metabolism during human cytomegalovirus infection. PLoS Pathog 2013;9:e1003497.
25. Zhang J, Liu YY, Sun HL, Li S, Xiong HR, Yang ZQ, Xiang GD, Jiang XJ. High human cytomegalovirus IgG level is associated with increased incidence of diabetic atherosclerosis in type 2 diabetes mellitus patients. Med Sci Monit 2015;21:4102-10.
26. Chen S, de Craen AJ, Raz Y, Derhovanessian E, Vossen AC, Westendorp RG, Pawelec G, Maier AB. Cytomegalovirus seropositivity is associated with glucose regulation in the oldest old. Results from the Leiden 85-plus Study. Immun Ageing 2012;9:18.
27. Roberts BW, Cech I. Association of type 2 diabetes mellitus and seroprevalence for cytomegalovirus. South Med J 2005;98:686-92.
28. Haeseker MB, Pijpers E, Dukers-Muijrers NH, Nelemans P, Hoebe CJ, Bruggeman CA, Verbon A, Goossens VJ. Association of cytomegalovirus and other pathogens with frailty and diabetes mellitus, but not with cardiovascular disease and mortality in psycho-geriatric patients: a prospective cohort study. Immun Ageing 2013;10:30.
29. Blum A, Peleg A, Weinberg M. Anti-cytomegalovirus (CMV) IgG antibody titer in patients with risk factors to atherosclerosis. Clin Exp Med 2003;3:157-60.
30. Kotton CN, Kumar D, Caliendo AM, Huprikar S, Chou S, Danziger-Isakov L, Humar A; The Transplantation Society International CMV Consensus Group. The Third International Consensus Guidelines on the management of cytomegalovirus in solid-organ transplantation. Transplantation 2018;102:900-31.
31. Gomes MB, Cobas RA. Post-transplant diabetes mellitus. Diabetol Metab Syndr 2009;1:14.
32. Leung Ki EL, Venetz JP, Meylan P, Lamoth F, Ruiz J, Pascual M. Cytomegalovirus infection and new-onset post-transplant diabetes mellitus. Clin Transplant 2008;22:245-9.
33. Kim JA, Yoon S, Kim LY, Kim DS. Towards actualizing the value potential of Korea Health Insurance Review and Assessment (HIRA) data as a resource for health research: strengths,

limitations, applications, and strategies for optimal use of HIRA data. J Korean Med Sci 2017;32:718-28.
34. Liu J, Kong J, Chang YJ, Chen H, Chen YH, Han W, Wang Y, Yan CH, Wang JZ, Wang FR, Chen Y, Zhang XH, Xu LP, Liu KY, Huang XJ. Patients with refractory cytomegalovirus (CMV) infection following allogeneic haematopoietic stem cell transplantation are at high risk for CMV disease and non-relapse mortality. Clin Microbiol Infect 2015;21:1121.
35. Lee YH, Han K, Ko SH, Ko KS, Lee KU; Taskforce Team of Diabetes Fact Sheet of the Korean Diabetes Association. Data analytic process of a nationwide population-based study using national health information database established by National Health Insurance Service. Diabetes Metab J 2016;40:79-82.
36. Wang H, Peng G, Bai J, He B, Huang K, Hu X, Liu D. Cytomegalovirus infection and relative risk of cardiovascular disease (ischemic heart disease, stroke, and cardiovascular death): a meta-analysis of prospective studies up to 2016. J Am Heart Assoc 2017;6:e005025.
37. American Diabetes Association. 3. Comprehensive medical evaluation and assessment of comorbidities: standards of medical care in diabetes-2018. Diabetes Care 2018;41(Suppl 1):S28-37.
38. Yoneda S, Imagawa A, Fukui K, Uno S, Kozawa J, Sakai M, Yumioka T, Iwahashi H, Shimomura I. A histological study of fulminant type 1 diabetes mellitus related to human cytomegalovirus reactivation. J Clin Endocrinol Metab 2017;102:2394-400.
39. Numazaki K, Goldman H, Wong I, Wainberg MA. Viral infection of human fetal islets of Langerhans. Replication of human cytomegalovirus in cultured human fetal pancreatic islets. Am J Clin Pathol 1988;90:52-7.
40. Eckhard M, Martin I, Eich T, Weimer R, Zinn S, Bretzel RG, Brendel MD. Incidence of cytomegalovirus infections after immunosuppression induction in clinical islet transplantation and impact on graft function. Transplant Proc 2002;34:1922-4.

Supplementary Table 1. Comparison of clinical characteristics between adult individuals with and without new-onset T2DM after cohort entry in the matched cohort of total 3,456

Characteristic	New-onset T2DM		*P* value
	Yes (*n*=95)	No (*n*=3,361)	
Male sex	52 (54.74)	1,838 (54.69)	0.992[a]
Age, yr-old	58.02±12.70	50.75±16.02	<0.001[b]
≥60	46 (48.42)	938 (27.91)	<0.001[a]
Lower income status	23 (24.21)	801 (23.83)	0.932[a]
Hypertension	51 (53.68)	746 (22.20)	<0.001[a]
Dyslipidemia	28 (29.47)	427 (12.70)	<0.001[a]
COPD	22 (23.16)	504 (15.00)	0.029[a]
Ischemic heart diseases	24 (25.26)	373 (11.10)	<0.001[a]
Cerebral infarction	8 (8.42)	158 (4.70)	0.095[a]
Heart failure	7 (7.37)	114 (3.39)	0.038[a]
End-stage renal disease	12 (12.63)	139 (4.14)	<0.001[a]
CMV diseases	32 (33.68)	544 (16.19)	<0.001[a]
Follow-up duration, yr	1.38±1.19	3.04±1.42	<0.001[b]

Values are presented as number (%) or mean±standard deviation.
T2DM, type 2 diabetes mellitus; COPD, chronic obstructive pulmonary disease; CMV, cytomegalovirus.
[a]McNemar test, [b]Paired *t*-test.

Supplementary Table 2. Multivariate logistic regression models to examine the effect of refractory cytomegalovirus disease on new-onset T2DM in various subgroups

	CMV group		Total no.	T2DM no.	Total FU duration, yr	IR[a] of T2DM	Model 1	Model 2	Model 3	*P* value for interaction
Total	No	-	2,880	63	8,664.4	7.27	1 (reference)	1 (reference)	1 (reference)	-
	Yes	Non-refractory	488	24	1,461.2	16.42	2.26 (1.39–3.57)	2.26 (1.38–3.56)	1.77 (1.07–2.82)	
		Refractory	88	8	220.2	36.34	4.80 (2.12–9.43)	4.84 (2.14–9.53)	4.01 (1.76–7.96)	
Sex										0.256
Male	No	-	1,575	33	4,737.1	6.97	1 (reference)	1 (reference)	1 (reference)	
	Yes	Non-refractory	261	15	769.4	19.49	2.80 (1.48–5.05)	2.80 (1.48–5.07)	2.05 (1.06–3.78)	
		Refractory	54	4	146.3	27.35	3.85 (1.15–9.69)	3.78 (1.13–9.50)	2.75 (0.81–6.70)	
Female	No	-	1,305	30	3,927.3	7.64	1 (reference)	1 (reference)	1 (reference)	
	Yes	Non-refractory	227	9	691.8	13.01	1.72 (0.77–3.47)	1.69 (0.76–3.43)	1.46 (0.64–3.03)	
		Refractory	34	4	73.9	54.14	6.42 (1.91–16.30)	6.88 (2.04–17.53)	6.89 (2.02–17.87)	
Age, yr-old										0.114
<60	No	-	2,060	26	6,357.6	4.09	1 (reference)	1 (reference)	1 (reference)	
	Yes	Non-refractory	345	17	1,062.3	16.00	3.93 (2.09–7.18)	4.04 (2.15–7.38)	3.22 (1.65–6.13)	
		Refractory	67	6	166.6	36.01	8.27 (3.08–18.80)	7.44 (2.77–16.94)	5.72 (2.07–13.52)	
≥60	No	-	820	37	2,306.8	16.04	1 (reference)	1 (reference)	1 (reference)	
	Yes	Non-refractory	143	7	399.0	17.55	1.10 (0.45–2.31)	1.10 (0.45–2.32)	0.98 (0.40–2.07)	
		Refractory	21	2	53.6	37.35	2.25 (0.37–7.36)	2.36 (0.38–7.88)	2.03 (0.33–6.83)	

Values are presented as number or odds ratio (95% confidence interval). Model 1, non-adjusted; Model 2, age- and sex-adjusted; Model 3, age-, sex-, lower income-, hypertension- and dyslipidemia-adjusted.
T2DM, type 2 diabetes mellitus; CMV, cytomegalovirus; FU, follow-up; IR, incidence rate.
[a]Per 1,000.

Supplementary Table 3. Comparison of incidence rate of new onset T2DM between adult individuals with and without cytomegalovirus disease in the matched cohort of 1:3 ratio with age, sex, cohort entry year, lower income status, hypertension, and dyslipidemia

CMV disease	Total no.	T2DM no.	Total FU duration, yr	IR[a] of T2DM	OR (95% CI)
No	1,488	41	4,403.6	9.31	1 (reference)
Yes	496	27	1,444.7	18.69	2.01 (1.22–3.24)

T2DM, type 2 diabetes mellitus; CMV, cytomegalovirus; FU, follow-up; IR, incidence rate; OR, odds ratio; CI, confidence interval.
[a]Per 1,000.

Supplementary Table 4. Distribution for incidence of cytomegalovirus disease or infection according to ICD-10 codes in the entire-population database

ICD-10 codes	Year				
	2010	2011	2012	2013	2014
B25 (cytomegalovirus disease)	2	0	1	0	0
B25.0 (cytomegaloviral pneumonitis)	14	14	14	16	17
B25.1 (cytomegaloviral hepatitis)	20	10	10	19	26
B25.2 (cytomegaloviral pancreatitis)	0	0	0	0	0
B25.8 (other cytomegaloviral diseases)	56	77	93	87	123
B25.9 (cytomegaloviral disease, unspecified)	67	105	87	137	193
Total (n=1,188)	159	206	205	259	359

Data were number from the Korean Health Insurance Review and Assessment Service claims data warehouse.
ICD-10, International Statistical Classification of Diseases and Related-Health Problems 10th Revision.

Supplementary Table 5. Frequencies of solid organ transplantation recipients with CMV disease or infection according to ICD-10 codes between 2010 and 2015 in the total database

ICD-10 codes	Patients with CMV disease	
	Total individuals	SOT recipients
B25 (cytomegalovirus disease)	3	0
B25.0 (cytomegaloviral pneumonitis)	89	6 (6.7)
B25.1 (cytomegaloviral hepatitis)	101	4 (4.0)
B25.2 (cytomegaloviral pancreatitis)	4	0
B25.8 (other cytomegaloviral diseases)	485	80 (16.5)
B25.9 (cytomegaloviral disease, unspecified)	621	193 (31.1)
Total	1,303	283 (21.7)

Values are presented as number (%).
CMV, cytomegalovirus; ICD-10, International Statistical Classification of Diseases and Related-Health Problems 10th Revision; SOT, solid organ transplantation.