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Original Article

The effects of sphingosine-1-phosphate (S1P) and CD69 on the pathogenesis of type 2 diabetes complicated by coronary heart disease

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Abstract: The goal of this study was to examine the factors, particularly S1P concentrations and expression of CD69 on $CD3^+CD4^+$ T lymphocytes, affecting the pathogenesis of type 2 diabetes mellitus complicated by coronary heart disease (T2DM-CHD). Samples were collected from 68 people with T2DM-CHD consecutively and 81 people with T2DM but without CHD as control. Enzyme-linked immunosorbent assay (ELISA) kits were used to measure plasma S1P levels, and the expression of CD69 was measured via immunofluorescence staining and flow cytometry. Biochemical parameters such as high-sensitivity C-reactive protein (hsCRP), blood glucose, and lipid indices were also collected. The levels of plasma S1P, HDL, and $ApoA_1$ were significantly lower among the persons with T2DM-CHD compared with the control participants ($P<0.01$), whereas $CD3^+CD4^+CD69^+$ T cell content ($P<0.01$), hsCRP, FPG, 2hPG, HbA_1c, TC, TG, LDL, ApoB, LP (a) and prior history of CHD were each significantly increased ($P<0.05$). Multiple stepwise logistic regression analysis demonstrated that the plasma S1P level was an independent protector against the development of T2DM-CHD, whereas the CD69 antigen, hsCRP, 2hPG, LDL, and Lp (a) were each separate risk factors for its development. Among type 2 diabetics, decreased S1P levels, increased $CD3^+CD4^+CD69^+$ T cells' content, as well as hsCRP, 2hPG, LDL and Lp (a) levels each increase the risk of developing CHD, which suggests that changes in these parameters are tied to its underlying pathophysiology.

Keywords: T2DM-CHD, immune inflammation, S1P, CD69 antigen

Introduction

T2DM is a non-insulin-dependent metabolic inflammatory disease and is a risk factor for both cardiovascular disease and other types of vascular pathology. In addition to increased blood glucose levels, several other factors such as lipid metabolism disorders, high blood pressure, chronic systemic inflammation, and endothelial damage also correlate with the development of coronary artery lesions in the setting of T2DM [1]. However, mechanisms underlying the disease's development and progression remain controversial.

S1P is a biologically active lipid secreted by a wide variety of cell types and plays a vital role in regulating diverse cellular processes, including both cell survival and migration, vessel maturation, and angiogenesis [2]. As an important signaling molecule within the cardiovascular system, S1P is also involved in the regulation of fundamental immune functions such as the trafficking of $CD4^+$ T lymphocytes and the proliferation of T cells, as well as their positions within lymph nodes [3, 4]. HDL, the primary carriers of S1P in the plasma, exert several important cardioprotective effects as follows: anti-inflammatory effects, antioxidant effects, and cholesterol-efflux effects. The absence of these effects may result in the development of vascular diseases such as atherosclerosis, which is mediated by T2DM [5, 6]. Moreover, previous studies have demonstrated that T2DM complicated by atherosclerosis is characterized by lower levels of HDL-S1P compared with T2DM not complicated by atherosclerosis, which indicates that up-regulation of peripheral blood HDL-S1P may exert protective effects within the vasculature in the setting of T2DM [7].

However, whether a correlation exists between these effects and plasma total S1P concentrations has not been studied in type 2 diabetics.

Recent studies have demonstrated that the occurrence of T2DM may be related to the development of autoimmune disease. CD69 is an important signal transduction molecule and is expressed on the surfaces of activated T lymphocytes. Previous studies have indicated that S1P inhibits the activation of $CD4^+$ T lymphocytes, which release proinflammatory factors, by decreasing expression of CD69 in mouse models with type 1 diabetes as a means of modulating the immune responses associated with diabetes and by preventing subsequent development of the vascular complications associated with diabetes [8]. Here, expression of CD69 was studied on the surface of $CD3^+CD4^+$ T lymphocytes in type 2 diabetics with and without CHD in order to understand the mechanisms underlying both the development and the progression of these diseases as a means of developing new therapies.

Patients and methods

The present study included 149 patients with T2DM who were evaluated by the Departments of Cardiology and Endocrinology of the Second Hospital, affiliated with Chongqing Medical University between October 2013 and June 2014. Sixty-eight persons (69.12±9.43 years, 71.8% male) with CHD were assigned to the T2DM-CHD group, and 81 age- and gender-matched persons (mean age 71.02±9.50 years, 71.02% male) who underwent coronary examinations but did not meet the criteria for the diagnosis of CHD were assigned to the T2DM group. Each person met the study's inclusion and exclusion criteria, as follows:

Inclusion criteria: 1) people diagnosed with T2DM based on the criteria developed by the ADA, as follows: polydipsia, polyphagia, polyuria, and weight loss in combination with a random plasma glucose ≥ 11.1 mmol/l, a FPG ≥ 7.0 mmol/L, or a 2hPG ≥ 11.1 mmol/L on an OGTT, as well as a HbA_1c ≥ 6.5% [9]. Persons receiving treatment for hyperglycemia with either insulin or oral drugs also met the diagnostic criteria, 2) people with confirmed CHD via either coronary arteriography or coronary computed tomographic angiography. The severity of coronary stenosis was assessed via a visual estimation of vascular diameter and judged independently by two experienced cardiologists or radiologists. CHD was diagnosed if vessel diameter stenosis was ≥ 50% [10], and 3) patients with either hypertension or hyperlipidemia receiving appropriate pharmacologic therapy.

Exclusion criteria: 1) people with type 1 diabetes or forms of diabetes mellitus, 2) people suffering from recent acute diabetes complications (within six months), including diabetic ketoacidosis, hyperglycaemic hyperosmolar state, lactic acidosis, or hypoglycemic coma, 3) people suffering from kidney failure (serum creatinine > 106 μmol/L), proteinuria, liver injury (ALT > 80 U/L), heart failure, or retinopathy, and 4) people suffering from diseases known to affect either S1P or CD69 levels, including malignant tumors, connective tissue disease, autoimmune disease, thyroid dysfunction, bacterial and viral infection, Chlamydia, parasitic infection, recent surgery and peripheral vascular disease complicated by either ischemia or thrombosis.

The study protocol was approved by the Ethics Committee of Chongqing Medical University and the Scientific Research Department of our hospital. All of our experimental procedures were performed in accordance with the principles of the Declaration of Helsinki. Each person provided written informed consent.

The following clinical data were collected: 1) Risk factors for CHD, including blood glucose and lipid levels, hsCRP levels etc.; and 2) medication history, including drugs used to treat hyperglycemia, hyperlipidemia, and hypertension, as well as antiplatelet drugs and vasodilators.

Measurement of the relevant biochemical indices

Detection of CD69 antigens via flow cytometry: We randomly selected fifty-five people (thirty-five from the experiment group and twenty from another group) for the measurement of CD69 antigen. Peripheral blood lymphocytes were subsequently isolated using lymphocyte isolation liquid. The lymphocyte concentration was adjusted to $1 * 10^6$ cells per millilitre using RPMI-1640 culture medium containing 10% heat-inactivated fetal bovine serum. PHA was

Table 1. Baseline clinical characteristics of the people

	T2DM (n=81)	T2DM-CHD (n=68)	*P* values
Gender (male/female)	42/37	40/28	0.491
Age (years)	71.02±9.50	69.12±9.43	0.223
Drinking (%)	40.70%	30.90%	0.212
Smokers (%)	42.00%	45.60%	0.658
Hypertension (%)	59.30%	66.20%	0.385
Systolic blood pressure (mmHg)	135.72±18.56	137.99±16.39	0.434
Diastolic blood pressure (mmHg)	79.89±10.46	78.71±10.96	0.502
BMI (kg/m^2)	23.71±3.84	26.81±24.79	0.269
FPG (mmol/l)	7.39±3.20	7.42±1.80	0.03*
2hPG (mmol/l)	13.47±3.62	16.98±3.01	0*
RBG (mmol/l)	12.52±5.40	10.97±4.05	0.051
HbA_1c (%)	7.63±1.76	8.13±1.27	0.046*
TC (mmol/l)	4.52±1.38	4.99±1.43	0.047*
TG (mmol/l)	1.89±2.14	2.53±1.66	0.047*
LDL (mmol/l)	2.44±1.75	3.07±0.95	0.009*
HDL (mmol/l)	1.11±0.36	0.97±0.25	0.008*
$ApoA_1$ (g/l)	1.52±0.35	1.36±0.27	0.002*
ApoB (g/l)	0.89±0.33	0.99±0.28	0.049*
Lp (a) (mg/l)	158.15±117.66	249.08±219.37	0.003*
S1P (pg/ml)	1327.43±119.65	1083.18±161.55	0*
$CD3^+CD4^+CD69^+$ T cell content (%)	3.12±2.00	16.58±9.23	0*
hsCRP (mg/L)	3.06±3.09	24.17±47.09	0*
ACEI/ARBs (%)	59.30%	85.30%	0*
B-blockers (%)	22.20%	55.90%	0*
CCB (%)	37.00%	57.40%	0.013*
Diuretics (%)	14.80%	35.30%	0.004*
Insulins (%)	54.30%	47.10%	0.377
Glucosides (%)	23.50%	29.40%	0.41
Glitinides (%)	27.20%	23.50%	0.613
Sulfonylureas (%)	16.00%	10.30%	0.305
Thiazolidinediones (%)	3.70%	7.40%	0.325
Biguanides (%)	23.50%	16.20%	0.27
Statins/Fibrates (%)	76.50%	80.90%	0.521
Nitrates (%)	11.10%	54.40%	0*
Anti-platelet agents (%)	22.20%	75%	0*

Continuous variables were described as mean ± SD. N (%) for categorical variables. *Significant differences between groups $P<0.05$. T2DM, type 2 diabetes mellitus; T2DM-CHD, type 2 diabetes mellitus complicated by coronary heart disease; BMI, body mass index; FPG, fasting plasma glucose; 2hPG, postprandial 2 h plasma glucose; RBG, random blood glucose; TC, total cholesterol; TG, triglycerides; LDL, low-density lipoprotein; HDL, high-density lipoprotein; $ApoA_1$, apolipoprotein A_1; ApoB, apolipoprotein B; Lp (a), lipoprotein (a); S1P, sphingosine-1-phosphate; hs-CRP, high-sensitivity C-reactive protein; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker. The incidence of CHD within different quartile ranges of CD69 expression on $CD3^+CD4^+$ T lymphocyte subsets, and S1P levels.

used at a concentration of 20 μg/ml. The T lymphocytes were incubated at 37°C in a 5% CO_2-humidified atmosphere for 20 hours.

The activated T lymphocytes were harvested as described above, the samples were subsequently co-stained with 10 μl each of PE-labelled anti-human CD69 or PE-labelled CD69 isotype control rat-IgG1, FITC-labelled anti-human CD4, APC-labelled anti-human CD3 antibodies (each purchased from BD Biosciences) and incubated at room temperature for 20 minutes,

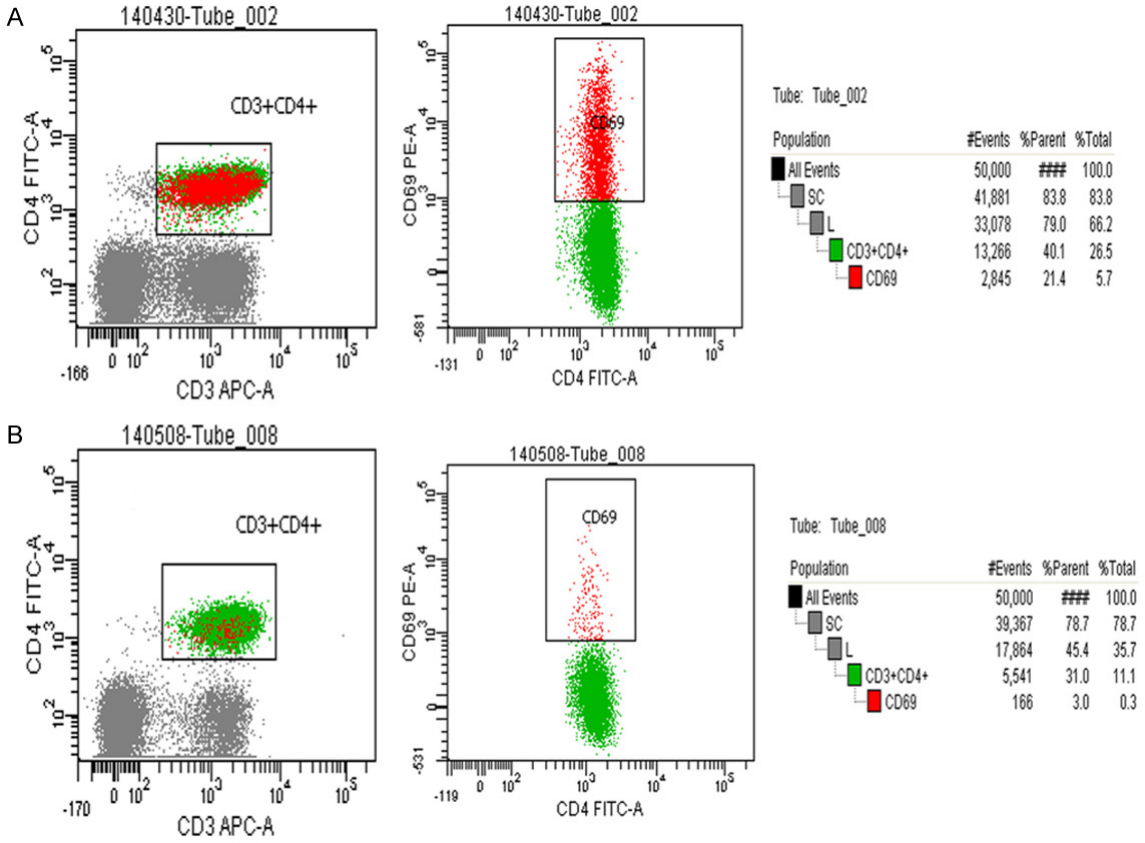

Figure 1. Expression of CD69 on $CD3^+CD4^+$ T lymphocytes with fluorescence cytometry in T2DM with or without CHD. (A) shows the expression level of the CD69 antigen on the $CD3^+CD4^+$ T lymphocyte subset is 21.4% in type 2 diabetes with CHD and (B) indicated the expression content of the CD69 antigen on the $CD3^+CD4^+$ T lymphocyte subset is 3.0% in type 2 diabetes without CHD. *T2DM* type 2 diabetes mellitus, *CHD* coronary heart disease.

shielded from light. The samples were then resuspended for flow cytometry. The data collection, measurement and analysis were accomplished using a FACS-Calibur (BD Biosciences).

Total plasma S1P detection: Fasting peripheral venous blood samples were taken from each person before being centrifuged at 3000 r/min at 4°C in order to separate the plasma from the remainder of the sample. The samples were subsequently numbered and frozen at -80°C. Following collection, each sample was thawed, and S1P levels were measured via ELISA (Shanghai Jinma Science & Technology Co., Ltd.) according to the manufacturer's instructions.

The assessment of biochemical variables: 1) Serum hsCRP concentrations were quantified using an immune turbidimetric test, suing a Hitachi7600 Automatic Biochemistry Analyser; 2) the level of HbA_1c was measured via high performance liquid chromatography; 3) both FPG and 2hPG levels were tested using a Beckman CX3 Automatic Biochemistry Analyser; 4) TC, TG, LDL, HDL, $ApoA_1$, ApoB, and Lp (a) were each measured using a Hitachi7600 Automatic Biochemistry Analyser.

Statistical analysis

All statistical analysis was performed with SPSS software, version 20.0. Difference of measurement data were compared with Independent-Samples T Test. Comparisons of count data were made by Chi-square test. Multiple stepwise logistic regression analysis and odds ratios were used to determine independent predictors of cardiovascular events. Receiver operating curve (ROC) analysis was adopted to analyze the accuracy of CD69 and S1P in predicting CHD complication in type 2 diabetics. Two sided $P<0.05$ were considered statistically significant.

Results

General clinical characteristics of the study population were as follows (**Table 1**): 1) The content of peripheral blood $CD3^+CD4^+CD69^+$ T lymphocyte subpopulation and the concentrations of hsCRP, 2hPG, LDL, and Lp (a) among the people with T2DM-CHD were much higher compared with persons with T2DM alone, with evident statistical significance ($P<0.01$). The flow cytometry results are included in **Figure 1**. Total plasma S1P, HDL, and $ApoA_1$ were significantly lower in the CHD group compare with those in the control group ($P<0.01$). FPG, HbA_1c, TC, TG, and ApoB levels, as well as the use of ACEI/ARBs, beta-blockers, CCB, diuretics, nitrates, and anti-platelet agents were more common among the members of the CHD group than that of the control group ($P<0.05$). However, a comparison between the two groups demonstrated that several risk factors for macroangiopathy, including gender, age, alcohol history, smoking history, hypertension, BMI, and random blood glucose, as well as the use of medications to treat hyperglycemia and hyperlipidemia, including various types of insulins, glucosides, glitinides, sulfonylureas, thiazolidinediones, biguanides, statins, and fibrates, were not significantly different.

People were divided into quartiles based on CD69 expression as follows (**Figure 2A**): Quartile 1, 2, 3 and 4 (0.4-3.0, 3.8-8.2, 10.1-21.3 and 21.4-41.0). Three of the four sets included fourteen individuals, and the fourth set included thirteen individuals. The incidence of CHD within each group was 21.4%, 35.7%, 100% and 100%, respectively, and increased in conjunction with the expression of the CD69 antigen. Persons within the highest quartiles exhibited an approximately five-fold increase in the incidence of CHD compared with the persons within the lowest quartile. Therefore, people with T2DM within the top quartiles of CD69 antigen expression had a greater risk of developing coronary lesions than the people within the remaining two quartiles ($P<0.001$).

Contrasting results were observed among people stratified into quartiles based on S1P levels as demonstrated (**Figure 2B**): Quartile 1, 2, 3 and 4 (786.453-1067.451, 1069.951-1245.190, 1245.190-1373.387 and 1375.434-1525.960). Thirty-seven persons were included in groups one through three, and the fourth group included thirty-eight persons. The incidence of CHD in each group was 86.5%, 67.6%, 13.5% and 15.8%, respectively, and decreased as the level of S1P increased. The probability of developing CHD was lower among individuals within the third quartile compared with quartiles 1, 2 and 4 ($P<0.001$). Therefore, we concluded that increases in CD69 expression and decreases in S1P levels were both associated with an increased risk of CHD.

An analysis of independent predictors of CHD in the setting of T2DM

The parameters directly related to the occurrence of CHD among type 2 diabetics (**Table 1**) were investigated using a multiple stepwise logistic regression analysis, the results of which are included in **Table 2**. The levels of 2hPG, LDL, Lp (a), hsCRP, as well as $CD3^+CD4^+CD69^+$ T cell content, were each an independent risk factor for CHD. Increases in the levels of these determinants exerted a statistically significant effect on the incidence of coronary lesions among persons with T2DM and increased the risk of a subsequent cardiovascular event. However, the level of S1P was an independent protector against CHD. Therefore, down-regulation of total plasma S1P levels was responsible for development of CHD in the setting of T2DM.

Based on the results of the logistic regression analysis, we subsequently evaluated the ability of both CD69 expression on the $CD3^+CD4^+$ T lymphocyte subpopulation and total plasma S1P levels to predict CHD among type 2 diabetics using ROC and the areas under curve (AUC). The results are included in Supplementary Figure 1A and 1B and indicate that the AUC of the CD69 antigens was 0.937 ($P<0.01$), and the cut-off value was 5.75%, with a sensitivity of 91.4% and a sensitivity of 95%. The AUC of S1P was 0.874 ($P<0.01$), and the cut-off value of 1194.619 pg/ml exhibited a sensitivity and specificity of 87.7% and 82.4%, respectively. When either the $CD3^+CD4^+CD69^+$ T cell content was greater than 5.75% or the total plasma S1P level was below 1194.619 pg/ml, the persons with T2DM were more vulnerable to the complications of CHD.

Discussion

Among people with T2DM, the occurrence of macrovascular complications such as CHD

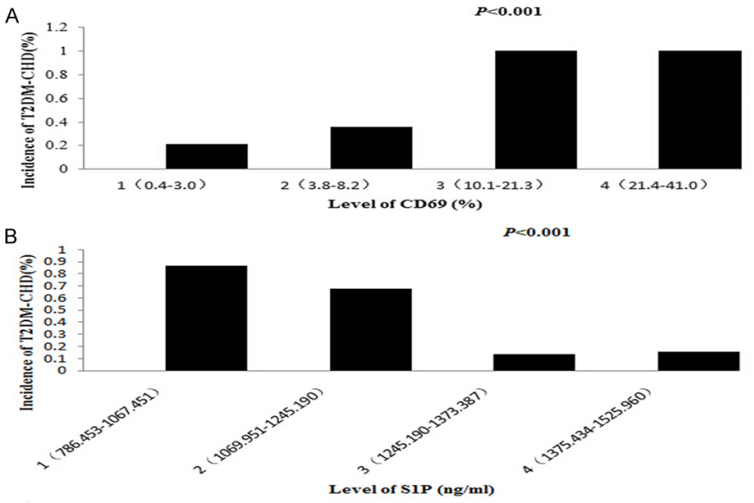

Figure 2. The incidence of T2DM-CHD stratified by quartile of $CD3^+CD4^+CD69^+$ T lymphocyte expression and S1P level. A. Shows the incidence of CHD within each group (Quartile 1, 2, 3 and 4) were 21.4%, 35.7%, 100%, and 100%, respectively, and increased in conjunction with the expression of the CD69 antigen. B. Indicated the incidences of CHD in each group (Quartile 1, 2, 3 and 4) were 86.5%, 67.6%, 13.5%, and 15.8%, respectively, and decreased as the level of S1P increased. *T2DM-CHD* type 2 diabetes mellitus complicated by coronary heart disease, *S1P* sphingosine-1-phosphate, *CHD* coronary heart disease.

Table 2. The predictors of CHD in the setting of type 2 diabetes as determined via a multiple stepwise logistic regression analysis

Variables	95% CI	OR	*P* values
FPG (mmol/l)	0.887-1.133	1.003	0.965
2hPG (mmol/l)	1.213-1.543	1.368	0*
HbA_1c (%)	0.995-1.523	1.213	0.056
TC (mmol/l)	0.998-1.617	1.27	0.052
TG (mmol/l)	0.988-1.475	1.207	0.066
LDL (mmol/l)	1.083-1.944	1.451	0.013*
HDL (mmol/l)	0.124-4.523	0.748	0.751
$ApoA_1$ (g/l)	0.044-1.426	0.249	0.119
ApoB (g/l)	0.993-8.424	2.892	0.052
Lp (a) (mg/l)	1.001-1.006	1.003	0.004*
S1P (pg/ml)	0.986-0.993	0.989	0*
$CD3^+CD4^+CD69^+$ T cell (%)	1.223-2.291	1.674	0.001*
hsCRP (mg/l)	1.044-1.206	1.122	0.002*

*Significant differences between groups P<0.05. OR, odds ratio; CI, confidence interval; FPG, fasting plasma glucose; 2hPG, postprandial 2 h plasma glucose; TC, total cholesterol; TG, triglycerides; LDL, low-density lipoprotein; HDL, high-density lipoprotein; $ApoA_1$, apolipoprotein A_1; ApoB, apolipoprotein B; Lp (a), lipoprotein (a); S1P, sphingosine-1-phosphate; hs-CRP, high-sensitivity C-reactive protein. The accuracy of CD69 and S1P in predicting CHD in the setting of T2DM via ROC curves.

remains one of the primary reasons for the reduced quality of life quality and increased mortality. An endeavour may require both development and the implementation of novel therapies targeting macrovascular lesions, including coronary artery lesions.

Over the past decade, an extensive body of research has demonstrated that the vast majority of people with T2DM have suffered from obesity-associated insulin resistance, hyperglycemia, and hyperlipidemia, and that both the development and the progression of all these diseases results in changes to the immune system and subsequent proliferation of several chemical mediators produced by proinflammatory cells such as hsCRP, IL-6, and TNF-α [11, 12]. Proinflammatory environments have long been linked with imbalances in T lymphocytes. In the setting of obesity-associated insulin-resistant T2DM, the release of T and B lymphocytes and the secretion of proinflammatory cytokines may enhance either local or systemic inflammatory reactions, findings indicating that in type 2 diabetics, immune cells are the driving force underlying the disease's inflammation [13, 14].

CD69 is a member of the type II C-group lectin-like receptor family, as well as an inducible primed leukocyte receptor. Although CD69 is infrequently produced by static lymphocytes, its expression may be rapidly induced in activated cells. Due to its ability to be expressed on the surfaces of different activated leukocyte subsets, this receptor may also influence cellular differentiation, interfere with cytokine synthesis, and regulate inflammatory reactions, as well modulate the responses of the immune system. It often presents in the setting of chronic inflammatory disease and modulates the migration of effector T cells and dendritic cells derived from the bone marrow, a process mediated by chemokine S1P [15]. Our research indicates that when people with T2DM develop CHD, T cells in peripheral blood become active and may proliferate and

subsequently participate in various systemic inflammatory reactions. We have determined that $CD69^+$ T lymphocytes are most likely involved in the pathogenesis underlying the vascular injury associated with T2DM, an idea supported by the findings of Khallou-Laschet et al. [16]. By contrast, Cruz-Adalia et al. hypothesized that the absence of the C69 receptor may facilitate the inflammation and subsequent myocardial tissue damage mediated by Th17 lymphocytes, promoting the eventual progression of heart failure, via their establishment of a "lack of CD69" autoimmune myocarditis model [17]. Similar views have been expressed by de la Fuente and Martin as follows: CD69 selectively binds to its ligand, galactin-1, on surface of dendritic cells and activates the Jak3/STAT5 signaling pathway, inhibits Th17 cell differentiation, and weakens the proinflammatory response mediated by these helper cells, which results in decreased susceptibility to different inflammatory diseases via the attenuation of this inflammatory reaction [18]. These results do not conform to those of our study. However, we determined that among persons with T2DM complicated by coronary lesions, expression of CD69 may be activated inappropriately, which may have profound implications for the pathogenesis of CHD.

S1P is an intermediate of sphingomyelin metabolism and has multiple functions such as protecting the vascular endothelium, suppressing the migration of smooth muscle cells, ameliorating myocardial ischemia-reperfusion injury, and inhibiting the expression of adhesion molecules by attaching to specific receptors on the surfaces of various cells linked to various metabolic functions, including tumorigenicity, cardiovascular events and immunity. In the immune system, it regulates both the transportation and the distribution of lymphocytes within lymphoid organs, controls lymphocytic egress from lymph nodes via the S1P/S1P1 receptor axis, and modulates the retention of T cells in the peripheral tissues. CD69 also decreases the expression of S1P1 receptors on T lymphocytes in order to induce lymphocytes to return to lymph nodes [19-21]. Our results indicate that total plasma S1P concentrations are associated with the vascular protective effects observed in type 2 diabetics, findings analogous to those pertaining to the HDL-S1P relationship.

Most clinicians and researchers believe that diabetes is associated with systemic damage to both microcirculatory and macrocirculatory vascular beds [22], effects caused by immune abnormalities and inflammatory reactions; macroangiopathy represents one of the most common chronic complications of diabetes. Previous studies have observed that hsCRP, an inflammatory mediator, acts directly on vascular endothelial cells in order to break down protective polysaccharide-protein complexes, down-regulate both the activity and the expression of nitric oxide synthase, and inhibit vasodilation. Its elevation not only correlates with obesity, insulin resistance, diabetes and cardiovascular disease but is also an independent risk factor for cardiovascular disease and may predict its developing and progressing [23]. Moreover, an elevated 2hPG level is also one of the risk factors underlying the high mortality associated with cardiovascular disease, as acute hyperglycemia may cause microvascular endothelial dysfunction via the generation of free radicals and the formation of a proinflammatory environment [24]. Lp (a), a subtype of LDL-C and a carrier of Apo (a), is part of the plasminogen family and also has the potential to promote both atherosclerosis and thrombosis [25]. The $ApoB/ApoA_1$ ratio is believed to be a better predictor of the risk of cardiovascular disease than LDL-C [26]. Our research has provided evidence that both high blood sugar and elevated lipid levels may promote vascular inflammatory reactions and exacerbate vascular damage. However, decreases in these parameters may decrease the risk of developing CHD and are of profound significance where the diagnosis and treatment of it are concerned. Furthermore, increasing the plasma level of S1P may decrease the likelihood of developing this disease. In conclusion, prompt implementation of multiple interventions may prevent people with T2DM from developing CHD [27], a hypothesis supported by our findings.

We hypothesize that the relationship among CD69, S1P, and CHD in the setting of T2DM may be enhanced by inflammatory immune responses. However, additional research and discussion are necessary in order to bolster our viewpoints and elucidate the mechanisms underlying the pathophysiology of CHD.

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Disclosure of conflict of interest

None.

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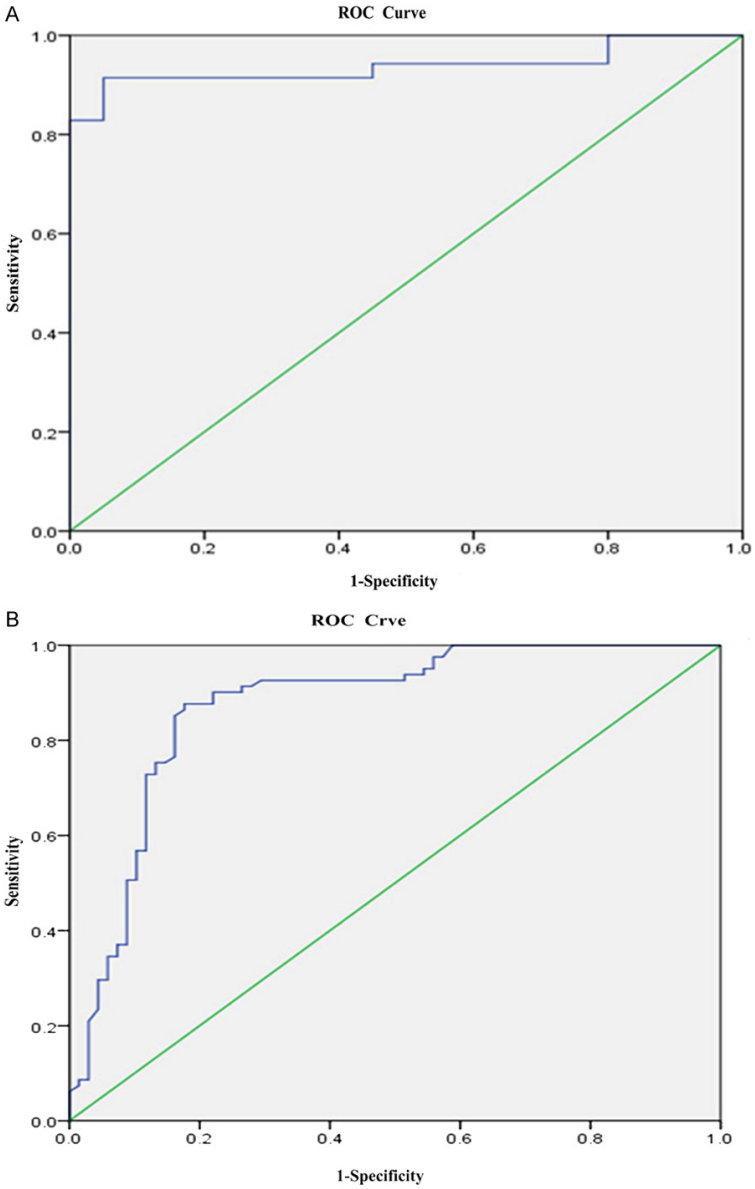

Supplementary Figure 1. A receiver operating characteristics curve of $CD3^+CD4^+CD69^+$ T lymphocytes and S1P for predicting T2DM-CHD. A. Shows that the AUC of the CD69 antigens was 0.937 (95% CI=0.869-1.000, P<0.001), and the cut-off value was 5.75%, with a sensitivity of 91.4% and a sensitivity of 95%. B. Indicated the AUC of S1P was 0.874 (95% CI=0.813-0.934, P<0.001), and the cut-off value of 1194.619 pg/ml exhibited a sensitivity and specificity of 87.7% and 82.4%, respectively. *S1P* sphingosine-1-phosphate, *T2DM-CHD* type 2 diabetes mellitus complicated by coronary heart disease, *AUC* areas under curve, *CI* confidence interval.