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CD51/CD61$^+$ Endothelial Microparticles Decrease in Diabetes Patients with Hypertension

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Abstract

Backgrounds: Type 2 Diabetes Mellitus (T2DM) and hypertension are commonly co-occurred and both diseases are related to endothelial dysfunction. Endothelial microparticles (EMPs) are shed from endothelial cells and can be found in condition of endothelial dysfunction. This study aimed to evaluate the circulating endothelial MPs (CD51/CD61$^+$) levels in T2DM patients with or without hypertension and the correlation between endothelial MPs and clinical parameters. **Methods and Results:** 20 healthy control, 16 T2DM patients without hypertension and 11 T2DM patients with hypertension were recruited. CD51/CD61$^+$ EMPs from all subjects were analyzed by flow cytometry. We found that, in the group of T2DM patients with hypertension, the absolute median number of CD51/CD61$^+$EMPs was significantly decreased, compared with that in the healthy control and T2DM without hypertension groups. Furthermore, we conducted receiver operating characteristics (ROC) analysis to examine the accuracy of CD51/CD61$^+$EMPs in the discrimination between T2DM patients with hypertension and healthy control, showing the accuracy was 76.4%. In addition, we also found that the accuracy of CD51/CD61$^+$EMPs was 83.5% in the discrimination between T2DM patients with hypertension or without hypertension **Conclusion:** These findings identify CD51/CD61$^+$EMPs as a potential biomarker to monitor endothelial dysfunction in T2DM patients with hypertension.

Keywords: Diabetes mellitus; Hypertension; Endothelial microparticles; Endothelial dysfunction.

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1. Introduction

Over two-thirds of type 2 diabetes mellitus (T2DM) patients are found to coexist with hypertension [1]. Accumulating evidence shows that patients with these two diseases have an enhanced risk for cardiovascular disease [2]. Current knowledge shows that endothelial dysfunction is one of the initial event in the pathogenesis of cardiovascular diseases and is also associated with the pathology in diabetes and hypotension patients [3, 4]. Therefore, examining the endothelial function in patients with diabetes and hypertension might provide an alternative route to diagnose these diseases at very early stage. Endothelial microparticles (EMPs) are derived from endothelial cells in response to endothelial activation and apoptosis. EMPs are closely associated with endothelial dysfunction in diabetes or hypertension [5,6]. However, there is a lack of specific biomarkers to monitor endothelial dysfunction in condition of diabetes and hypertension. The current study aimed at studying CD51/CD61^{+}EMPs in diabetes patients with or without hypertension to identify a biomarker to monitor endothelial function in diabetes patients with hypertension.

2. Methods

2.1 Study subjects

In total, 27 T2DM patients were recruited, including 16 T2DM patients without hypertension and 11T2DM with hypertension patients. Diagnose of diabetes was based on 1999 World Health Organization diagnostic criteria for diabetes mellitus. Hypertension was diagnosed as either a systolic BP≥140 mmHg or diastolic (DBP) ≥90 mm Hg supine arterial blood pressure (BP) (after 10 minutes of rest), and was measured twice using a mercury sphygmomanometer. Patients with a history of chronic renal failure requiring dialysis, hepatic or hematologic disorders, or inflammation, autoimmune, malignant diseases, type 1 diabetes and coronary heart diseases were excluded. 20 healthy subjects were recruited. Healthy subjects were included if they had no known history of medical illness, normal blood pressure (<140/90 mmHg) and normal blood glucose, and appeared healthy in a physical examination. The protocol regarding this study was approved by the Institutional Review Board of the medical institution (Ethics committee at institute of Microcirculation Peking Union Medical College &Chinese Academy of Medicine Science) and verbal informed consent was received from each study subject before entering the study. Clinical and laboratory data were collected from all subjects as shown Table 1.

2.2 Isolation and measurement of endothelial microparticles (EMP)

For microparticles isolation and measurements, the protocol was applied as previous experiments [7]. For the endothelial microparticles flow cytometry assay, platelet-poor plasma (50 µl) was incubated with fluorescein isothiocyanate-labelled (FITC) anti-CD51/CD61 (integrin alpha v beta3, 110519. eBioscience), the samples were incubated at room temperature for 20 min, diluted with 1 ml of phosphate-buffered saline, and analyzed by flow cytometry (Accuri C6, Accuri Cytometers). An isotype control antibody was used as a negative control in all measurements. EMP was defined as CD51/CD61^{+}positive particles. Values are reported as counts per µl of peripheral blood. The laboratory personnel that performed these assays were blinded to all clinical data and the study participants.

2.3 Statistical analysis

GraphPad Prism version 8 was used for statistics calculation and plots. D'A gostino-Pearson test was used to measure data normality. One-way ANOVA testing followed by Tukey test was applied for normal distributed data while one-way Kruskal-Wallis analysis followed by Manny-Whitney U test was applied to data which was not normally distributed among three groups. The chi-square test was used to compare quantitative and categorical variables. Univariate correlation analysis was performed by Spearman's rank correlation coefficients (r). Receiver Operating Characteristic (ROC) analysis was performed to assess the accuracy of the diagnostic test of CD51/CD61^{+}EMP in DM with hypertension patients. P values<0.05 were considered significant. All data is expressed as mean ± SD or otherwise identified in the table.

3. Results and Discussion

3.1 Clinical characteristics and CD51/CD61^{+}EMPs analysis of study subjects

Clinical data of both health control and patients' groups are described in Table1. The groups are matched by gender. Healthy control group and T2DM without hypertension group also are matched by BMI status. The mean age of healthy control was younger than that of the T2DM patients groups ($p<0.0001$) including T2DM patients with or without hypertension. The BMI index was higher in T2DM with hypertension groups ($p<0.001$) than that of the healthy control and T2DM without hypertension group ($p<0.05$). As figure 1 shows the numbers of CD51/CD61^{+}EMPs were lower in the T2DM with hypertension group than that of the healthy control ($p<0.05$) and T2DM without hypertension group ($p<0.01$). There was no significantly statistics of CD51/CD61^{+}EMPs between healthy control and T2DM patients without hypertension.

Table 1: Clinical data of experimental subjects

Characteristics	Control	DM-HP(-)	DM-HP(+)
Age	36.00±8.53	55.69±9.11^{a}P	59.18±11.81^{a}P
Gender(Female/male)	13/7	7/9	4/7
BMI(kg/m^2)	22.49±1.77	24.05±2.76	26.67±3.02 ^{a}P ^{b}P
Blood Glucose(mmol/l)	-	9.77±3.28	10.5±3.73
Hemoglobin A1C%	-	9.64±2.29	8.7±1.84
Medicine No			
Insulin	-	5	5
Metformin	-	1	1
Sulfonylurea	-	-	2
Acarbose	-	2	2
Repaglinide	-	-	37
B-blocker	-	0	1
Diuretic	-	0	1
ACE inhibitor/ARB	-	0	2
CCB	-	0	2

Values are mean ± SD, or number. BMI, body mass index; ACE-I/ARB, angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker; CCBs, calcium channel blockers; DM-HP(-),diabetes without hypertension; DM-HP(+), Diabetes with hypertension; A value of $P < 0.05$ was considered significant. ^{a}P compared with control, ^{b}P: compared with diabetes without hypertension.

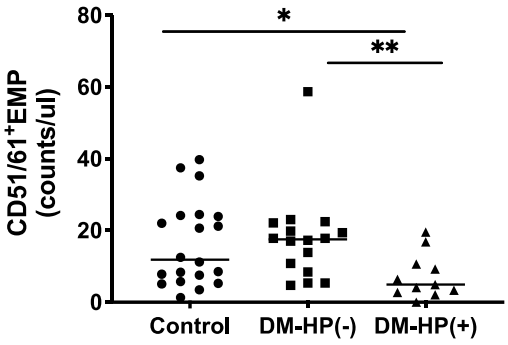

Figure 1: Diabetic patients with hypertension had significantly decreased number EMPs compared to healthy control and Diabetic patients without hypertension. The difference between groups was analysed using a Mann-Whitney U test. (* $p<0.05$, ** $p<0.01$).

3.2 Correlations between the number of CD51/CD61⁺EMP and clinical parameters

To investigate the correlation among the CD51/CD61⁺EMP levels and age and BMI (Figure 2). All the subjects were included (healthy control and two patients groups), no correlation were observed between CD51/CD61⁺EMP levels and age or BMI (all $p>0.05$). Considering only patients groups, no correlation were observed between CD51/CD61⁺ EMP levels and blood glucose level or hemoglobin A1c level (all $p>0.05$).

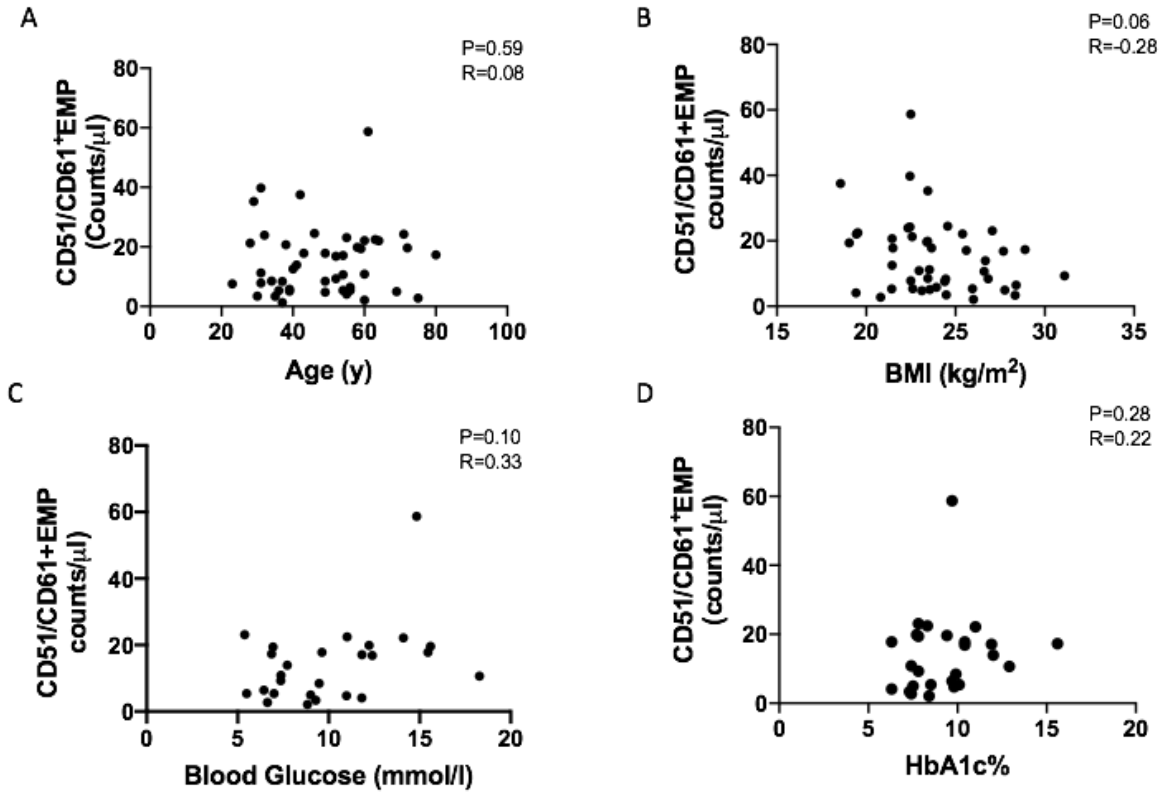

Figure 2: The correlation between CD51/CD61⁺EMP and clinical parameters. (A, B, C, D) no significant association was detected between CD51/CD61⁺EMP and age, BMI, blood glucose and HbA1c%.

3.3 Usefulness and Accuracy of CD51/CD61⁺EMP as a monitor endothelial dysfunction factor in DM with hypertension

To assess the accuracy of CD51/CD61⁺ EMP to monitor endothelial dysfunction in T2DM with hypertension patients, ROC curve was performed and shown in Figure 3. To diagnose patients between healthy control and T2DM with hypertension patients, the area under the ROC curve was 0.764 (95% CI, 0.592-0.935, P=0.02). The optimal cut off value was 20.14 counts/µl, with sensitivity and specificity values of 100% and 45%, respectively. To distinguish between T2DM with hypertension and T2DM without hypertension patients, the area under the ROC curve was 0.835 (95% CI, 0.679-0.991, P=0.0036). The optimal cut off value was 10.76 counts/µl, with sensitivity and specificity values of 81.82% and 75%, respectively.

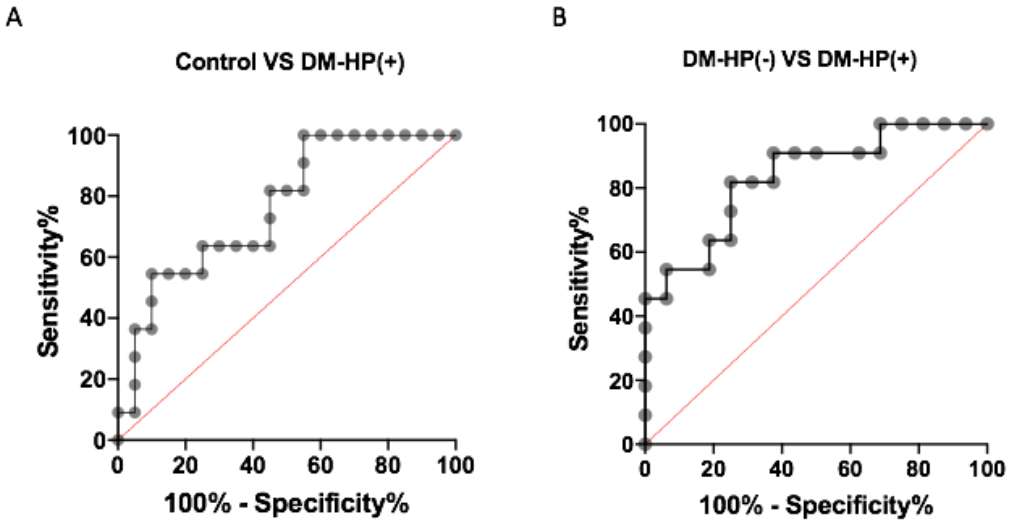

Figure 3: ROC curve analysis to determine the threshold of CD51/CD61⁺EMPs. (A). CD51/CD61⁺EMPs at a cut off 20.14 counts/µl ,p<0.05, could be discriminated between healthy control and T2DM patients with hypertension with 76.4% accuracy. (B) CD51/CD61⁺EMPs at a cut off 10.76 counts/µl ,p<0.01, could be discriminated between healthy control and T2DM patients with hypertension with 83.5% accuracy.

4. Discussion

We demonstrated that the decreased level of circulating CD51/CD61⁺ EMP is associated with DM patients with hypertension. According to ROC analysis, CD51/CD61⁺ EMPs show high sensitivity to discriminate T2DM with hypertension patients from healthy control. Furthermore, CD51/CD61⁺ EMP shows high sensitivity and specificity to discriminate between T2DM patients with or without hypertension. CD51/CD61⁺ EMPs are not correlated with age, BMI, blood glucose and hemoglobin A1c, which shows CD51/CD61⁺ EMPs are an independent biomarker to monitor endothelial dysfunction in T2DM with hypertension patients. Compared with previous studies, these studies show CD144, CD105, CD31, CD62E EMP significantly increased in T2DM patients [5, 8]. In our study, there is no significant difference of CD51/CD61⁺ EMP between healthy control and DM patients, the possible reason is that EMP is a heterogenous population [9] and EMP shows the ambivalent role in vascular diseases (8). Our study suggests the different role of CD51/CD61⁺ EMP in T2DM compared with

other EMP biomarkers. Our previous study shows [10] that there is no significant difference of CD51/CD61$^+$ EMPs numbers between healthy control and hypertension patients without diabetes, which indicates that CD51/CD61$^+$ could be a specific biomarker to monitor endothelial dysfunction in T2DM with hypertension patients. In our previous study [10], we also demonstrated that the number of CD51/CD61$^+$EMP was significantly high in obese hypertension patients, which could be indicating that CD51/CD61$^+$EMP is involved in different pathophysiological roles in hypertension patients with obesity or with T2 DM.

5. Conclusion

The findings of this study indicate that CD51/CD61$^+$ EMP can be used a specific EMP biomarker to monitor endothelial dysfunction in T2DM with hypertension patients.

6. Limitations of the study

There are some limitations in our study: 1) It is necessary to recruit large numbers of diabetes, hypertension and diabetes with hypertension patients to validate our results.2) The pathophysiological role of CD51/61$^+$ EMP in hypertension patients with diabetes or with obesity needs to be further investigated.

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