Platelet P-selectin(CD62P) and soluble TREM-like transcript-1(sTLT-1) are associated with coronary artery disease-A case control study

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Abstract

Background/Aims: Platelet activation plays a crucial role in the pathogenesis of coronary artery disease(CAD) . platelet P-selectin(CD62P)is a platelet classic activation indicator in platelet surface and soluble TREM-like transcript-1 (sTLT-1) is a new one,the relation between these two makers and acute coronary syndromes(ACS) has not been elucidated. This study aimed to investigate the expression of CD62P in platelet surface and sTLT-1 on serum and their relationship with CAD. **Methods:** We measured the levels of CD62P and sTLT-1 in 83 patients undergoing CAD compared to 49 controls.The associations with age, blood pressure,lipid profiles, body mass index and liver injury marker levels were also examined. **Results:** A stepwise increase in CD62P concentration was found based on the number of CAD patients ($P < 0.01$), especially in AMI ($P < 0.01$) . Serum sTLT-1 concentration of AMI and UAP were more higher than the NC group ($P < 0.01$). **Conclusions:** The consistent of sTLT-1 and CD62P expression levels in CAD,indicating that sTLT-1 level may be a new maker of platelet activating and positively related with CAD.

Introduction

With an increasing global burden of coronary artery disease (CAD), early detection and timely management of risk factors are crucial to reduce morbidity and mortality in such patients. Platelet activation plays a crucial role in the pathogenesis of CAD[1], Circulating-activated platelets are thought to trigger ischaemic complications after angiography, angioplasty and vascular surgery.The onset of CAD, especially in acute coronary syndromes(ACS),is closely associated with the enhanced platelet adhesion and aggregation,Therefore early detection and antiplatelet therapy are extremely important for the treatment of coronary heart disease.

P-selectin, also called CD62p, is a kind of glycoprotein stored in Weibel-Palade bodies of vascular endothelial cells and a-granules of platelets. Activated CD62P is a transmembrane protein expressed on the surface of platelets[2] . During platelet activation, the expression of CD62P on the surface of the cell membrane was dramatically increased, accompanied by the simultaneous increase of the expression level of plasma soluble CD62, which played an important role in the initiation, formation and expansion of thrombus. Therefore, it is agreed that the detection of the expression level of CD62P on the platelet membrane is one of the gold standards reflecting platelet activation.

TREM-like transcript-1 (TLT-1) , coexistence of CD62P in platelet alpha particles, is a new platelet activation secretory expression in platelet membrane surface [3,4]at the same time, releasing a soluble TLT - 1 (sTLT - 1) in the plasma, enhance the aggregation of platelet[2,5],therefore we hypothesised that detecting potentially physiologically active substances sTLT - 1 expression level can reflect the expression of platelet membrane TLT-1[6],and indirectly reflect the platelet activation status.Despite of the proof of the biological function of CD62P on platelets and sTLT-1 on serum, there has been little information about its role either in patients with with CAD.Therefore,our study was done to elucidate the

association between CD62P & sTLT-1 and CAD. Based on the potential link between CD62p, sTLT-1 and platelet activation, we systematically analyzed their correlation with CAD.

Materials And Methods

Clinical samples

This study was planned to enroll consecutive patients with CAD whose maximal coronary stenosis < 50% confirmed by coronary angiograms were included in the control group, of whom 29 had acute myocardial infarction (AMI), 26 had unstable angina pectoris (UAP), and 28 had stable angina pectoris (SAP). Patients Compared with 49 patients without CAD, patients with any evidence of tumors or systemic disease were excluded, had no recent history of acute disease, injury or surgery. They agreed to participate in this study and signed the informed consent. A detailed clinical history was collected from all patients, including clinical characteristics. This study was approved by Medical Ethics Committee of Xiangya Hospital of Central South University.

Information Collection

Clinical data on general condition (age, gender, height, and weight), medical history (hypertension, diabetes or stroke), personal history (smoking or drinking), medication history, family history and laboratory tests were obtained from all patients.

Measurement of plasma

Blood venous samples were collected directly into plastic tubes after an overnight fast, collected blood samples of about 5ml, placed in a vacuum blood collection vessel, centrifuged for 3min (1500r/min) to separate the upper platelet-rich plasma and placed in a sterile EP tube. The expression level of CD62P on the platelet surface was detected by flow cytometry (BD FACSCalibur, America). The concentration of sTLT-1 in serum was measured by enzyme-linked immunosorbent assay according to the manufacturer's instructions.

Statistical analysis

The statistical analysis was performed by IBM SPSS version 22.0 software. Quantitative data were presented as the mean $\pm$ SD deviation. Any differences among ≥ 3 groups were evaluated by ANOVA with the Scheffe's test for parametric variables and by the Kruskal-Wallis test with the Steel-Dwass test for nonparametric variables. Any differences between 2 groups were evaluated by the unpaired t-test for parametric variables. Correlations between sTLT-1 & CD62P and the severity of coronary atherosclerosis were evaluated by the Pearman's rank correlation test. P-value levels of <0.05 was considered statistically significance.

Result

2.1 Clinical population clinical characteristics of participants

Of the 132 study patients, the details of demographic, clinical and laboratory data are listed in Table 1. The results showed no significant differences were found with respect to age, sex, BMI, systolic blood pressure, DBP, and platelet count between the CAD and non-CAD groups. LDL and TG in ACS group were higher than those in NC group ($\Delta P < 0.05$). A stepwise decrease in HDL levels was found in AMI and UAP groups than those in control group ($\Delta P < 0.05$). BS of AMI group was higher than that of NC group ($P = 0.03$).

2.2 Levels of Plasma sTLT-1 & platelet CD62p in Patients with CAD and Healthy Controls

We found that the expression level of CD62P in platelet membrane in CAD group was significantly higher than that in NC group, and the difference was statistically significant (figure a-e and table1). The expression level of platelet CD62P in AMI group was significantly higher than that in SAP group ($P = 0.011$), and the difference was statistically significant ($P < 0.01$).

Given that elevated plasma levels of sTLT-1 are implicated in the activation of platelet, we measured plasma sTLT-1 in patients diagnosed with CAD. Soluble TLT-1 levels were significantly increased in patients with AMI/UAP compared to matched, healthy control participants; significant correlation was observed between patients with AMI versus SAP ($P < 0.01$)

2.3 Correlation analysis of serum sTLT, platelet membrane CD62P and other risk factors of coronary heart disease

Pearman regression was used to analyze the correlation between platelet membrane CD62P and serum sTLT-1 and other risk factors of coronary heart disease in ACS patients, including HDL, LDL and TG (Table3 & Table4). The results showed that serum sTLT-1 level was positively correlated with TG ($r = 0.42$), LDL ($r = 0.17$) and CD62P ($r = 0.49$), and negatively correlated with HDL ($r = -0.28$); Among them, there was positive correlation among BS ($r = 0.2$), CHO ($r = 0.47$), LDL ($r = 0.32$), body weight ($r = 0.21$), and sTLT-1 ($r = 0.49$), but negative correlation among HDL ($r = -0.29$)

Discussion

The world health organization (WHO) divides coronary heart disease into five clinical types: asymptomatic, angina pectoris, myocardial infarction, ischemic cardiomyopathy and sudden death. ACS (Acute Coronary Syndrome) refers to as Coronary atherosclerosis plaque rupture and thrombosis or vasospasm and clinical Syndrome, Acute or subacute myocardial ischemia including unstable angina pectoris (UAP) and Acute myocardial infarction (AMI), and its typical performance for myocardial

ischemia caused by chest pain, its development is associated with platelet activation. The adhesion molecule P-selectin (CD62P) plays a dominant role in modulating interactions between platelets and the endothelium, and involved in acute cardiovascular events[7], Studies agree that CD62P is one of the classic indicators of platelet activation. The activation of platelets is one of the targets of antiplatelet therapy.

The results of our study showed that the platelet CD62P level in AMI, UAP and SAP group was significantly higher than that in the normal control group, and the platelet CD62P level in AMI group was significantly higher than that in UAP and SAP group, which was consistent with previous research results. The results of this experiment showed that there was no significant difference between the UAP group and the SAP group, which may be related to the small amount of specimens collected in this experiment.

TLT-1 is a type-1 transmembrane protein [8,9,5] that is highly and exclusively expressed in megakaryocytes and platelets[10], stored in a-granules and possibly in another platelet compartment, and it is rapidly upregulated to the surface of activated platelets, with the most obvious increase in expression being CD62P[11] Upon platelet activation, TLT-1 transports to the membrane where it enhances Ca^{2+} influence and promotes platelet aggregation, TLT-1 is a new player in platelet aggregation.

It has been proved that the expression level of sTLT-1 in patients with chest pain is significantly higher than that in the control group[12,13] and in Acute Respiratory Distress Syndrome The results showed that the expression level was closely related to coronary heart disease and was also one of the indexes of platelet activation. Morales-Ortiz et al show in a murine model of acute lung injury (ALI) that TLT-1 mediates fibrinogen deposition in the lungs and facilitates platelet-neutrophil release during transmigration[14]. In mice, inhibition of TLT-1 has been shown to reduce thrombosis in carotid artery thrombosis models and to protect mice from excessive bleeding during pulmonary embolism[12]. It has been proved that the serum level of sTLT-1 is correlated with the incidence of acute coronary syndrome and has certain predictive value[15] The specific mechanism needs to be further studied.

Combined with our experimental results, the serum sTLT-1 level in AMI group and UAP group was significantly higher than that in NC group and SAP group. The expression level of sTLT-1 in AMI group was significantly higher than that in SAP group. Therefore, it is speculated that in the early stage of rapid platelet activation, TLT-1 may also have an obvious increase in expression as CD62P, which is also one of the indicators of platelet activation. The level of serum sTLT-1 in ACS patients can provide some reference for the diagnosis of coronary heart disease. Antiplatelet therapy is the cornerstone of the treatment of thrombotic diseases especially acute coronary syndrome. To better characterize sTLT-1 involvement in CAD, plasma levels of sTLT-1 should be evaluated in a larger cohort of patients and should place TLT-1 as an important factor to be considered to better correlate with cytokine levels, disease severity, and survival rate. This study undoubtedly demonstrates platelet's function in CAD. TLT-1 is a newly discovered indicator of platelet activation, and further study and understanding of this

indicator may provide new directions and targets for diagnosis of thrombotic related diseases and anti-platelet therapy.

Declarations

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DISCLOSURE

The authors declare no conflict of interest.

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Tables

Table 1. Information of clinical characteristics(Mean±SD)

		SAP [□] n=28 [□]		AMI [□] n=26 [□]
	NC(n=49)		UAP(n=29)	
Age [□] years [□]	55.10±9.19	56.89±8.59	57.28±8.69	58.62± 7.52
Men/female [□] n [□]	(27/22)	(15/13)	(16/13)	(15/11)
BMI [□] KG/m ² [□]	23.24±3.06	24.11±1.28	24.33±1.96	24.19±2.43
TG (mmol/L)	1.12±0.53	1.21±0.59	1.52±0.76 [△]	1.76±1.06 [△]
LDL(mmol/L)	2.24±0.55	2.38±0.65	2.81±0.43 ^{△□}	2.99±0.43 ^{△□}
HDL(mmol/L)	1.43±0.44	1.32±0.32	1.14±0.23 ^{△□}	1.10±0.26 ^{△□}
BS [□] mmol/L [□]	5.04±0.53	5.3±0.53	5.37±0.96	5.72±1.52 [□]
ALT(IU/L)	28.58±7.01	26.08±6.05	40.23±15.38	43.12±19.83
SBP [□] mmHg [□]	125.8±20.02	130.5±19.24	131.4±22.35	129.3±19.03
DBP [□] mmHg [□]	79.08±8.05	80.32±8.59	76.65±11.28	77.38±12.66
Platelet count(10 ⁹ /L)	193.1±44.47	191.2±46.55	185.93±45.24	187.12±42.48

Abbreviations: BMI: body mass index; TG: total triglycerides; LDL-C: low-density lipoprotein cholesterol;HDL-C: high-density lipoprotein cholesterol;Glu: fasting glucose;BS: blood glucose; ALT: alanine aminotransferase;SBP: systolic blood pressure;DBP: diastolic blood pressure;Data are presented as mean values ± standard or proportions (%). P-value represents the difference between the four groups: vs NC[□]△P<0.05;vsSAP:[□]P<0.05; vs NC[□]□P=0.03;

Table 2.Levels of Plasma sTLT-1[□]platelet CD62p in Patients with CAD and Healthy Controls

		SAP [□] n=28 [□]		AMI [□] n=26 [□]
	NC(n=49)		UAP(n=29)	
CD62P Positive Cells(%)	8.44±5.83	12.46±6.63 [△]	23.02±5.83 [△]	27.2±7.3 ^{△□□}
sTLT-1(pg/ml)	187.62±52.61	156.21±64.85	252.63±118.93 [△]	284.35±77.56 ^{△□}

Abbreviations: CD62P : The platelet P-selectin[□] sTLT-1[□]soluble TREM-like transcript-1[□] Data are presented as mean values ± standard or proportions (%). P-value represents the difference between the four groups: vs NC[□]

△P<0.05; vsUAP: [□]P=0.011; vs SAP[□]□P<0.01

Table 3. Correlation analysis of serum sTLT and other risk factors of CAD

	sTLT-1	
	r	P-value
BS	0.18	0.07
TG	0.42	0.00
LDL	0.17	0.05
HDL	-0.28	0.00
Pt	-0.077	0.34
Age	0.15	0.09
Weight	0.26	0.77
BMI	0.33	0.71
Sex	-0.15	0.14
CD62P	0.49	0.00

r: Pearman's correlation coefficients

Table 3. Correlation analysis of platelet membrane CD62P and other risk factors of CAD

	CD62P	
	r	P-value
BS	0.25	0.04
TG	0.38	0.65
LDL	0.32	0.00
HDL	-0.29	0.00
Pt	-0.27	0.61
Age	0.13	0.15
Weight	0.21	0.02
BMI	0.29	0.04
Sex	0.12	0.22
sTLT-1	0.49	0.00

r: Pearman's correlation coefficients

Figures

a. Platelet particles were selected on the FSC histogram

b. Positive rate of platelet CD62P in NC group (%)

c. Positive rate of platelet CD62P in SAP group (%)

d. Positive rate of platelet CD62P in UAP group (%)

e. Positive rate of platelet CD62P in AMI group (%)

Figure 1

A region (R1) is drawn around platelets, which identified according to their characteristic size (a); CD62P positive rate of platelet in NC, SAP, UAP, AMI group (%) (b-e).

Figure 2

Comparison of positive rate of CD62P in platelet membrane in each group(NC group, SAP group, UAP group, AMI group):The CD62P levels were significantly higher in CAD participants: NC vs SAP: $P=0.0068$ vs UAP: $P<0.001$; vs AMI: $P<0.001$;especially in AMI:vs SAP: $P<0.001$; vs UAP: $P<0.001$.

Figure 3

Serum sTLT-1 level was compared in four groups(NC group, SAP group, UAP group, AMI group):The sTLT-1 levels were significantly higher in CAD participants: NC vs SAP:P=0.0235; vs UAP:p=0.0014; vs AMI: P<0.001; especially in AMI: vs:SAP:P<0.001.