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The role of platelets CD40 ligand (CD154) in acute coronary syndromes

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ABSTRACT

Background: Despite of the proof of the biological function of CD154 on platelets, there has been little information about its role either in patients with stable angina or in those with acute coronary syndrome (ACS).
Objective: This study aimed to investigate the expression of CD154 on platelets and its role in ACS.
Methods: The study included 50 patients with ACS (24 patients with acute myocardial infarction (AMI) and 26 patients with unstable angina (UA)), 20 patients with stable angina (SA) and 18 healthy volunteers. CD154 and CD62 expression on platelets were analyzed by flow cytometry. Their relations to the clinical and laboratory data were assessed in the studied group.
Results: Patients with AMI and UA had higher levels of platelets CD154 and CD62 as compared to those with SA and among patients with AMI, UA and SA versus healthy volunteers. Platelets CD154 showed significant positive correlations with the studied pro-inflammatory markers (Ox-LDL, CRP and fibrinogen), segmental wall motion score and the studied risk factors. There were significant negative correlations between platelet CD154 and serum nitric oxide among patients.
Conclusions: CD154 may be used as a marker of thrombo-embolic events. Nitric oxide may have an anti-atherogenic effect. There is an association between platelet activation and severity of coronary artery disease among patients with ACS.

Introduction

Acute coronary syndrome (ACS) is a term used to describe a constellation of symptoms resulting from acute myocardial ischemia. Acute myocardial ischemia is usually but not always caused by atherosclerotic plaque rupture or erosion with superimposed intracoronary thrombosis [1]. Inflammation is known to be a major driving force underlying the initiation of coronary plaques, their unstable progression, and eventual disruption, and it contributes significantly to thrombotic complications that occur in ACS. Therefore, atherosclerosis is defined as a chronic inflammatory response to vascular injury that caused by a variety of agents that activate or injure endothelium and promotes lipoprotein infiltration, retention and modification combined with inflammatory cell entry, retention and activation [2].

In addition, endothelial cells (ECs), smooth muscle cells (SMCs), leukocytes and platelets express CD40 receptor and their ligand (CD40L and referred to CD154). Thus, CD40-CD40L interaction plays an important role in initiation and progression of atherosclerosis [3]. CD40 receptor is constitutively expressed on B-cells, monocytes, macrophages, ECs, and SMCs [4]. CD 40 L (CD154) is a transmembrane protein. It was originally identified on stimulated $CD4^+$ T-cells, and later on stimulated atheroma associated cells including ECs, SMCs and macrophages and on activated platelets [5].

Despite of the proof of the biological function of CD154 on platelets, there has been little information about its role either in patients with stable angina or in those with ACS.

The purposes of this work are to investigate whether patients with ACS show significant differences in the expression of platelet CD154 in comparison to patients with stable angina and healthy volunteers relating the results to those of platelet P-selectin (CD62-P) as a well established marker of platelet activation. Secondly, to study

Abbreviations: ACS, Acute coronary syndrome; ECs, endothelial cells; SMCs, smooth muscle cells; CD40L, CD40 ligand; CAD, coronary artery disease; AMI, acute myocardial infarction; UA, unstable angina; SA, stable angina; BMI, body mass index; WHR, waist hip ratio; SWMSI, segmental wall motion score index; C-RP, C-reactive protein; Ox-LDL, oxidized low density lipoprotein level; NO, serum nitric oxide; GMFI, geometric mean fluorescence intensity.

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the relations of platelet CD154 to the clinical, cardiographic and laboratory data in ACS. Finally, to study the relation between platelet CD154 with different potential risk factors for coronary artery disease (CAD).

Patients and methods

The study was conducted on 50 consecutive patients with ACS. Relying on a serial electrocardiogram (ECG) evaluation and serum cardiac enzymes measurement (creatine phosphokinase (CK), CK-MB iso-enzyme and troponin T), patients were categorized according to criteria of Antaman and Braunwald [6] as follows:

- Patients with ST elevation myocardial infarction (STEMI): They were 18 patients with: 1-Typical anginal pain of great severity, long duration and not relieved by sublingual nitroglycerine. 2-Serial ECGs showed ST segment elevation ⩾0.2 mV in adjacent chest leads and ⩾0.1 mV in adjacent limb leads with or without pathological Q waves. 3-Serum CK levels were twice the upper limit of normal and CK-MB and troponin T were elevated.
- Patients with non ST elevation myocardial infarction (NSTEMI): They were six patients characterized by: 1-Typical aforementioned anginal pain. 2-Serial ECGs showed ST segment depression 0.05 mV with T wave inversion and infrequently pathological Q waves. 3-Serum CK levels were twice the upper limit of normal and CK-MB and/or troponin T were elevated.

The small number of patients with NSTEMI (6 patients) that might yield no statistically significant results if they were considered as a separate group, had encouraged us to add them to patients with STEMI and all (24 patients) had evolved the group of acute myocardial infarction (AMI).

- Patients with unstable angina (UA): They were 26 patients who had the same characteristics of patients with NSTEMI except for the serial level of serum CK, CK-MB and troponin T over 24-72 hours period remained negative.
- Patients with stable angina (SA): They were diagnosed according to criteria of Schwartz and Goldberg [7]. They characterized by: 1-Anginal pain that occurred after a constant level of exertion for >60 days with no change in frequency, duration, severity, precipitating factors, or ease of relief. 2-Resting ECGs; in some patients they were normal, in others they showed T wave inversion or Q waves suggestive of a previous myocardial injury and in the remainders they showed ST segment depression with or without T wave inversion. 3-Diagnosis of SA in this group was confirmed with stress ECG test as described by Mark et al. [8] and/or myocardial perfusion scanning as described by Iskanadria and Verani [9].

Patients with non-cardiac diseases that may interfere with the findings such as infection, advanced liver disease, renal failure, diabetes mellitus, malignancy, collagen diseases, hyperthyroidism, inflammatory bowel disease and acute cerebrovascular stroke were excluded. Diagnosis of liver disease was done by means of physical, laboratory and radiological evaluation. Diagnosis of renal failure was defined when estimated glomerular filtration rate ⩾50 ml/min [10] and diagnosis of diabetes mellitus was defined as fasting blood sugar ⩾6.9 mmol/L, 2 hours postprandial blood sugar ⩾11 mmol/L, random blood sugar ⩾11 mmol/L or current intake of oral hypoglycemic medications or insulin [11].

Patients with cardiac diseases other than coronary artery disease, except for minor mitral regurgitation and patients with overt right or left ventricular failure, those who had subjected to coronary artery bypass graft or percutaneous transluminal coronary angioplasty and those who had treated by thrombolytic and antiplatelet agents within three months before the study were excluded as well.

All the study subjects were subjected to the following after informed written consent in accordance with Assiut University ethical committee guidelines.

- Thorough clinical history and examination that included estimation of systemic arterial blood pressure, body mass index (BMI) and waist hip ratio (WHR).
- Evaluation of standard 12 – leads electrocardiogram (ECP-2155 Fukuta Denshi, Japan)
- Evaluation of M- mode 2D- Doppler echocardiography (Agilent HP-Sonos 4500-USA):

Two-dimensional echocardiography was used to detect resting segmental wall motion (SWM) abnormalities. SWM score (SWMS) was calculated by summing the scores for each segment. SWMS index (SWMSI) was calculated by summing the scores for each segment and dividing them by the number of analyzed segments [12].

- Coronary artery angiography was performed in 30 patients (6 AMI, 8 UA and 16 SA) for diagnostic and therapeutic purposes using Philips Integris 3000 system. It could not be done to all patients because of lack of financial support, impaired left ventricular systolic function or patient's refusal.
- Laboratory investigations that included assessment of cardiac enzymes, serum blood sugar, kidney functions, complete lipogram, liver function tests, prothrombin time and concentration, complete blood count, erythrocyte sedimentation rate, serum antinuclear antibody, thyroid profile, serum C-reactive protein (C-RP), plasma oxidized low density lipoprotein level (Ox-LDL), Serum nitric oxide (NO), plasma fibrinogen level (Table 1). In patients with AMI and UA, peripheral venous blood sample was withdrawn immediately after hospital admission and before administration of thrombolytic or anticoagulant therapy.

Isolation of LDL

To prevent oxidation and proteolytic degradation of LDL; plasma was separated by low-speed centrifugation of blood sample with a single vertical spin in a vertical ultracentrifuge rotor using the modified method of Chung et al [13]. The isolated LDL was dialyzed for 48 hours against 0.15 mmol/NaCl containing EDTA. The protein content of the LDL was measured by the modified technique of Lowry et al [14]. LDL was sterilized by passage through a 22 um milli-pore filter, and used within two weeks of preparation.

Estimation of Ox-LDL

The following solutions were added in succession; 0.2 ml of isolated LDL solution, 0.2 ml of sodium dodecyl sulphate, 1.5 ml of 20% acetic acid adjusted to PH 3.5 with NaOH, and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid. The volume was then completed to 4 ml with distilled water. The mixture was heated at 95 °C for 60 minutes in a water bath and then cooled with tap water. One ml of distilled water and 5 ml of a mixture n-butanopyridine (1: 5, volume for volume) were added to the mixture.

The mixture was shaken vigorously and centrifuged at 4000 rpm for 10 minutes. The organic layer (n-butanol phase) was taken and its absorbance at 532 nm was measured. 1,1,3,3 tetramethoxypropane was used as an external standard to prepare standard concentrations of malonaldehyde and the procedure was repeated to prepare a standard curve. From this curve the peroxide concentration was deduced from the corresponding absorbance using the regression equation for the standard curve and Ox-LDL level was calculated [15].

Table 1
Demographic, clinical and laboratory data among patients and healthy volunteers.

Variables	AMI (n=24)	UA (n=26)	SA (n=20)	Healthy volunteers (n=18)	*P*-value				
					A	B	C	D	E
Age (years)	56.9±9.7	54.2±7.6	54.9±8.3	55.2±9.3	0.9	1.0	0.88	0.9	1.0
Male gender (%)	83.3%	61.5%	80%	72.2%	0.09	0.76	0.17	0.39	0.46
Smokers (%)	54.2%	11.5%	35%	18.9%	0.001*	0.2	0.06	0.03*	0.33
FH of CAD (%)	8.3%	7.7%	5%	0%	0.93	0.66	0.71	0.21	0.23
BMI (Kg/M2)	25.8±4.31	28.8±5.51	26.4±2.6	27.1±2.3	0.09	0.99	0.22	0.7	0.68
Waist hip ratio	0.94±0.05	0.93±0.06	0.92±0.05	0.88±0.05	0.82	0.56	0.95	0.009*	0.07
SBP (mmHg)	131.3±24.4	139.03±29.6	118.5±18.7	111.7±12.9	0.69	0.22	0.02*	0.06	0.003*
DBP (mmHg)	84.2±14.7	86.2±14.01	77.5±11.2	70±9.2	0.96	0.21	0.07	0.004*	0.001*
FBS (mmol/l)	5.5±0.78	5.8±1.52	4.5±0.85	4.5±0.6	0.97	0.01*	0.002*	0.001*	0.000*
2h PPBS (mmol/l)	7.27±1.6	7.9±2.3	6.4±1.7	5.9±0.66	0.83	0.35	0.06	0.046*	0.004*
Cholesterol (mg/dl)	189±39	193±46	190±42	157±20	1.0	0.94	0.94	0.056	0.049*
Triglyceride (mg/dl)	163±63	190±102	181±85	115±33	0.83	0.86	1.00	0.285	0.048*
LDL-C (mg/dl)	115±31	120±40	118±36	89±21	0.98	0.98	1.00	0.041*	0.015*
HDL-C (mg/dl)	45.2±11.2	44.6±11.7	43.8±11.8	50.3±6.6	1.00	0.95	0.95	0.38	0.36
Ox-LDL (nmol/mg protein)	0.56±0.2	0.53±0.2	0.53±0.2	0.38±0.1	0.92	0.97	0.99	0.015*	0.06
C-RP (mg/dl)	51.5±28.6	27.7±13.5	3.3±3.1	2±2.9	0.000*	0.000*	0.000*	0.000*	0.000*
Fibrinogen (mg/dl)	606±137	598±126	531±107	369±35	0.99	0.32	0.42	0.000*	0.000*
Nitric oxide (mmol/l)	14±3.2	13.9±4.03	14.1±3.02	15.9±3.3	0.99	1.00	0.99	0.44	0.3

Quantitative variables are expressed as mean±standard deviation and compared by using Post Hoc.
AMI: acute myocardial infarction; UA: unstable angina; SA: stable angina; FH of CAD = family history of premature coronary artery disease; BMI= body mass index; SBP= systolic blood pressure; DBP = diastolic blood pressure; FBS= fasting blood sugar; 2 h PPBS =2 hours postprandial blood sugar; LDL-C=low-density lipoprotein–cholesterol; HDL-C= high-density lipoprotein–cholesterol; Ox-LDL =oxidized low-density lipoprotein. C-RP =C-reactive protein; * = statistically significant result.
A= p-values when AMI group compared with US group.
B= p-values when AMI group compared with SA group.
C= p-values when US group compared with SA group.
D= p-values when AMI group compared with healthy volunteers group.
E= p-values when US group compared with healthy volunteers group.

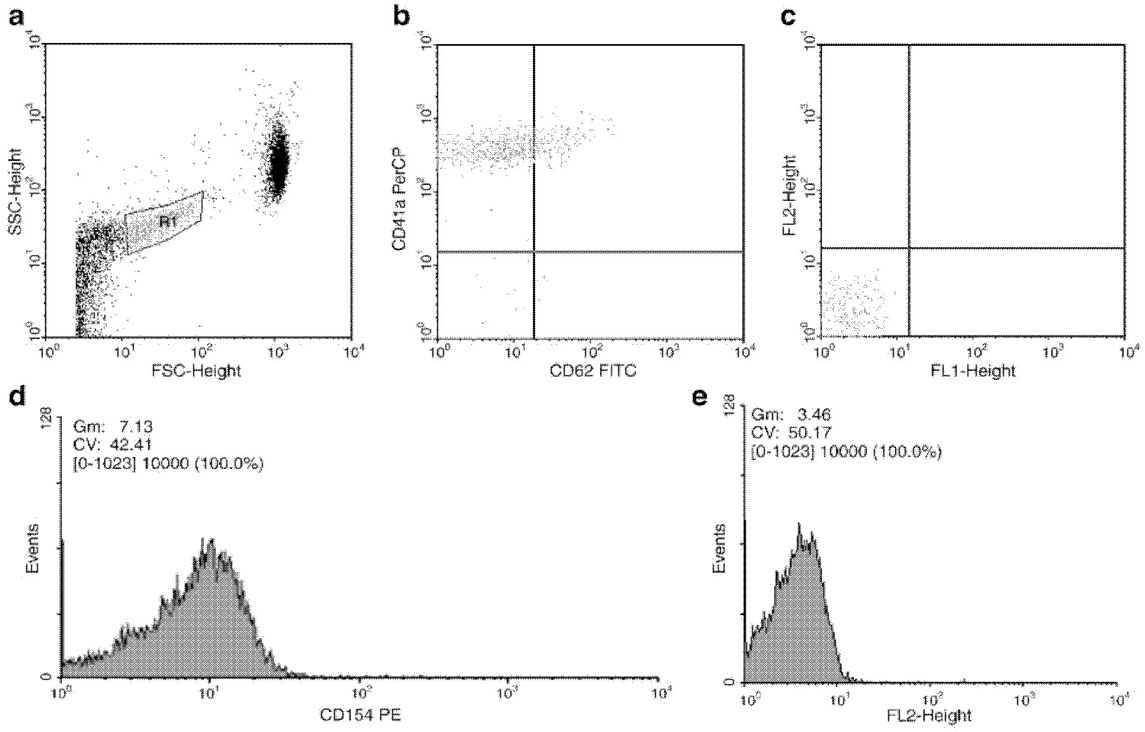

Fig. 1. A region (R1) is drawn around platelets, which identified according to their characteristic size (a). Platelet gate was adjusted such that >95% of the particles analyzed were anti CD41a positive (b). Cells in R1 are positive for CD62-P (b). Histograms for CD154 (d) show higher geometric mean (GM) comparing with their isotypic controls (e).

Serum NO Measurement

This assay determines total NO based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. The reaction is followed by a colorimetric detection of nitrite as an azo dye product of the Griess reaction [16].

Determination of Plasma Fibrinogen

Plasma was mixed with sodium sulphite solution and left to stand for exactly 10 minutes. The tube was shaken and read at 680 nm against sodium sulphite as a blank [17].

Determination of Platelet CD154 and CD62-P Selectin by Flow Cytometry (Fig. 1)

To minimize and standardize platelet activation during venesection, an atraumatic procedure was used. Whole blood anti-coagulated with EDTA was diluted 1:8 in HEPES buffer. Twenty-five μl of diluted blood were directly labeled with 5 μl of each CD62P-FITC, CD154-PE, CD41a Per-CP antibodies. CD41a (glycoprotein IIb/IIIa complex) is specific to platelets, thus, the stained platelets can be recognized and gated. Corresponding isotope controls were added to the appropriate tube. All antibodies are purchased from Pharmingen, BD, USA. Analysis was done by flow cytometry using FACSCaliber (BD, USA) by identifying platelets according to their characteristic size (log forward scatter) and granularity (log side scatter). Platelet gate was adjusted such that >95% of the particles analyzed were anti CD41a positive. The degree of platelet activation was expressed as a percentage and to determine the total amount of platelet surface antigen, geometric mean fluorescence intensity (GMFI) was recorded.

Statistical analysis

The clinical and laboratory data were collected, categorized and processed by using personal compatible computer by Statistical Package for Social Sciences (SPSS), version 15 software package. The quantitative variables were expressed as mean ± standard deviation (SD) and comparison between groups was done using One-Way ANOVA and Post Hoc tests. While, the qualitative variables were expressed as percentages and comparison between groups was done using chi square test and test of proportion. Pearson's correlation co-efficient "*r*" used to assess the correlation between each continuous variable in each group. In addition, regression analyses were done. *P*-value levels of <0.05 was considered statistically significance.

Results

The details of demographic, clinical and laboratory data of all patients and controls are listed in Table 1.

The current study reported that patients with AMI and UA had higher levels of platelets CD154 and CD62 P-selectin as compared to those with SA and among patients with AMI and UA versus healthy volunteers (Table 2). Patients with SA had significantly higher levels of platelet CD154 compared to healthy control ($p = 0.006$).

There were significant positive correlations between indicators of platelet CD154 (percentage of positive platelets, GMFI) and their congeners of CD62 P-selectin among patients with coronary heart disease ($r = 0.74$, $p = 0.0001$). These significant positive correlations were also confirmed by logistic regression analyses (β- coefficient = 0.52, $p = 0.0001$ for percentage of platelet CD145 and CD62-P and β- coefficient = 0.69, $p = 0.0001$ for CD145 and CD62-P GMFI).

Percentage of platelets expressed CD154 showed significant positive correlations with all studied pro-inflammatory markers (Ox-LDL, C-RP and plasma fibrinogen) among the studied patients (Table 3). In addition, C-RP had significant positive correlations with CD154 GMFI in these patients.

There were significant negative correlations between all indicators of platelet CD154 and serum NO among patients with coronary heart disease ($r = -0.44$, $p = 0.0001$ for platelets percentage; $r = -0.33$, $p = 0.006$ for GMFI). Similar relations were also detected by logistic regression analysis (β- coefficient = -0.28, $p = 0.001$ for platelets percentage and serum NO).

There were significant positive correlations ($r = 0.3$, $p = 0.009$) between platelet CD154 GMFI with SWMS among patients with coronary heart disease (Table 4).

There were significant positive correlations between percentage of platelet CD154 and age, WHR, systolic blood pressure, fasting and 2 hours post-prandial blood sugar, and total serum cholesterol among patients with coronary heart disease (Table 5).

Discussion

The present study revealed up-regulation of indicators of platelet CD154 and P-selectin in patients with ACS versus healthy volunteers and those with SA. These results were in accordance with many others [18–20]. As CD40-CD154 interaction plays an important role in initiation and progression of atherosclerosis [21], it was not surprising to find that patients with SA had significantly higher levels of platelet CD154 compared to healthy control. This subject was a point of debate, as some studies were in accordance with ours [18,22,23], while others found no significant differences of either platelet or monocyte CD154 in both groups [24,25]. These conflicting results may be due to a wide range of platelet reactivity among patients with chronic stable angina that assessed by different markers [26].

Garlich et al. [27] could not find any correlation between platelet CD154 and P-selectin among hypercholestrolemic patients and attributed their results to different storage site and different expression pattern of both markers. On the other hand, the present study found a significant positive correlation between indicators of platelet CD154

Table 2
Platelet CD154 and CD62-P among patients and healthy volunteers.

Variables	AMI (n = 24)	US (n = 26)	SA (n = 20)	Healthy volunteers (n = 18)	*P*-value				
					A	B	C	D	E
CD154 positive platelets (%)	46.25 ± 11.5	46.2 ± 13.3	34.9 ± 8.01	26.9 ± 5.56	0.99	0.004*	0.009*	0.000*	0.000*
CD154 GMFI	5.8 ± 2.4	5.2 ± 2.2	3.7 ± 0.65	3.6 ± 0.54	0.64	0.002*	0.05*	0.002*	0.047*
CD62-P positive platelets (%)	69.3 ± 13.2	69 ± 11.6	54.5 ± 6.7	40.85 ± 3.6	0.92	0.00*	0.007*	0.000*	0.000*
CD62-P GMFI	18.2 ± 10.3	12.8 ± 8.3	7.49 ± 1.21	4.32 ± 0.83	0.55	0.002*	0.082	0.000*	0.005*

Quantitative variables are expressed as mean ± standard deviation and compared by using Post Hoc.
AMI=acute myocardial infarction; US= unstable angina; SA= stable angina; GMFI= geometric mean fluorescence intensity; * = statistically significant result.
A= p-values when AMI group compared with US group.
B= p-values when AMI group compared with SA group.
C= p-values when US group compared with SA group.
D= p-values when AMI group compared with healthy volunteers group.
E= p-values when US group compared with healthy volunteers group.

Table 3
Correlations between platelet CD154 and some proinflammatory markers among patients with coronary heart disease.

Variables	CD154 positive platelets (%)		CD154 GMFI	
	r	P	r	P
Ox-LDL (nmol/mg protein)	0.28	0.01*	0.09	0.44
C-reactive protein (mg/ml)	0.26	0.02*	0.42	0.0001*
Fibrinogen (mg/dl)	0.29	0.01*	0.15	0.21
Nitric oxide (mmol/l)	-0.44	0.0001*	-0.33	0.006*
CD62-P positive platelets (%)	0.74	0.0001*		
CD62-P GMFI			0.74	0.0001*

GMFI = geometric mean fluorescence intensity; Ox-LDL = oxidized low-density lipoprotein, * = statistically significant result.

(percentage and GMFI) and their congeners of P-selectin among patients with CAD. Our results were in agreement with other previous reports [19,28] and confirmed by logistic regression analyses.

Ox-LDL is a more potent pro-atherosclerotic mediator than the native unmodified LDL. Both Ox-LDL and CD40–CD154 interaction have been identified to co-localize in atherosclerotic plaques [29]. This may account for the presence of a significant positive correlation between percentage of platelet CD154 and Ox- LDL levels in the present study. To the best of our knowledge, no available data about the relationship between those two markers in CAD patients, except, an in vitro study by Li et al [29].

The present study revealed a significant positive correlation between plasma fibrinogen levels and CRP with values of platelet CD154. This relation may be explained by the possibility that CD40/CD154 interaction may induce endothelial expression of adhesion molecules and release of interleukin-6 (IL6). IL6 stimulates hepatic gene that encode acute phase reactants including fibrinogen and C-RP. This concept was accepted by Tousoulis et al. [30] and refused by Ohashi et al. [31]. However, these relations could not be confirmed by logistic regression analysis.

Nevertheless, the present study reported a significant negative correlation between NO levels and all indicators of platelet CD154 and confirmed by logistic regression analysis. Unfortunately, there was no available data studied this relation among similar patients. However, Schäfer et al. [32] reported that intravenous injection of a NO synthesis inhibitor (nitroglycerin-monomethyl-L-arginine) in healthy volunteers resulted in enhanced markers of platelet activation (P-selectin and CD145) and sublingual administration of a NO donor (glyceryl trinitrate) restored these markers to baseline levels. In addition, Davis and Zuo [33] found that exposure of cultured human aortic endothelial cell to clinical relevant concentration of sCD154 led to dose dependent decrease of NO bioavailability. Therefore, we suggest that NO might have an anti-atherogenic effect.

Segmental wall motion abnormality that is detected by SWMS and SWMSI identifies myocardium at risk either stunned myocardium (transient dysfunction due to acute ischamia) or hibernated myocardium (poor functional myocardium secondary to chronic hypoperfusion). In addition, echocardiography is of tremendous value in risk

Table 4
Correlations between platelet CD154 and left ventricular ejection fraction, segmental wall motion score and segmental wall motion score index among patients with coronary heart disease.

Variables	CD154 positive platelets %		CD154 GMFI	
	r	P	r	P
Ejection Fraction (%)	-0.11	0.37	-0.13	0.27
Segmental wall motion score	0.07	0.52	0.3	0.009*
Segmental wall motion score index inindex	0.08	0.49	0.22	0.06

GMFI = geometric mean fluorescence intensity; * = statistically significant result.

Table 5
Correlations between platelet CD154 and some conventional cardiovascular risk factors among patients with coronary heart disease.

Variables	CD154 positive platelets (%)		CD154 GMFI	
	r	P	r	P
Age (years)	0.27	0.02*	0.27	0.02*
Body mass index (kg/m2)	0.17	0.14	0.11	0.36
Waist hip ratio	0.33	0.006*	0.12	0.3
Systolic blood pressure(mmHg)	0.25	0.03*	0.19	0.1
Diastolic blood pressure (mmHg)	0.1	0.13	0.1	0.3
Fasting blood sugar(mmol/l)	0.3	0.01*	0.18	0.13
2h PP blood sugar (mmol/l)	0.3	0.01*	0.14	0.25
Cholesterol(mg/dl)	0.24	0.04*	0.12	0.31
Triglyceride(mg/dl)	0.15	0.15	0.08	0.5
Low density lipoprotein (mg/dl)	0.18	0.22	0.06	0.59
High density lipoprotein (mg/dl)	-0.31	0.8	0.06	0.58

GMFI = geometric mean fluorescence intensity; 2 h PP blood sugar: 2 hours postprandial blood sugar; * = statistically significant results.

stratification [34]. The present study found a significant positive correlation between platelet CD154 GMFI with SWMS. These findings indicated that there was an association between platelet activation and the severity of coronary artery disease among patients with CAD.

Concerning the cardiovascular risk factors, obesity in particular visceral obesity is associated with elevated markers of platelet activation [35]. In consistence with the facts concerning the impact of visceral obesity rather than diffuse obesity in inducing platelet activation, the present study revealed that platelet CD154 correlated well with WHR but not with BMI.

Enhanced platelet activation among hypertensive patients is linked to the presence of hypertension-related microvascular changes [36]. The presence of a significant positive correlation between percentage of platelet CD154 and systolic blood pressure but not diastolic blood pressure in our study may be explained by the fact that the former is strongly associated with increased incidence of CAD than the latter [37]. Yan et al. [38] reported slight statistically significant associations between sCD154, but not membrane bound, and blood pressure among hypertensive patients and among multiethnic population-based study. They also found that good control of blood pressure led to marked decrease of soluble form but no significant effect on platelet bound form. On the contrary, Sonmez et al. [39] reported that sCD154 failed to correlate significantly with blood pressure among non-obese young hypertensive men.

There is a significant positive correlation between platelets CD154 and others risk factors as fasting and 2 hours post-prandial blood sugar and total cholesterol levels among patients with CAD in the present study. These results are in agreement with Izzi, et al. [40] who reported a relation between platelet activity and blood glucose level among general population and with Pignatelli et al. [41] who reported an evidence of oxidative stress-mediated platelet CD154 up-regulation in hypercholesterolemic patients.

Lastly, in the present study, the percentage of platelet CD154 had significant positive correlation with some studied cardiovascular risk factors while CD154 GMFI correlated to SWMS. Hence, it could be claimed that the percentage of platelet CD154 is well correlated with the risk of CAD and can predict it, while CD154 GMFI is superior in assessing its severity.

However, the weak "*r*" value in the most of this relations and the inability to detect any reliable correlation between the platelet CD154 and the rest of risk factors for CAD in our study could be attributed to the small sample size. Another limitation of this study is the small number of patients with NSTEMI which had obliged us to add them to patients with STEMI although they are with different clinical conditions. The uni-variant analysis and the lack of follow up to trace platelet bound CD154 level consider as other limitations in this study.

In conclusion: Up-regulation of CD154 may be used as a marker of thrombo-embolic events. In addition, CD154 may play a role in initiation and progression of atherosclerosis and consequently in pathphysiology of CAD as patients with SA had up-regulation of platelet CD154 compared with healthy subjects. CD154 represents a bridge links inflammation and thrombosis based on presence of close relations between it and both CD62-P selectin and many pro-inflammatory markers. There is an association of platelet activation and severity of CAD based on presence of close relations between CD154 GMFI with SWMS.

Conflict of interest statement

We declare no conflict of interest.

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