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Enhanced P-selectin expression on platelet-a marker of platelet activation, in young patients with angiographically proven coronary artery disease

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Abstract P-selectin (CD62p) exposure is an established marker for platelet activation. P-selectin exposure can trigger variety of thrombotic and inflammatory reactions. In patients with coronary artery disease (CAD), platelets are activated, and hence, there is increased P-selectin exposure. The role of P-selectin exposure in patients on treatment with statins and anti-platelets is conflicting. A case–control study was performed to determine P-selectin exposure in consecutively recruited 142 patients (age $\leq$ 55 years) with angiographically proven CAD on treatment and 92 asymptomatic controls. P-selectin exposure was determined by flow cytometry. Data on conventional risk factors were obtained along with estimation of levels of thrombotic [fibrinogen, lipoprotein (a), tissue plasminogen activator, plasminogen activator inhibitor-1, homocysteine and von Willebrand factor] and anti-thrombotic factors (antithrombin III). The P-selectin exposure was compared among patient groups who had different modes of presentation of CAD and categories of CAD disease severity. The patients were followed up for a period of 26 months. The results indicate that P-selectin exposure was significantly elevated in patients (mean $\pm$ SD 9.24 $\pm$ 11.81) compared to controls (mean $\pm$ SD 1.48 $\pm$ 2.85) with $p < 0.0001$. Similarly, conventional risk factors were significantly elevated in patients. P-selectin exposure showed significant negative correlation with antithrombin III levels. P-selectin exposure was higher in patients who presented with acute coronary syndromes than those who presented with effort angina. Cardiovascular event rate was 6 % on follow-up. The study establishes that thrombotic–inflammatory pathways enhancing P-selectin exposure unrelated to treatment might be activated in patients, while the event rate remained lowered, and hence, treatment strategies should be inclusive to control these factors.

Keywords Coronary artery disease · Platelet activation · P-selectin · CD62p · Antithrombin · Flow cytometry · India

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Introduction

Coronary artery disease (CAD) being a worldwide health epidemic involves the bidirectional processes of thrombosis and inflammation. During thrombosis and inflammation, platelets are activated [1]. Platelet activation is followed by platelet adhesion which is characterized by expression of variety of glycoproteins on platelet surface. During the process, there is exposure of CD62p (P-selectin) on the surface of platelets, which are released from alpha granules to the surface by open canalicular system of platelets. Exposed P-selectin is a receptor for activated monocytes which actively bind with it by P-selectin glycoprotein ligand-1 (PSGL-1). P-selectin also triggers monocyte

rolling on endothelium and actively involved in endothelial activation and smooth muscle proliferation. These processes enhance the release of more thrombotic–inflammatory molecules (tissue factor, other coagulation factors and matrix metalloproteinase to name a few) along with chemokines [2]. Thus, P-selectin is an important marker for thrombosis and inflammation [3].

There is established evidence of heightened platelet activation in conditions such as tobacco smoking [4], diabetes mellitus [5], family history to premature CAD [6], low HDL-C [7] and hypertension [8]—all being important conventional CAD risk factors.

Moreover, among all ethnic groups, Indians have one of the highest rates of CAD especially in the younger population [9]. Many of the circulating thrombotic risk factors are elevated in young Indians with angiographically proven CAD even though they are on treatment with anti-atherosclerotic drugs [10]. There are only limited studies on the role of platelet activation and even fewer studies on the role of P-selectin exposure on platelets in Indians with cardiovascular disease [11].

There is an evidence of recurrence of acute coronary events in patients on treatment with anti-atherosclerotic drugs (statins and anti-platelet drugs) [12] even though their lipid profile are under the recommended target levels. The presence of the recently identified markers such as P-selectin expression may be responsible for the continued risk of events in those who are on guideline based treatment. Thus, identification of P-selectin exposure, occurring as a result of thrombotic–inflammatory response, could be an important marker along with other conventional and non-conventional risk factors, which can improve risk assessment of young CAD patients and may open new avenues to improve the efficacy of current treatment methods.

In this background, we performed this case–control study to assess P-selectin exposure, a marker for platelet activation in young patients with angiographically proven CAD, who are on anti-platelets drugs and statins for at least 3 months. The data on conventional risk factors and thrombotic risk factors were simultaneously obtained and the follow-up events were collected.

Materials and methods

Subject selection

The study subjects included 142 patients with angiographically proven CAD, who were admitted to the Department of Cardiology at the Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), a tertiary care hospital in the southern state of Kerala, India, during the period: October 2011–December 2013. The study was a collaborative work done by the Departments of Cardiology, Biochemistry and Thrombosis Research Unit. Patients were selected from an existing database of 5500 young CAD patients. Patients who were admitted at the hospital for elective procedures after at least 3 months of the presentation of symptoms (after a mean period of 3 months of treatment) were enrolled. Patients were on mandatory treatment with cholesterol-lowering drugs, statins (atorvastatin) and standard anti-platelet agents (aspirin and clopidogrel). Beta blockers, angiotensin-converting enzyme inhibitors, calcium channel blockers and nitrates were prescribed wherever indicated. The patients were provided standard dose of aspirin and clopidogrel as indicated by ACC/AHA guidelines [13]. The dose of aspirin was 150 mg daily and is recommended for the whole life, while 75 mg of clopidogrel is advised for a period of 1 year after ACS or following coronary stent implantation. All the patients were on statins and dual anti-platelet agents.

Healthy volunteers who were free living subjects without any evidence of CAD were recruited as controls. Ninety-two subjects were selected as controls. The laboratory investigations were carried out at Department of Biochemistry and Thrombosis Research Unit of SCTIMST. The study was approved by the Institutional Ethics Committee of the Institute (IEC/014; November 28, 2009), and written informed consent was obtained from all the participants before blood sample collection.

Data on conventional risk factors

Conventional risk factor data were collected from all patients and controls. Data on patients were collected from discharge summary and hospital records and entered into a structured questionnaire, whereas data regarding controls were obtained using a structured questionnaire. Those with fasting blood glucose level above 127 mg/dl and taking anti-diabetic medication were considered as those with diabetes mellitus [14]. Dyslipidemia [15] and hypertension was defined on the basis of present accepted clinical guidelines [16]. Data regarding tobacco smoking (current and ex-smoking) were collected. Family history of premature CAD was considered when there is an occurrence of CVD in parents or sibling before 60 years of age [17].

Sample collection

Blood samples were collected from patients and controls after an overnight fasting for 12 h. A total of 10 ml blood was collected from each subject. Fresh EDTA blood (2 ml of blood transferred to K2 EDTA 3.6 mg vacutainer tube) was used for the assay of platelet activation. Rest of the

blood samples were transferred to appropriate vacutainer tubes for assay of biochemical parameters and other related thrombotic factors.

Laboratory investigations

Platelet activation assay

Platelet activation was performed by flow cytometry based on the protocol described by Michelson [18] with some modifications. Fluorescent antibody used against P-selectin was anti-CD62p/P-selectin conjugated with phycoerythrin/PE [Beckman Coulter (Ref: IM1759U)]. Fresh EDTA blood was appropriately diluted using phosphate buffered saline (PBS) to adjust the platelet count to 2–2.5 $\times$ 10^5 cells/100 µl. To this 5 µl of CD62p, PE antibody was added and incubated in dark for 1 h. The sample was then diluted in 750 µl of PBS and analyzed immediately. The sample was read in Beckman Coulter flow cytometer (COULTER Epics XL) using 488 nm laser. When platelet is activated, P-selectin is exposed on to the platelet surface to which PE-conjugated antibody binds. P-selectin exposure was expressed as percentage (%) fluorescence. P-selectin was monitored directly from blood without any external stimuli. Unstained cells were used to gate the negative population of cells to eliminate auto-fluorescence of the cells.

Biochemical assays

Total cholesterol level was determined by cholesterol esterase, cholesterol oxidase and horse radish peroxidase method (CHOL method, Flex Reagent Cartridge, SIEMENS, Dimension Clinical Chemistry System), and triglycerides were assayed using lipoprotein lipase, glycerol kinase and glycerol-3-oxidase method (TGL method, Flex Reagent Cartridge, SIEMENS Dimension Clinical Chemistry System). HDL-C was analyzed using enzymatic end point method (direct method using ASPEN HDL-C KIT), and LDL-C was derived using Friedewald equation [19]. Fasting blood glucose was estimated by hexokinase and glucose-6-phosphate dehydrogenase method (GLU method, Flex Reagent Cartridge, SIEMENS, Dimension Clinical Chemistry System).

Thrombotic risk factor assay

Thrombotic risk factors analyzed were fibrinogen (Clauss method), lipoprotein(a)/Lp(a) (Turbidimetry method), von Willebrand factor (v-WF), tissue plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1) (by ELISA), homocysteine (enzymatic assay) and anti-thrombotic antithrombin III (chromogenic assay) as described in the earlier study [10].

Follow-up and event rate

Patients were followed up regularly during their follow-up visits to the hospital or over telephone. Mean follow-up duration was 26 $\pm$ 5.4 months. The combined end point of mortality, re-admissions with acute coronary syndromes, need for repeat revascularization and stroke was estimated to determine the event rate during follow-up.

Statistical analysis

Statistical analysis was carried out using Microsoft Office Excel, GraphPad Prism demo 5 and STATA/IC 11.2 version software. Mean along with standard deviation (SD) and/or median along with the range (lowest value to highest value) were used for the expression of continuous variables. Unpaired Student's *t* test was used to compare the difference between mean values. Chi-square analysis/Fisher's exact test (Binary logistic regression analysis) and multiple logistic regression analysis were used for group comparisons. $p < 0.05$ was defined significant. Pearson's correlation analysis was used for the correlation of different parameters.

Results

Basic characteristics

Males constituted majority of the patients (84 %). Patients had significantly low total cholesterol, LDL-C and triglycerides compared to controls (Table 1). Conventional risk factors such as diabetes mellitus, tobacco smoking, hypertension and low HDL-C were significantly high among patients compared to controls (Table 1). Patients were on lipid-lowering therapy, and hence, total cholesterol, LDL-C and triglycerides were significantly lower compared to controls. But in the case of HDL-C, the levels were significantly high in controls compared to patients.

Flow cytometric analysis of P-selectin (CD62p) expression in blood

P-selectin expression is expressed as percentage of fluorescence, measured by flow cytometry as fluorescently conjugated CD62p-PE antibody bind to the P-selectin (CD62p) exposed on activated platelets [Fig. 1 (lower rate of expression) and Fig. 2 (higher rate of expression)].

Platelet activation as a risk factor for CAD

Platelet activation analyzed by flow cytometry based on the exposure of P-selectin revealed that significantly more

Table 1 Basic features, conventional risk factors and biochemical analysis–lipid profile and fasting blood sugar

(a) Basic demographical features			
	Patients	Controls	'p' value
Number (*N*)	142	92	
Males (%)[a]	119 (84)	67 (73)	0.0478
Age: mean ± SD	45.46 ± 6.68	43.67 ± 9.29	0.089
Conventional risk[a] factors, *N* (%)			
Positive family history to CAD	41 (29 %)	16 (17 %)	0.06
Smoking	78 (55 %)	15 (15 %)	<0.0001
Diabetes mellitus	78 (55 %)	16 (17 %)	<0.0001
Hypertension	71 (50 %)	4 (4 %)	<0.0001
Low HDL	107 (75 %)	44 (48 %)	<0.0001
(b) Biochemical analysis (Student 't'Test) [b]			
Parameters (mg/dl):Mean ±SD	Patients	Controls	'p' value
Fasting blood sugar	159.2 ± 88.85	99.55 ± 33.74	<0.001
Total cholesterol	143.9 ± 40.58	219.3 ± 45.07	<0.001
Triglycerides	129.3 ± 58.01	145.1 ± 64.59	0.0580
HDL-C	33.99 ± 7.84	42.77 ± 9.16	<0.001

[a] Percentage of males and conventional risk factors: '*p*' value—Chi-square (χ^2) analysis

[b] Lipid profiles of patients were normal compared to controls as they were on treatment with statins, while controls were free living subjects, without any treatment. '*p*' value assessed using Student's '*t*' test

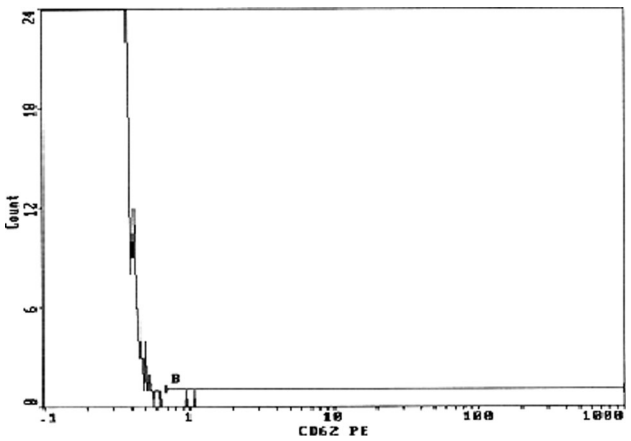

Fig. 1 Representative flow cytometry histogram for CD62 PE expression. Low P-selectin expression (0.01 % platelet activation)

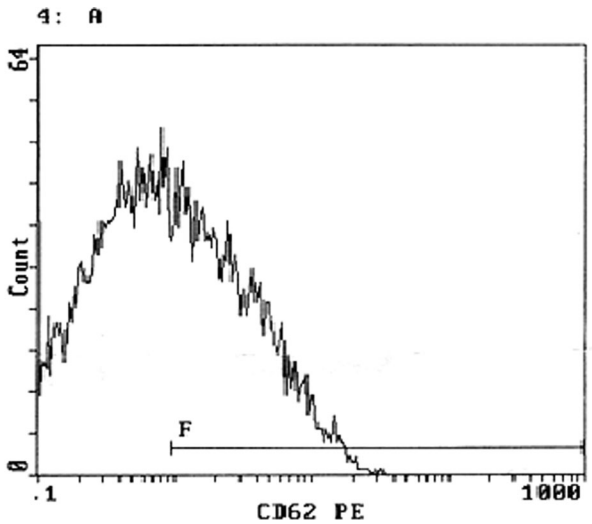

Fig. 2 Representative flow cytometry histogram for CD62 PE expression. High P-selectin expression (35.7 % platelet activation). (Figs. 1, 2 X-axis represents PE fluorescence and Y-axis represents platelet count. In Fig 2 there are more platelets showing PE fluorescence and hence higher P-selectin expression compared to Fig. 1)

number of patients had increased rate of expression of P-selectin compared to that of controls. Mean P-selectin expression was also high in patients compared to controls. 84 % of the patients and 40 % of the controls had high platelet activation (Table 2).

There was a wide range of variation in patients and controls on the exposure of P-selectin on platelets. Patients also showed a high median value compared to controls (Fig. 3).

Platelet activation and thrombotic risk factors

P-selectin and relationship with other thrombotic risk factors were evaluated by correlation analysis. P-selectin exposure showed significant negative correlation with anti-

Table 2 Platelet activation (P-selectin exposure) profile of patients and controls: (a) P-selectin expression in patients and controls, (b) elevated P-selectin exposure in subjects with elevated conventional risk factors[c]

(a) P-selectin expression in patients and controls i) Chi-Square analysis[a]			Odds ratio	95 % CI
	Patients N (%)	Controls N (%)		
	119 (84 %)	37 (40 %)	8.04	4.3–14.9
ii) Student's *t* test[b]	Patients	Controls		*p* value
Mean ± SD	9.24 ± 11.81	1.48 ± 2.85		<0.001
Median (range)	3.44 (0.06–54.9)	0.23 (0.01–15)		

(b) Elevated P-selectin exposure in subjects with conventional risk factors [c]			
	Patients	Controls	Student's t test 'p' value
i) Positive family history and elevated P-selectin			
N	31	15	
Mean ± SD	10.85 ± 10.82	3.12 ± 1.45	0.0089
Median (range)	6.73 (0.5–30)	3.03 (0.53–7.2)	
ii) Smoking and elevated P-selectin			
N	66	14	
Mean ± SD	13.46 ± 13.54	2.72 ± 1.03	0.0042
Median (range)	8.24 (0.5–54.9)	2.78 (0.51–4.56)	
iii) Diabetes mellitus and elevated P-selectin			
N	67	7	
Mean ± SD	11.09 ± 13.43	5.27 ± 3.52	0.2596
Median (range)	3.38 (0.08–54.9)	3.95 (0.42–12.1)	
iv) Low HDL-C and elevated P-selectin			
N	87	19	
Mean ± SD	10.43 ± 11.43	2.924 ± 2.73	0.0055
Median (range)	6.72 (0.5–54.9)	1.4 (0.5–8.7)	

N represents number of subjects in the group

[a] Chi-square analysis with odds ratio and confidence interval of entire population

[b] P-selectin exposure in the entire population with mean, median and *p* value by Student's '*t*' test

[c] P-selectin exposure in patients and controls with conventional risk factors with mean, median and *p* value by Student's '*t*' test

thrombotic factor antithrombin III in patients ($r = -0.4$; $p < 0.001$) and controls ($r = -0.5$, $p < 0.001$). Further correlation analysis revealed no relation between P-selectin exposure and other thrombotic risk factors [fibrinogen, Lp(a) v-WF, PAI-1, t-PA and homocysteine].

Multiple logistic regression analysis of increased percent expression of P-selectin exposure and other thrombotic risk factors [high fibrinogen, low antithrombin III, high lipoprotein(a), high von Willebrand factor and plasminogen activator inhibitor-1], showed an odds ratio of 11.5 at 95 % CI 0.93–142.27 ($p < 0.057$) for increased P-selectin exposure, indicating it as an important risk factor along with antithrombin III Lp(a) and fibrinogen (Table 3).

Detailed results of the levels of thrombotic risk factors [fibrinogen, lipoprotein(a), PAI-1, t-PA, v-WF, homocysteine and antithrombin III and its variation among patients vs. controls] were published earlier [10].

Platelet activation and conventional risk factors

There was significant difference in conventional risk factor profile between patients and controls (Table 1a, b). When platelet activation in patients and controls were compared, 70–87 % of patients with conventional risk factors had high platelet activation. Controls with conventional risk factors also showed a trend toward increased P-selectin exposure, but median values were lower compared to

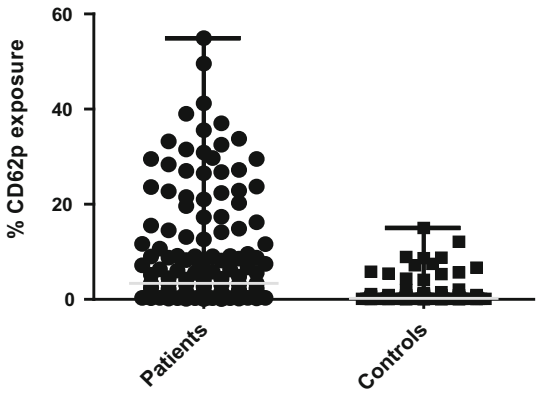

Fig. 3 Percentage CD62p exposure in patients versus controls: median (range): 3.44(0.06–54.9) versus 0.23(0.01–15). *Gray line* represents the median value

patients, but in the case of diabetes subjects there was no significant difference between P-selectin exposure in both patients and controls and median values were similar in patients and controls (Table 2b).

Platelet activation and mode of presentation of CAD

A trend of difference in platelet activation was observed, when the mode of presentation of CAD was compared. Majority of the patients (75 %) presented in our group with acute coronary syndromes (ACS), and rest of the patients presented with effort angina (EA). Mean and median P-selectin exposure was elevated in those who presented with ACS [mean ± SD 11.43 ± 12.4; median (range):6.54 (0.5–54.9)] compared to EA [mean ± SD 10.31 ± 12.27; median (range):3.2 (0.52–39)], but this did not reach statistical significance ($p = 0.69$). Similarly, when coronary angiograms were classified into mild (normal to single vessel disease) and severe (double to multi-vessel disease) CAD, P-selectin exposure levels were found high in those with severe CAD [mild CAD-mean ± SD: 9.7 ± 12.24, median (range): 4.54 (0.5–54.9); severe CAD: mean ± SD 12.11 ± 12.4, median (range):7.2 (0.52–49.6)]. Here also difference did not reach statistical significance ($p = 0.3$).

The total event rate was calculated as 6 % in the patient group with two deaths, one admission with ACS, four coronary artery bypass graft (CABG) surgery and three patients requiring repeat angiography. Correlation between event rate and P-selectin expression was not practicable as the event rate was only 6 % at a mean follow-up period of 26.23 ± 5.4 months with a median of 26 months.

Discussion

Platelet activation is a critical component in the pathogenesis of atherosclerotic CAD [1]. Various components released by activated platelets which include cytokines, chemokines, matrix degrading enzymes and thrombotic factors may eventually cause plaque rupture and atherothrombosis. Activated platelets also contribute to arrhythmias, myocardial dysfunction and vasoconstriction by the release of reactive oxygen species. Variety of markers for platelet activation have been established and CD62p (P-selectin) is one of them [20].

P-selectin is the largest of the selectins (140 KD), which are generally stored in the alpha granules of the platelets. Subsequent to platelet activation, exposed P-selectin can bind with P-selectin glycoprotein ligand-1 (PSGL-1) of monocytes. These aggregates can further enhance activation of monocyte, facilitating activation of inflammation and thrombosis [1]. Platelet rolling and platelet endothelial adhesion are also mediated by P-selectin expression upon platelet activation [21].

Soluble P-selectin in serum/plasma [22] and exposure of P-selectin on platelets upon induction by platelet activating agents and platelet reactivity [23] have been reported to be enhanced in Indians. A study on hematological, immunological and cardiovascular changes on people residing in Delhi (one of the most polluted cities in India) revealed an increase in P-selectin exposure compared to non-polluted regions in the country [11]. In our study, percentage expression of P-selectin on platelets without any stimulation with platelet activating factors

Table 3 Multiple logistic regression analysis of P-selectin expression and thrombotic risk factors with adjusted odds ratio

Parameters	Odds ratio	*p* value	95 % CI
High fibrinogen	7.92	0.058	0.93–67
High P-selectin exposure/platelet activation	11.50	0.057	0.93–142.27
Low antithrombin III	6.16	0.046	1.03–36.73
High lipoprotein(a)	8.49	0.044	1.058–68.14
High V-WF	1.81	0.227	0.69–4.76
High PAI-1	0.09	0.025	0.01–0.74

Homocysteine and t-PA were not analyzed for multiple logistic regression as their sample size was low

CI confidence interval

(ADP, collagen and thrombin) was higher in patients compared to controls.

Platelet activation as evidenced by exposure of P-selectin on platelets was significantly elevated in our patients, even though they were on anti-platelet drugs and statins. There was also a trend towards increased platelet activation among patients who presented with acute coronary syndrome compared to those who presented with effort angina, though the difference was not significant. A study on platelet function of aspirin-treated patients undergoing cardiac catheterization by Linden et al. [24] showed similar trends as in our study.

Many of our patients had percentage platelet activation above 10 %, while the normal value is near to zero. The levels of platelet activation in controls were much lower, indicating the severity of inflammatory and thrombotic process, which might be prevalent in the patients.

The results from the present study indicate that platelet activation by P-selectin exposure was high in patients who had conventional risk factors. The platelet activation levels were lower in controls versus patients who had conventional risk factors. But number of controls with conventional risk factors was low and there may be a possibility of some amount of bias in the analysis.

In subjects with diabetes, whether they were patients or controls, platelet activation were higher. In a study from Chennai (India) on patients with CAD and diabetes, collagen-induced GP IIb/IIIa binding (another marker for platelet activation) was significantly higher [5].

Patients with low HDL-C in our study had elevated P-selectin exposure which supports recent reports which substantiated the role of HDL-C in cardio-protection as well as reducing platelet activation [25].

Smoking is also an important contributor to elevated platelet activation as almost all smokers (both patients and controls) had elevated P-selectin exposure, though the percentage of P-selectin exposure was lower in controls. Elevated P-selectin was reported in smokers who were on low dose aspirin by an earlier study [26]. In our study, P-selectin exposure was also elevated in patients with positive family history of CAD and hypertension, in support of previous data [8, 9]. Thus, our results indicate that conventional risk factors have an influence on P-selectin exposure.

Now we shall be discussing the relation between thrombotic risk factors and P-selectin exposure. Correlation analysis of P-selectin exposure with thrombotic risk factors showed that P-selectin was negatively correlated with the levels of antithrombin III (which is an antithrombotic factor having inhibitory effects on thrombosis and inflammation) among our subjects. This provides evidence to the fact that increased P-selectin levels and low antithrombin III levels, act synergistically in the progression of thrombotic–inflammatory process [27, 28].

No correlation was observed between other thrombotic risk factors (fibrinogen, Lp(a), PAI-1, t-PA, homocysteine and v-WF) and P-selectin exposure. Treatment with anti-platelet drugs, statins, beta blockers and angiotensin-converting enzyme inhibitors (ACEI)/angiotensin receptor blockers (ARB) might have resulted in attaining similar levels of the thrombotic risk factors such as PAI-1, v-WF, homocysteine and t-PA between patients and controls. This could be the probable reason for lack of correlation between P-selectin exposure and the above markers.

A trend towards increased platelet activation was seen in patients presented with ACS versus stable angina as well as patients with multi-vessel disease versus single vessel disease, though the difference was not significant. A previous study on relation of platelet activation to coronary angiographic severity also could not establish a significant difference between platelet activation and coronary angiographic severity [29].

Stellos et al. [30] have also reported that platelet-bound P-selectin expression was heightened in patients with CAD. They also showed that those who presented with ACS had higher levels of platelet activation compared to stable angina. The results were independent of age, gender and baseline medications of aspirin and clopidogrel received by the patients. The patients in that study were followed up for a period of 3 months and they also reported very low event rates. The study by Stellos et al. [30] showed many similarities to our study even though the follow-up period was longer in our study.

The study also found that molecular markers of myocardial necrosis and infarct size correlated with P-selectin expression [30]. This explains the clinical relevance of P-selectin expression in patients. The low event rate of 6 % in our patients may be due to meticulous follow-up they were undergoing as they were part of a study and were in regular contact with physicians. All these ensured regular monitoring and control of risk factors and also ensuring drug compliance.

A study on platelet function profiles on diabetic and non-diabetic patients with CAD on combination therapy with aspirin and clopidogrel undergoing elective percutaneous intervention revealed that diabetic patients had increased platelet reactivity and many of them had unresponsiveness to clopidogrel [31]. Majority of our patients were diabetic and our patients also showed and heightened P-selectin expression.

There is also a report of influence of the anti-cholesterol drug, statin on P-selectin expression. Statins can attenuate increase in P-selectin expression produced by exercise [32]. Though our patients were on statins, the platelet reactivity was still high indicating the need for further therapeutic avenues.

The study was novel as it was the first study from the region which evaluated P-selectin expression as a marker

of platelet activation in patients with angiographically proven CAD on treatment with anti-platelets and statins. Also in this study, we correlated the levels of P-selectin expression with conventional coronary risk factors and thrombotic markers. P-selectin is reported as a trigger for many thrombotic and inflammatory pathways and its role in adverse coronary events is also established [30].

There is a clear-cut evidence on the role of P-selectin in thrombotic and inflammatory pathways, mainly contributed by thrombin activation with its effects on platelets and leukocytes [33]. Thus, our study substantiate the fact that platelet mediated thrombotic–inflammatory processes are operational in CAD, even in patients who are on treatment with anti-platelet drugs and there is a need for newer therapeutic strategies that specifically target P-selectin exposure which forms a bridge between platelet and inflammatory cells, especially endothelium and monocytes as indicated by other studies [34].

Conclusion

P-selectin exposure, a marker for platelet activation, was elevated in patients on treatment compared to controls. The exposure of P-selectin on platelets was related to conventional risk factors and showed negative correlation with anti-thrombotic factor antithrombin III. It had its influence on angiographic severity and acute coronary syndrome indicating the clinical relevance of the marker, even though the event rate was low for the follow-up period. The relation of P-selectin with adverse coronary events and lack of effect of optimal anti-atherosclerotic medications on P-selectin exposure on platelets points to the need for new therapeutic avenues especially targeting platelets and its activation mechanisms.

Future directions

Since the role of current treatment regimes in the control of platelet activation is limited as we found in our study, we should explore newer therapeutic avenues targeting platelets. Monocyte-platelet aggregates are considered to be a more reliable marker for platelet activation, which needs to be evaluated in future studies. Additionally role of thrombin activation on platelet function and its therapeutic prospects has to be elaborated further.

Strengths and limitations of the study

Our study evaluated platelet activation, indicated by P-selectin exposure in CAD patients on treatment in comparison to healthy controls, along with other thrombotic and conventional risk factors. We also compared angiographic profile and mode of presentation, a novel approach compared to other studies from the region.

Major limitation of the study was that we have not compared the effect of P-selectin exposure on various inflammatory pathways. We also did not have patients who are not on anti-platelet drugs and statins as it could be unethical to withhold these drugs under our hospital circumstances. This is a case–control study; some of the results might be influenced by the selection of subjects as the sample size was small. There was wide range variability in P-selectin exposure in both patients and controls. Patients were having multiple risk factors, both thrombotic and conventional, while controls had very few risk factors. Gender bias was evident in our sample selection as majority of the patients were males, this prevented us from performing gender specific studies. The period of follow-up was also very minimal which prevented authors to reach definite conclusion regarding event rate and P-selectin exposure.

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Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest in any form.

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